Australian Government
Department of Health and Ageing
Office of the Gene Technology Regulator

The Biology of *Brassica napus* L. (canola)

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This document provides an overview of baseline biological information relevant to risk assessment of genetically modified forms of the species that may be released into the Australian environment. Cover photo courtesy of Brian Weir.

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PREAMBLE

This document describes the biology of canola (*Brassica napus* L.) with particular reference to the Australian environment, cultivation and use. Information included relates to the taxonomy and origins of cultivated *B. napus*, general descriptions of its morphology, reproductive biology, biochemistry, and biotic and abiotic interactions. This document also addresses the potential for gene transfer to occur to closely related species. The purpose of this document is to provide baseline information about the parent organism for use in risk assessments of genetically modified canola that may be released into the Australian environment.

The term 'canola' refers to those varieties[1] that meet specific standards on the level of erucic acid and glucosinolates. The word 'canola' is derived from '**Can**adian **o**il, **l**ow **a**cid' and was registered in Canada in 1970. The canola name is now used for three *Brassica* species: *B. napus* also known as 'Argentine variety', *B. rapa* also known as 'Polish variety' and *B. juncea* or mustard. Canola has been grown in Australia since 1969, however, rapeseed or oilseed rape (also *B. napus*), which did not meet the current canola standards, had been grown in Australia from the early 1960's. Canola is grown primarily for its seeds, which yield between 35 % to over 45 % oil. Its main use is as cooking oil, but it is also commonly used in margarine. Canola meal is produced as a by-product during extraction of oil from canola seed and is widely used as a high protein feed source in animal nutrition.

SECTION 1 TAXONOMY

The Brassicaceae family (formerly Cruciferae) consists of approximately 375 genera and 3200 species of plants, of which approximately 52 genera and 160 species are present in Australia (Jessop & Toelken 1986). Of the 160 species of Brassicaceae present in Australia, several species are important weeds of the southern Australian cropping zone. Genera of economic importance in Australia are *Brassica* as a crop and *Raphanus*, *Sinapis*, and *Brassica* as weeds. In Australia, other important cropping weeds from the Brassicaceae family include *Hirschfeldia incana*, *Diplotaxis* spp. and *Sisymbrium* spp. (Rieger et al. 1999).

The *Brassica* genus consists of approximately 100 species, including species *Brassica napus* L., spp. *oleifera*, commonly known as oilseed rape, rapeseed or canola. *B. napus* is not native to Australia, and originated in either the Mediterranean area or Northern Europe. It is thought to have originated from a cross where the maternal donor was closely related to two diploid species, *B. oleracea* and *B. rapa* (OECD 1997).

The botanical relationship between the Brassica oilseed species was first established as a result of taxonomic studies carried out in the 1930s (U 1935) (Fig 1). It was proposed that the three species with higher chromosome numbers, *B. juncea*, *B. napus* and *B. carinata*, are amphidiploids (double the number of chromosomes) derived

[1] The terms *variety* and *cultivar* are often used interchangeably in literature to designate a group of cultivated plants of significance in agriculture, forestry or horticulture, which have distinct and heritable characteristics. The term *cultivar* is a contraction of "**culti**vated **var**iety" and is synonymous with the term *variety* (Hartmann & Kester 1975). The term *variety* is used throughout this document.

from the diploid species, *B. nigra* (L.) Koch, *B. rapa* (syn *B. campestris*) and *B. oleracea* L.

The cytogenetic relationships of Brassica species show that:

- *B. carinata* is an amphidiploid (BBCC[2], n=17) probably arising from *B. oleracea* (CC, n=9) and *B. nigra* (BB, n=8);
- *B. napus* is an amphidiploid (AACC, n=19) of *B. oleracea* and *B. rapa* (AA, n=10), and
- *B. juncea* is an amphidiploid (AABB, n=18) of *B. rapa* and *B. nigra*.

The cytogenetic relationship between the Brassica species established by (U 1935) was later confirmed by chromosome pairing and artificial synthesis of amphidiploids, nuclear DNA content, DNA analysis and the use of genome-specific chromosome markers. Although it was proposed that the three diploid species have originated from one common ancestor, recent molecular investigations indicate a common origin for *B. rapa* and *B. oleracea*, with *B. nigra* having evolved from a separate progenitor (Paterson et al. 2006; Sabharwal et al. 2006)

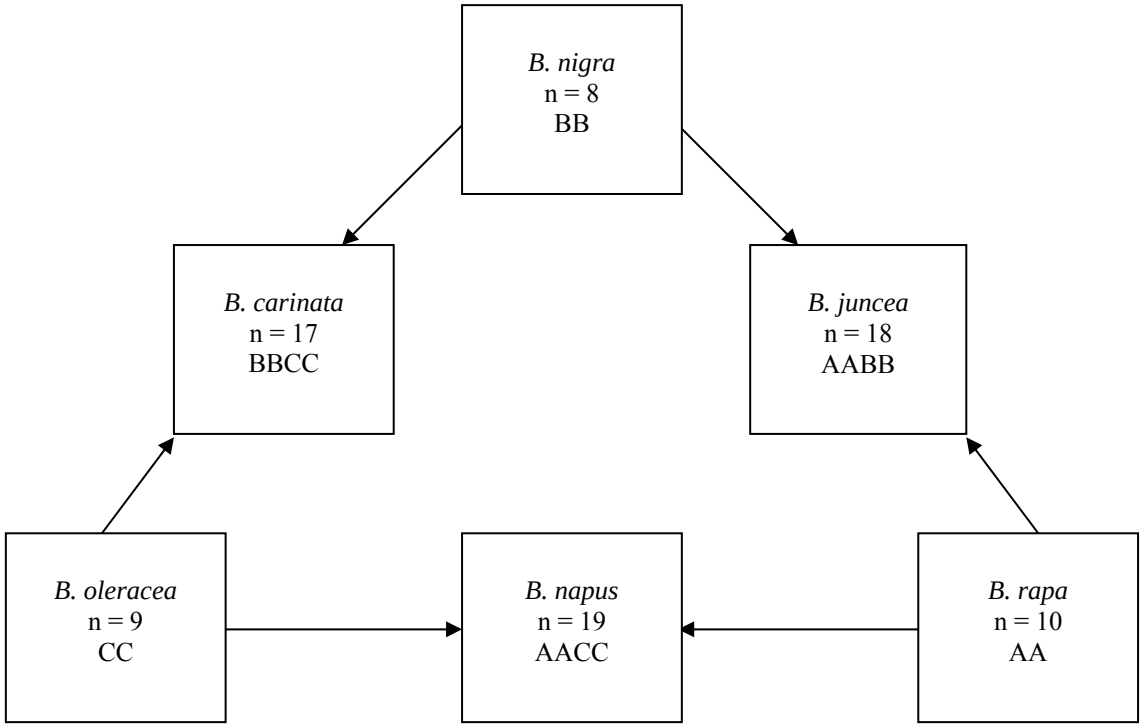

Fig.1 Genomic relationship of main cultivated *Brassica* species.

SECTION 2 ORIGIN AND CULTIVATION

2.1 Centre of diversity and domestication

Brassica napus was cultivated by ancient civilisations in Asia and the Mediterranean. Its use has been recorded as early as 2000BC in India (Colton & Potter 1999) and has been grown in Europe since the 13th century, primarily for its use as oil for lamps

[2] Genomic designation

(Colton & Sykes 1992). *B. napus* was first grown commercially in Canada in 1942 as a lubricant for use in war ships. It was first grown commercially in Australia in 1969.

Traditionally, in western countries, *B. napus* was considered unsuitable as a source of food for either humans or animals, because the seed naturally contains erucic acid and glucosinolates, which are toxic to humans and other organisms (see Section 5). However, it was widely used as an edible oil in Asia for thousands of years (OECD 1997). In the 1970s, very intensive breeding programs in several countries including Australia produced high quality varieties that were significantly lower in these two toxicants. The term 'canola' refers to those varieties of *B. napus* that meet specific standards on the level of erucic acid and glucosinolates. These varieties must yield oil low in erucic acid and meal low in glucosinolates and are often referred to as double low varieties.

2.2 Commercial uses

Canola has become more important to the western world, through breeding for better oil quality and improved processing techniques (OECD 1997). Edible oil was first extracted in Canada in 1956 (Colton & Potter 1999). Canola is now grown primarily for its seeds, which yield between 35 % to over 45 % oil. Its main used is as cooking oil, but it is also commonly used in margarine. Although normally grown as an oilseed crop, it may be profitable for canola to be cut for hay in spring if demand is high (Pritchard et al. 2007).

Canola meal is produced as a by-product during the extraction of oil from canola seed and is widely used as a high protein feed source in animal nutrition. Full fat canola seed may also be used directly as animal feed (Roth-Maier 1999). Industry standards require canola meal to be low in glucosinolates (total glucosinolates of 30 μmoles g^{-1}) in toasted oil free meal (OECD 2001). The maximum level for erucic acid is 2% in the oil fraction (CODEX 2001). Note that oil from varieties of *B. rapa* or *B. juncea,* which also meet these standards, may also be referred to as canola.

2.3 Cultivation in Australia

2.3.1 Commercial propagation

Canola reproduction is through seed production. Generally seeds of a canola variety can be saved and used to plant subsequent crops, with the exception of hybrid canola (see Section 2.4.2). However, saving seed can result in poor seed viability and establishment failure in subsequent crops. Outcrossing in canola can result in slight genetic change from year to year and considerable change over a number of years. Experiments have shown that over time, farmer-retained seed can have reduced oil quality, yield and other agronomic performance (Marcroft et al. 1999).

Canola seed of high varietal or genetic purity is produced following a seed certification scheme based on the Rules and Directives of the OECD Seed Schemes and International Seed Testing Association (ITSA) (Smith & Baxter 2002). There are two main production classes for pure seed, either certified or basic seed. The production of basic seed requires an isolation of 200 m from other varieties or any other Brassica or cruciferous crop or weed species. Additionally, the land used for basic seed production must not have grown or been sown to canola or another *Brassica* or Cruciferous crop species for the previous five years, unless it was the

same variety or certification class. The production of certified seed requires an isolation distance of only 100 m and the same land use restrictions as for basic seed production, but only extending for the previous three years. Seed must be at least 99% pure (by mass), have a minimum germination of 85% (by count) and contain no more than 20 other seeds per kg (Smith & Baxter 2002).

2.3.2 Scale of cultivation

Canola production grew significantly in Australia from approximately 100,000 ha in the early 1990s to an estimated total area of 1.4 Mha in 2000 (Colton & Potter 1999). The five year average (to 2004/2005) area planted to canola was 1.335 Mha, with an average production of 1529 kt. Typically 400 to 500 kt are used domestically, nearly all of this is crushed for oil production, with seed production accounting for 5 to 6 kt (ABARE 2007). Current production estimates for the 2007/8 crop year are for 1399 kt (ABARE 2007). Internationally, production by the largest producers in 2004, was Australia 1.55, United Kingdom 1.61, Poland 1.63, France 3.97, Germany 5.23, Canada 7.73, and China 13.18 million tonnes (FAO 2006). Australia accounts for < 5% of the world's canola production, but it is second only to Canada as an exporter of canola seed (Carr 2005).

Canola occupies approximately 6 % of the cropped area in New South Wales, Victoria, South Australia and Western Australia (Norton et al. 1999) and these states account for more than 99% of Australia's total canola production (based on the 5 year average to 2004/2005) (ABARE 2007). Production in Australia by state was 38% WA, 22% NSW, 22% Victoria, and 17% SA (based on the 4 year average to 2005/006). Total production over this 4 year period was 5,383,000 tonnes on 4,056,000 ha, for an average of 1.327 tonne per ha (AOF 2007b).

In Australia, canola is an established crop in the medium and high rainfall (400 mm and above) areas of southern Australia, which represents the winter production cereal belt (see Table 1, Figure 2). However the development of early maturing varieties is expanding growing areas of canola into the low rainfall areas of the wheat belt.

Table 1. Climatic/soil type data for areas where canola is grown

	Wagga Wagga (NSW)	Hamilton (VIC)	Mt Gambier (SA)	Minnipa (SA)	Merredin (WA)
Average daily max/min temperature[a] at planting (April-May)	19.9°C/7.5°C	17.2°C/7.8°C	17.8°C/8.0°C	17.1°C/10.7°C	22.9°C/10.9°C
Average daily max/min temperature (winter)	13.6°C/3.3°C	12.6°C/4.9°C	13.7°C/5.4°C	16.7°C/6.8°C	16.9°C/5.9°C
Average daily max/min temperature (spring)	21.3°C/7.8°C	17.9°C/8.6°C	18.5°C/8.0°C	23.9°C/10.1°C	24.4°C/9.7°C
Average Annual rainfall	568.4 mm	686.7 mm	774.9 mm	327.3 mm	327.3 mm
Rainfall May-November (% of Annual Rainfall)	363.9 mm (64%)	481.7 mm (70%)	574.0 mm (74%)	244.5 mm (75%)	239.8 mm (73%)
Soil type	reddish sandy loam	Acid basaltic clay	Volcanic sands/ sandy loam	reddish brown sandy loam, highly alkaline	Red-brown sandy loam to sandy clay loam

[a] Temperature and rainfall from Bureau of Meteorology: http://www.bom.gov.au/climate/averages/

Each state has an appropriate government agency (eg Department of Primary Industry), which tests and recommends varieties suitable to the canola growing regions of the state. For example, the "2005 Crop Variety Sowing Guide for Western Australia" lists characteristics of 41 major canola varieties comprising 14 triazine tolerant, 7 imidazolinone–tolerant, 16 conventional and 3 hybrid varieties, for the grainbelt of WA. Variety characteristics include flowering class (early to late), height, blackleg resistance, oil content and suitability for various rainfall zones. Information on new canola varieties being trialled in Australia can be found at the National Variety Trial – Online website (NVT Online 2007).

2.3.3 Potential for expansion of the Canola growing region

Canola has been grown in northern NSW and southern Qld, reaching approximately 15,000 ha in the early 1990s, but then due to frost damage and several drought years the area declined. The problems facing canola production in this area were reported to be variable climatic conditions (particularly frost during early pod filling), poorly adapted varieties, poor establishment and inadequate nutrition. Growers' perception was that canola was poorly adapted to these northern areas and it was noted that canola suppressed establishment and growth of subsequent sorghum and other summer crops. However, a decade later canola production had risen to 25-30,000 ha (most of this in NSW) – due to increased grower experience and greater variety choice, which allowed a reduction in the risk of frost loss at flowering time. Limitations to further canola increases for this area were identified as the need to design methods of harvest management to overcome large harvest losses (up to 90%) and the distance to market. (Holland et al. 2001)

Further expansion of up to 150,000 ha in northern NSW and an additional 50,000 to 175,000 ha in southern Queensland may be possible through the introduction of improved canola and Indian mustard varieties with higher oil contents, virus tolerance in mustard, a better understanding of nutritional requirements and reaction to frost, and rotation implications for following summer crops or winter cereals. A strong desire to have more rotation crops to help overcome crown rot and other disease problems in wheat is one of the main motivators for expansion of canola an/or Indian mustard in these areas. (GRDC 2007b)

Canola has typically been grown in areas of at least 450 mm rainfall in WA, but experience in WA has shown that canola can also be grown profitably in the lower rainfall (approximately 325 mm) areas of the northern grainbelt (Carmody & Cox 2001) Profitability depended upon a number of interrelated factors; the most limiting being the timing of opening rainfall and high temperature during pod fill. Other factors
included weed competition, soil acidity, fertiliser timing, blackleg disease, insect pests and harvest management. Managing the main limiting factors were the key to profitable canola production in the northern grainbelt of WA (Carmody & Cox 2001).

Canola has not been grown commercially in the Northern Territory (NT) but has recently been trialled at the Katherine Research Station as one of nine crops to identify bio-fuel crops agronomic adaptation to the area. These crops, including canola, were selected because they had either never been grown in the NT, or on clay loam soils or established under dry season irrigated conditions in Katherine (Bennett et al. 2006).

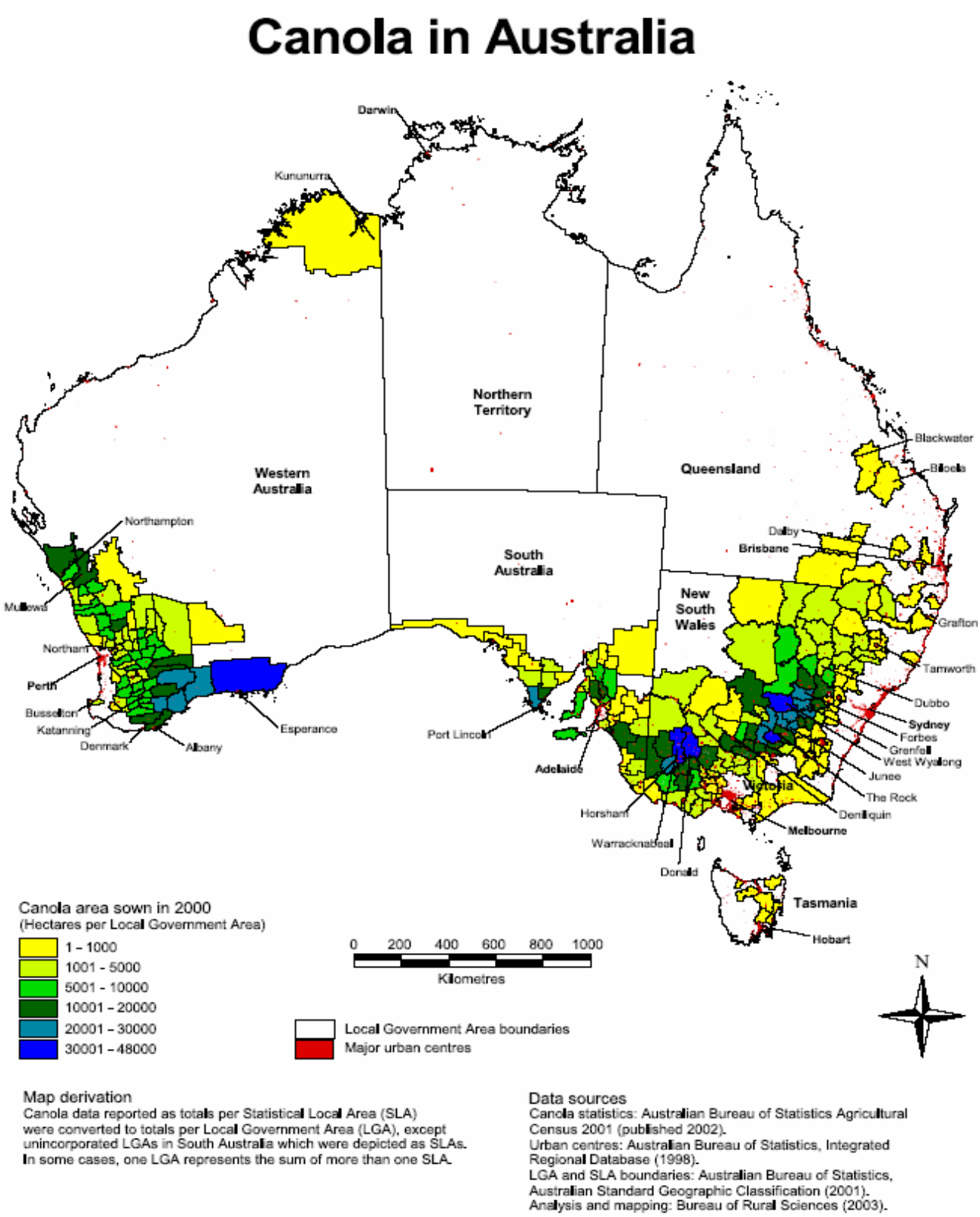

Figure 2. Map showing canola areas sown in Australia for year 2000 (BRS 2003). Local government areas (LGAs) are coloured according to the amount of canola sown. Typically, canola is not sown in northern WA and production in Queensland and Tasmania accounts for <1% of total Australian production.

2.3.4 Cultivation practices

Canola is mostly grown as a winter annual in winter dominant rainfall environments between 30°S and 38°S (Norton et al. 1999). Only spring type canola varieties are grown in Australia and unlike winter varieties, do not need vernalisation (winter chilling) to flower, although vernalisation speeds up flowering. Rain-fed crops are sown with the onset of significant rain in April or May. Canola varieties flower for a 6-week period with crops ripening in late spring or early summer, after a 5 to 7 month growing season (Walton et al. 1999). This compares to a considerably longer growing season in Europe, which lasts for 12 months (due to a vernalisation requirement) and a rather short growing season (due to long day length and warm temperatures) in Canada, which extends for less than 4 months (Walton et al. 1999).

Small areas of canola are sown in late spring-early summer in more temperate regions. These crops are located in areas that receive reliable rainfall, or have access to irrigation during summer as well as experiencing cool to mild temperatures at flowering (Norton et al. 1999). Summer grown canola crops are harvested in early autumn.

The average sowing rate tends to be between 4 and 6 kg ha^{-1}, with hybrid seed sown at 3 kg ha^{-1}. These sowing rates are used to achieve a plant population of approximately 50 to 70 plants m^{-2} (Walton et al. 1999). Under optimal soil moisture for germination, canola seed is sown at 2 to 4 cm depth, which gives rapid emergence. When soil moisture is dry and soil temperatures high, seed can be sown into more moist areas of the soil, at depths up to 6 cm (Walton et al. 1999). However, this depth can result in poor emergence, growth and yield. When sufficient moisture is not available at 5 cm, a common practice is to 'dry seed', that is to sow at a shallow depth and wait for rain (Oilseeds WA 2006). Emergence depends upon temperature, soil moisture and seeding depth (see section 4.4.1).

The growth of canola and its seed yield in Australia is almost always limited by the amount of water available to the crop, particularly during seed maturation (Walton et al. 1999). In Australia, yields for broad acre production average 1 to 2 tonnes ha^{-1} but range up to approximately 5 tonnes ha^{-1} in areas with a long, cool growing season and adequate moisture (Walton et al. 1999). Average yield in Australia over the 4 year period to 2005/06 was 1.327 tonne per ha (AOF 2007b).

Canola has a higher requirement for nitrogen, phosphorus and sulphur than cereals and other crops and will not produce high yields unless all three elements are adequately supplied. Canola needs approximately 40 to 50 kg of nitrogen (30% more than wheat), 8 kg phosphorus and 10 kg sulphur per tonne of grain produced (Colton & Sykes 1992).

Australian canola varieties are reasonably frost tolerant. Seedling losses may occur due to frosts and unusually late frosts after flowering can result in aborted seeds and reduced yields (Walton et al. 1999).

The canola crop is harvested in early summer when the seeds have reached their maximum dry weight and the crop can be windrowed (swathed). The majority of canola crops are swathed whereby the crop is cut and placed in rows. This process hastens the drying rate of the crop, reduces the possibility of seed losses from wind or

hail and ensures even ripening. At this time, seeds have good storage characteristics due to low moisture, and are of high quality due to low chlorophyll and free fatty acids (Walton et al. 1999). Swathing occurs when approximately 40 to 70% of seeds start to change from green to their mature colour and seed moisture is approximately 35 % (Oilseeds WA 2006). Pick up of the swath and threshing of the canola occurs approximately 7 to 10 days after swathing, when the moisture content of the black seed is 8.5% or less (Walton et al. 1999).

As an alternative to swathing, the canola crop can be direct harvested. Direct harvesting works well in small or low-yielding areas with uniform maturity and moisture content. The crop will be ready when the majority of pods are dry and rattle when shaken. Direct harvest can also occur after application of chemical desiccants. Chemical desiccation may be an option for canola harvest, in cases where herbicide resistant weeds are a problem, where there is uneven ripening of the crop, or where access to a swather is limited. However, direct harvesting may result in reduction of yield and/or quality and the best option for maximising yield and quality is swathing. (Carmody & Cox 2001)

Canola can be one of the most profitable crops available to grain growers in southern Australia and provides the opportunity for farmers to use more diverse cropping rotations. Canola is usually grown in rotation with wheat as the follow on crop. Like many other broadleaf crops, canola provides an important disease break during which the inoculum of cereal pathogens such as the take-all fungus (*Gaeumannomyces graminis*) decline (Norton et al. 1999). Canola root exudates have been reported to have biofumigation effects on fungal inoculum (Kirkegaard et al. 1994; Kirkegaard et al. 1998). Studies have shown that the root system of canola has beneficial effects on soil structure and soil moisture infiltration, resulting in higher yield and protein levels in the following cereal crop (Norton et al. 1999).

Canola is often the first crop grown following a pasture and benefits from the nitrogen fixed by legumes during the pasture phase. The subsequent crops following canola are generally wheat followed by a second wheat crop or a pulse, and then another cereal. The chosen pulse could be lupins (e.g. Western Australia) or field peas (e.g. western Victoria and South Australia). Due to the poor returns from pulses, alternating crops of canola and wheat are becoming more common, particularly in some regions of NSW (Norton et al. 1999).

2.4 Crop Improvement

The major issues facing *Brassica* oilseed production in Australia over the next few years include maintaining blackleg resistance and the development of additional fungicidal controls; enhancing understanding and developing controls for sclerotina; developing resistance or control measures for white rust (mainly western Australia); maintaining or increasing canola yields in low rainfall areas; developing *juncea* canola or mustard in lower rainfall areas; determining if hybrid canola can increase production over open-pollinated varieties; and reducing input cost for growers (nutrient and/or water use efficient varieties) (Potter et al. 2007; Amjad et al. 2007). Recently the National Brassica Germplasm Improvement Program (NBGIP) was established for the development of germplasm incorporating new or enhanced traits for the Australian canola industry. The key traits currently identified by the NBGIP are alternative sources of blackleg resistance, water use efficiency (drought tolerance),

shatter resistance, frost tolerance during seed improvement, and oil content stability/increased protein (Salisbury et al. 2007).

2.4.1 Breeding

Public programs for rapeseed breeding began in the early 1970s and focused on development of *B. napus* and *B. rapa* for Australia, with the earlier maturing *B. rapa* targeted at the lower rainfall areas. Initial goals were to develop open-pollinated varieties that were high yielding and blackleg resistant. By the mid-1970s work on *B. rapa* was discontinued and was replaced in the late 1970s by breeding programs incorporating *B. juncea*. These programs were aimed at producing canola quality *B. juncea* for lower rainfall areas (Salisbury & Wratten 1999) and was considered an important step in addressing the general decline of canola in southern Australian grain production (GRDC 2006b). The first canola quality *B. juncea* variety "Dune" for Australia was released in 2007 (Burton et al. 2007).

Early canola varieties were introduced into Australia from Canada and were poorly adapted to the short days of the winter-spring growing season. One of the earliest aims of Australian breeders was to understand the flowering response and delay the onset of flowering until after a satisfactory leaf canopy had developed (Walton et al. 1999). Breeders also recognised that growth and yield of canola would almost always be limited by water availability, particularly during seed set and maturation. Thus, developing ways to measure and improve water use efficiency was and is a major focus in canola breeding (GRDC 2007c).

Canola varieties introduced toward the end of last century have glucosinolate levels less than half the maximum value for canola quality. Since the introduction of canola quality, the oleic acid content of Australian canola varieties has remained relatively constant at approximately 60%. However, selection has been under way to further enhance oleic acid levels and reduce linolenic acid, which would increase oil stability for specific applications. Selection for reduced saturated fatty acid content is also underway (Salisbury & Wratten 1999). Specialty canola oils with high oleic acid (70%) and low linolenic acid (<3.5%) content have been developed with enhanced oil stability for frying compared to standard canola oil (Gororo 2007). Although international standards for canola oil require low levels of erucic acid, specialty *Brassica* varieties with high erucic acid levels are being developed for use as condiment mustard and biofumigation. In addition, breeders have applied additional selection pressure to increase oil and protein content to remain competitive in the export market. This increased selection pressure has resulted in the release of several mid-season varieties and early varieties targeting the low rainfall areas with high oil and protein content (Salisbury & Wratten 1999).

In the early 1970s, a blackleg epidemic made it clear that resistant varieties would have to be developed if the industry were to survive. Since the late 1970s Australian breeders have release a number of resistant lines. By the mid-1990s, Australian mid-season varieties had the highest levels of blackleg resistance of any spring canola varieties in the world. When grown with appropriate crop rotation, losses to blackleg became negligible. The source of the blackleg resistance came from Japanese spring and French winter material. Breeders continue to look for resistance in related species (*B. nigra, B. juncea* and *B. carinata*). Attempts to introduce resistance from a range of more distantly related wild crucifer species into *B. napus* has so far been

unsuccessful due to lack of introgression of the resistance genes into the *B. napus* genome (Salisbury & Wratten 1999). Breeders are currently looking for resistance to other diseases such as sclerotinia and white rust in *B. napus* and *B. juncea* germplasm from India and China (GRDC 2007a).

Breeders have also developed varieties which are shorter and more resistant to lodging and shattering than the earlier canola varieties. Reduced plant height decreases the risk of lodging and makes windrowing easier. Selection for shattering resistance would enable direct heading of canola (Salisbury & Wratten 1999). Improvements in all these agronomic traits would increase yield, as considerable seed loss can occur due to lodging, shattering and the extra handling during windrowing.

Development of hybrid canola

Traditional plant breeding selects for plants with characteristics of agronomic value, but repetitive self-pollination can produce inbred plants that may display lowered fitness or vigour as compared with their non-inbred counterparts. The converse of this, hybrid vigour, can occur when the progeny from crosses of genetically distinct parents outperform the parental lines in yield, increased resistance to disease and enhanced agronomic performance.

An historic example of hybrid seed production is hybrid corn seed, which is produced by crossing inbred corn lines. The technology to produce hybrid corn seed has been available to farmers in the US for more than 80 years. Corn is monoecious, with separate male and female flowers, which physically facilitates hybrid seed production. Mechanical removal of the male flower (tassel) from one inbred line (female) allows for wind-borne cross pollination from another inbred line (male) and the production of hybrid seed. Mechanical removal of the tassel was no longer required when genes for cytoplasmic male sterility (CMS) and fertility restoration of corn were identified in the 1940's.

Cytoplasmic male sterility (CMS) has been identified in many other crops including green beans, sorghum, beet, carrot, *B. napus*, sunflower and wheat. Several CMS lines have been developed in the crop *Brassica* species through the production of alloplasmic types of cultivated species following wide hybridisation (Malik et al. 1999).

The first conventional (non-GM) canola hybrids based on the CMS system were released in Australia in 1988 – by Pacific Seeds. An initial limitation to their cultivation has been that they have not consistently out yielded conventional varieties sufficiently to justify the higher seed costs. However, a number of hybrid canola varieties, with better or equal yields to conventional varieties are currently available to growers (Potter et al. 2005; McCaffery et al. 2006).

Bayer CropScience has utilised gene technology techniques to develop hybrid seed from two distinct parents. Bayer's hybrid system comprises a male sterile (MS) line and a fertility restorer (Rf) line. Cross pollination through conventional breeding of the MS and Rf lines results in hybrid lines (GM InVigor® canola), which are fully fertile (also see section 2.4.4 below). InVigor® canola hybrids have been reported to demonstrate significant yield advantages of 10 to 20% over open-pollinated varieties in Australian and Canadian trials (GRDC 2006a).

Herbicide tolerance

Triazine tolerant (TT) canola was developed to allow growers to plant canola on fields infested with cruciferous weeds (eg wild mustard, stinkweed, ball mustard) and a number of other weed species many of which cannot be controlled by herbicides used in conventional canola. Unfortunately the triazine resistance from the *B. rapa* weed is due to a cytoplasmic mutant, which meant that TT canola varieties yield considerably less compared to conventional canola varieties under weed-free conditions[3] (CCC 2005). The first TT variety, Siren, released in Australia in 1993, had a significant yield (15 to 20%) and oil content penalty compared with conventional varieties. Despite the penalty, TT varieties have been widely accepted (Salisbury & Wratten 1999). Since the mid-1990s, newer TT canola varieties, encompassing early to late season and low rainfall areas, have been released (NVT Online 2007).

In 1995, the first imidazolinone–tolerant (IT) canola *B. napus* variety "45A71" was registered. This variety and others were developed through mutagenesis by Cyanamid (now BASF) and are now called "Clearfield". In 2006, 11 new canola varieties were released in NSW (a total of 39 available), including two Clearfield, three TT and 6 conventional canola varieties (McCaffery et al. 2006).

During 2005/6, non-GM herbicide-tolerant canola varieties comprised approximately 90 to 95% of Western Australia's canola crop, with most of this being triazine tolerant varieties. In eastern Australia (SA, Vic and NSW) approximately 80 to 85% was herbicide-tolerant, with 60 to 70% triazine tolerant and 15% imidazolinone tolerant varieties (Trent Potter [4] personal communication, 2006).

Other

Work is underway in Australia to develop super *Brassica* varieties at the hexaploid level. Canola and Indian mustard are tetraploids (amphidiploids consisting of 2 distinct genomes, see Section 1 and Fig. 1 above) derived from diploid species (*B. rapa*, *B nigra* and *B. oleracea*). Researchers are attempting to create a hexaploid comprised of these three species and potentially gain yield and quality advantages as demonstrated in other hexaploids such as between bread wheat (hexaploid) and durum wheat (tetraploid) (Yan & Weerakoon 2007). Additionally, combining the various species may result in new varieties with increased tolerance to abiotic stresses such as drought or salinity and diseases such as blackleg or sclerotinia (Pradhan et al. 2007).

2.4.2 Genetic modification

Techniques for the genetic modification of *Brassica napus* have been available since the late 1980s and early 1990s. A wide range of target explants have been utilised such as hypocotyls, cotyledons, stem segments, microspores and protoplasts. Genetic transformation has included *Agrobacterium*-, biolistic-, or PEG[5]-mediation. A number of agriculturally important traits have been transferred into *B. napus* including resistance to herbicides, viruses, fungi and insects; composition of oils or proteins in

[3] Early work on triazine-tolerant canola took place at the University of Guelph (Canada), with the first *B. napus* variety, OAC Triton, registered in Canada in 1984.
[4] Trent Potter, at time of publication was senior research officer at South Australian Research and Development Institute (SARDI) Naracoorte.
[5] PEG = polyethylene glycol, which is often used with electroporation to facilitate uptake of DNA by protoplasts.

seeds; levels of secondary metabolites; and male or female fertility (Wang et al. 2005). Canola oil composition is of particular importance and has been modified so that the seeds accumulate stearidonic acid[6] (SDA), an omega-3 fatty acid, which has been positively associated with health and the prevention and treatment of heart disease, arthritis, inflammatory and autoimmune diseases, and cancer (Ursin 2003). Similarly, silencing of the endogenous *oleate desaturase* genes have resulted in substantial increases in oleic acid[7] levels, up to 89% in *B. napus* and 73% in *B. juncea* (Green & Salisbury P 1999).

Canola has also been genetically modified for resistance to glyphosate or glufosinate ammonium herbicides. In 1999, several GM bromoxynil-resistant canola varieties (295 BX, Armor BX, and Zodiac BX) were developed by the University of Manitoba, Canada. Canola and other *Brassica* sp. containing some of these traits have been tested under Australian conditions (see Table 2 below).

Table 2. **Summary of planned releases (PR) conducted under GMAC[8] and dealings involving intentional release (DIR) approved under the OGTR of GM *Brassica* sp. in Australia.**

Trial	Proponent	Species	Trait development
PR-14	Pacific Seeds	*B. napus*	Canola lines developed through protoplast fusion
PR-60	Monsanto	*B. napus*	Increased lauric acid
PR-62	AgrEvo	*B. napus*	Glufosinate ammonium tolerant canola
PR-63	AgrEvo	*B. napus*	Glufosinate ammonium tolerance and a new hybrid breeding system
PR-77	Monsanto	*B. napus*	Glyphosate tolerant GM canola
PR-79, 93, 110,119 & 133	AgrEvo	*B. napus*	Development of fungal resistant canola
PR-85	AgrEvo	*B. rapa*	Glufosinate ammonium tolerance and a new hybrid breeding system
PR-90	AgrEvo	*B. juncea*	Glufosinate ammonium herbicide tolerant hybrid Indian mustard
PR-111 & 132	AgrEvo	*B. napus*	Photoperiod insensitive canola
PR-120	AgrEvo	*B. napus*	Reduced anti-nutritional factors (glucosinolates)
PR-121	AgrEvo	*B. napus*	Modified plant architecture (dwarf stature)
PR-122	AgrEvo	*B. napus*	Reduced pod shattering
DIR010	Aventis	*B. napus*	Glufosinate ammonium tolerance and hybrid breeding system
DIR011	Monsanto	*B. napus*	Glyphosate tolerance
DIR020[9]	Monsanto	*B. napus*	Glyphosate tolerance
DIR021[10]	Bayer	*B. napus*	Glufosinate ammonium tolerance and hybrid breeding system
DIR032, 057 & 069	Bayer	*B. napus* *B. juncea*	Herbicide tolerance and hybrid breeding system

[6] Stearidonic acid is an omega-3 essential fatty acid, sometimes called moroctic acid. It is biosynthesized from alpha-linolenic acid by the enzyme delta-6 desaturase

[7] Oleic acid is a monounsaturated omega-9 fatty acid found in various animal and vegetable sources. The saturated form of this acid is stearic acid.

[8] Genetic Manipulation Advisory Committee was established in 1987 to oversee gene technology research and development in Australia. GMAC was replaced by the OGTR in 2001.

[9] Commercial release of GM Roundup Ready® canola (glyphosate herbicide tolerant)

[10] Commercial release of GM InVigor® canola (glufosinate ammonium herbicide tolerant)

SECTION 3 MORPHOLOGY

3.1 Plant morphology

A well grown canola plant produces approximately 10 to 15 mainly glabrous (smooth) leaves (Colton & Sykes 1992), with lower leaves lyrate-pinnatifid (leaves divided transversely into lobes with an enlarged terminal lobe and smaller lateral lobes, division between lobes nearly or to the mid-rib), sparsely bristly and petioled (leaf with stem attaching to main stalk). The middle and upper leaves are oblong-lanceolate, thicker, clasping and sessile (without a petiole) (Bailey 1976). Leaf colour is a dark bluish green (glaucous). A single leaf is attached to the stem at each node, with approximately15 to 20 internodes per plant at a spacing of approximately 5 to 10 mm. In the early 1990s, canola variety heights varied from approximately1.2 to 1.5 m depending upon variety and environmental conditions (Colton & Sykes 1992). A current search of the Plant Breeders Rights Database (Australian Government - IP Australia 2007) indicated that modern canola varieties are shorter, within the range of 70 to 110 cm (eg Tranby, Karoo, AG-Muster and AG-Outback) while others are taller, in the 150 to 170 cm range (eg Skipton and Rocket CL).

3.2 Reproductive morphology

Canola flowers are bisexual and develop in terminal racemes. The flowers are regular with 4 sepals and 4 petals (see Figure 3 below). The diagonally opposite, yellow petals narrow at the basal end and form a cross, which accounts for the original family name, Cruciferae (now Brassicaceae)(OECD 1997). The flowers also contain 6 stamens (2 of which are shorter and inserted lower than the others); a pistil of 2 carpels and a superior ovary (ie positioned above the receptacle). Seeds develop in a 2-celled, elongated capsule called a silique (or pod) with a prominent mid-vein (Bailey 1976).

SECTION 4 DEVELOPMENT

4.1 Reproduction

The normal means of canola reproduction is through seeds. There are no reports of vegetative reproduction under field conditions (*in vitro* asexual reproduction is possible, see Section 2.4.2).

4.2 Pollination and pollen dispersal

Canola has entomophilous flowers capable of both self- and cross- pollination (Treu & Emberlin 2000). Fertilisation of ovules usually results from self-pollination since in a flowering crop, each flower produces a large amount of pollen, which usually out competes the pollen from adjacent flowers. However, outcrossing can also occur between adjacent plants at levels of approximately 30% (see Section 9.1). The level of out-crossing varies depending on the availability of insect pollinators, variety and weather.

4.2.1 Pollen characteristics

Most insect pollinated plants have relatively large (32 to 33 μm), sticky grains that do not become airborne readily (Treu & Emberlin 2000). In contrast, grains from wind pollinated plants are generally light, dry and easily airborne. Canola pollen grains are an exception and intergrades in these situations. Under field conditions, canola has the ability to cross pollinate through physical contact between neighbouring plants

and/or be insect pollinated (OECD 1997) and whose pollen can also become airborne and potentially travel at least several kilometres downwind (Treu & Emberlin 2000).

Figure 3. Flowering raceme of canola (*B. napus*).
Photo courtesy of Brian Weir, Sept 2007

4.2.2 Pollen movement

Wind

In general, wind-borne pollen plays a minor role in long-distance pollination. The vast majority of pollen travels less than 10 m and the amount of pollen decreases as the distance from the pollen source increases (Scheffler et al. 1993; Timmons et al. 1995; Thompson et al. 1999). The dispersal range of canola pollen is variable; from a few metres to 360 m, or in extreme cases there is evidence of wind transfer up to 1.5 km (Timmons et al. 1995). Further discussion regarding pollen dispersal distances and outcrossing can be found in Section 9.

Most pollen grains released to the air flow will travel at least some way from the anthers but distances will depend on the dispersal processes operating. Air-borne pollen dispersal distances are variable in response to environmental and topographical

conditions. Pollen movement will depend on wind direction, wind speed, topography (eg hills, slopes, valleys) and surrounding vegetation (Gliddon et al. 1999; Thompson et al. 1999). Longer distance pollen transfer (termed 'regional pollen') occurs when pollen grains are caught by upward air movements and are transported above the height of vegetation and the local air currents created by surface features. No research has been carried out on movement of canola pollen in atmospheric conditions such as convection currents, turbulent conditions and weather fronts. However, research on pollen from other species has demonstrated that dispersal can occur over considerable distances (eg 380 km for arboreal pollen) (Tyldesley 1973).

Insect pollinators

Canola plants are mainly self-pollinating with pollen that is relatively heavy and sticky (OECD 1997; Harker et al. 2002). The flowers of canola produce nectar with relatively high concentrations of sugars and have a colour and structure which makes them attractive to insects, particularly bees. In Australia, honeybees (*Apis mellifera*) are believed to play a major role in the transfer of pollen over long distances. However, other beneficial insects such as hoverflies (*Simosyrphus grandicornis*), which prey on aphids in canola, may inadvertently transfer pollen between plants. Bumblebees (*Bombus* spp.) play a major role in the transfer of pollen in the Northern Hemisphere (Cresswell 1999). Since bumblebees only occur in Tasmania and are geographically discrete, these insects play a minor role in the pollination of canola crops in Australia.

Pollinator behaviour

Insect foraging behaviour is complex, being dependent on a number of factors including spatial arrangement of plants, environmental conditions, plant density and availability of pollen (Rieger et al. 2002). Given abundant flowers, such as in a cultivated field, individual honeybee foragers tend to collect nectar and pollen from flowers in the same or immediately adjacent plants. Bee hives are commonly introduced into canola crops to facilitate pollination and maximise seed set. In this situation, most foraging is carried out close to the hive and between neighbouring plants, which may be a few dozen square metres in size (Nieuwhof 1963). Studies have determined that a large proportion (up to 80 %) of bee flights are less than 1 m in distance, with the majority of pollen transported less than 5 m (Cresswell 1999; Ramsay et al. 1999; Pierre 2001). Occasionally however, bees may travel much further and studies have measured flight distances of 1 to 2 km (Eckert 1933), up to a maximum distance of 4 km (Ramsay et al. 1999; Thompson et al. 1999). Loose pollen grains can be picked up from within a hive, so with the majority of honeybee colonies foraging up to 2 km in all directions from a hive, some pollen transfer and fertilisation up to 4 km may occur (Ramsay et al. 1999).

While bees will search a larger region for food during flowering, honeybees will only forage during daylight and are unlikely to carry viable pollen grains to effect fertilisation beyond 12 hours (Kraai 1962). The mean distance of pollen dispersal is dependent not only on pollinator behaviour but also plant density and sparse areas of plants receive far fewer pollinator visits (Kunin 1997).

4.2.3 Pollen viability

The distance and success to which pollen-mediated gene flow is likely to occur is dependent not only on its dispersal in space by either wind or insect action, but also on the length of time the pollen grain retains its potential for pollination. Pollen

viability varies with environmental conditions, particularly temperature and humidity. Under controlled conditions in the laboratory, canola pollen can remain viable for between 24 hours and one week (Mesquida & Renard 1982). Under natural conditions pollen viability gradually decreases over 4 to 5 days (Ranito-Lehtimäki 1995). In Australia, canola crops flower in spring when temperature increases and humidity declines; under these conditions, pollen viability may be reduced to 24 to 48 hours (Salisbury 2006).

4.3 Fruit/seed development and seed dispersal

4.3.1 Fruit and seed development

The fruiting bodies produced by the Brassicaceae family are siliques, commonly called pods (Buzza 1979). In canola, between 15 and 25 seeds are produced per pod. Seed development is first seen on the lowest one third of the branches of the main stem. Seeds are translucent when they reach full size and then become green and finally black and hard (Colton & Sykes 1992). Seeds develop in a pod approximately 6 to 9 cm long, which includes a 1 to 2 cm beak or tip (Australian Government - IP Australia 2007), ascending on rather slender pedicels (Bailey 1976). Each canola plant produces hundreds of small (1 to 2 cm diameter), spherical, light brown to black seeds (Buzza 1991). Mature seed colour is dark brown to black, with approximately 280,000 to 300,000 seeds per kg (Colton & Sykes 1992). Individual canola seeds are released from the plant as the seed pods dry out and open (dehisce or shatter). Due to the significant number and small size of canola seed, individual seeds may be transferred by small to large vectors (discussed below).

4.3.2 Seed dispersal

Seed movement can be an important factor in overall spatial and temporal gene movement. Seed-mediated gene flow typically occurs over shorter distances although it can occasionally over very long distances (Raybould & Gray 1993). Dormancy is a mechanism for dispersing seed temporally (see Section 4.4.2). Mechanisms for the potential dispersal of canola seed are discussed below.

Wind

Widespread natural dispersal of canola seeds does not generally occur in the field. The small size of canola seeds and their high numbers on post harvest fields may facilitate some dispersal by wind (Lutman 1993). While pod shattering can disperse seeds over short distance, it is possible that windrows of canola plant material including seed could be blown into adjacent fields. The dispersal distance will depend on the wind strength, the amount of trash on the ground and the moisture content of the material. Although no data exists on wind dispersal of canola windrows, it is reasonable to expect, that seeds and pods of low moisture content, may be transported within the field or to adjacent fields during periods of unusually high winds.

Water

No data is known on seed transport rates by water of canola and other Brassica species. However, the specific gravity of canola seed of 1.15 (Jayas & Cenkowski 2006), which is slightly higher than that of water, thus it is probable that most of the seed would tend to sink, especially after soaking. It is likely however that seeds would be transported relatively easily as bed load sediment in rivers and creeks. There is a high likelihood that the majority of seed being transported by water would

be carried to positions unfavourable for establishment. Prolonged exposure to water would likely render canola seed unviable. Under flooding or waterlogged conditions, there would not be sufficient oxygen present for cell respiration to provide energy for germination to proceed (that is for emergence of the radicle from the seed). In poor germinating conditions, such as waterlogging, the seed is more susceptible to decay from soil micro-organisms (CCC 2007a).

As seeding of canola is generally into moist soil at depths ranging from 2 to 6 cm (see Section 2.3.3), flooding would have to be sufficient to transport considerable amounts of topsoil on basically level land to move canola seed any great distance. Heavy rains or flooding could transport residual canola seed on the soil surface lost during harvest. If the flooding was not prolonged and displaced seed did not become waterlogged, as canola seed have no dormancy they would likely germinate. However, without continued irrigation or rainfall, the seedlings would be unlikely to persist.

Humans (clothing, vehicles)

The greatest potential for the movement of canola seeds is from post harvest spillage by agricultural machinery or during transportation away from the production areas. Seed may also be moved on electrostatically charged containers (eg tarpaulins and bags) in storage bins. It is also possible that small amounts of seed could be transported on or in clothing (eg pockets and pant cuffs) or boots (especially muddy boots) of workers.

Animals

Given the large number and small size of seed produced by each plant, a number of animals other than humans may disperse canola seed (eg ants, birds, and grazing animals). Birds, such as cockatoos and sparrows can shred or remove pods during development and maturity (Stanley & Marcroft 1999). Mice can also climb plants and feed on the seeds and pods, or feed on un-germinated seed sown close to the surface.

The viability of canola seed after passing through the digestive gut of animals is poorly understood. Anecdotal evidence from Canada suggests canola seed (Roundup Resistant® canola), mixed with wheat, remained viable and subsequently emerged after being fed to chickens and distributed as chicken manure spread on a field 12 months later (Martens 2001).

In an Australian study, sheep fed canola seed as part of their diet excreted approximately 1 to 1.5% of the canola seed and a portion of this was able to germinate. Germination rates of the excreted seed were highest (approximately40%) on the first day after feeding of canola seed began, but then dropped by approximately an order of magnitude thereafter. The percentage of viable seed excreted daily was therefore in the order of 0.1% of daily intake. Sheep continued to excrete viable canola seed for 6 days after canola was removed from the diet (Stanton et al. 2003). The results from the feeding study demonstrate that ingestion of canola seed by sheep reduces the viability of excreted seed, but that a small portion of seed remains viable. Further research is needed to elucidate the effect of digestion on the viability of canola seed in other animals.

4.4 Seed dormancy and germination

4.4.1 Viability/germination

Optimal germination conditions for canola are 20°C, high water availability (eg –0.2 MPa) and exposure to light (Pekrun et al. 1998). However, canola seed will germinate under a variety of temperatures both at seeding and after harvest. Generally, soil temperatures below 10°C result in progressively poorer germination and emergence. Both *B. napus* and *B. rapa* canola will imbibe water and germinate at constant temperatures of 2°C. Sustained low temperatures for both *B. napus* and *B. rapa*, however, damage the seed embryo, which reduces germination and growth. Low temperature impairs the production of proteins required for proper germination and early seedling development. The number of days to 50% germination in *B. napus* was only three days at 8°C compared to nearly 13 days at 2°C. This low temperature effect of slower germination was even more pronounced with *B. rapa* canola. In *B. rapa*, there was greatly reduced germination at 3°C, and at 2°C, even after 20 days, 50% emergence was not reached. Germination is also influenced by the genetics of the variety, growth conditions as the seed matures, how the seed was stored and seed treatments (CCC 2007b).

4.4.2 Dormancy

Canola seed shows virtually no signs of dormancy at maturity (Lutman 1993; Pekrun et al. 1998). However, non-dormant canola seed may enter dormancy if environmental conditions are unfavourable for germination (referred to as 'secondary dormancy'). Induction of secondary dormancy in canola occurs in response to sub optimal germination conditions such as large temperature fluctuations, low soil water availability (eg –2.0 MPa), long exposure to darkness (Pekrun et al. 1997b), and sub-optimal oxygen supply (Pekrun et al. 1998). Far-red absorbing phytochrome is required to induce light sensitivity in non-dormant seed of many species and research by Lopez-Granados and Lutman (Lopez-Granados & Lutman 1998) suggests that phytochrome is involved in the induction of secondary dormancy in oilseed rape. Temperatures greater than 20°C can also induce dormancy in some genotypes (Linder 1998; Gulden et al. 2000). Once dormant, seeds will remain dormant if kept at a relatively constant temperature (eg 12° or 20°C) (Pekrun et al. 1997a). Secondary dormancy can be removed at low temperatures (2 – 4°C) (Gulden et al. 2000) or by alternating warm and cold temperatures (Pekrun et al. 1998).

During prolonged exposure to darkness and water stress, canola seeds develop light sensitivity (Pekrun et al. 1997b). Light sensitivity enables seed to germinate in response to very short exposure to light (Schlink 1995), as might be experienced during soil cultivation. The presence of large quantities of crop residues (eg in zero tillage systems) can also create shaded conditions that allow seed to develop light sensitivity (Legere et al. 2001). Studies have shown that development of secondary dormancy and persistence of canola varies among European (Schlink 1995; Pekrun et al. 1997c) and Canadian canola varieties (Gulden et al. 2000). For example, the response to laboratory conditions of osmotic stress and darkness to induce secondary dormancy ranged from 2 to 50% among 47 varieties (Pekrun et al. 1997c).

Persistence of canola seed is considerably longer in undisturbed soils compared to cultivated soils (Chadoeuf et al. 1998). Studies in the Northern Hemisphere suggest that viable seeds of canola may persist in disturbed soils for at least 5 years and

possibly up to 16 years in undisturbed soil (Masden 1962). Masden (1962) examined dormancy of *B. napus* var *napobrassica* seed (common names: rutabaga, swede or Swedish turnip), which is closely related to canola. After 16 years buried at a depth of approximately 20 cm, 1% of the seed germinated under conditions that would break dormancy. There was no seed germination after 17 or more years of burial (limit of study was 26 years).

Crawley et al (1993) determined that at 12 different habitats at 3 sites in the UK, GM herbicide tolerant canola had no remaining viable seed after burial (at 2 and 15 cm depth) for 2 years, while non-GM canola seed was 0.5% viable. Chadoeuf et al (1998) determined that that after 3 years of burial (at 30 cm depth) at two different sites in France, canola seed viability ranged from 0.03 to 0.08%, and that at 41 months the canola seed was non-viable.

Lutman et al (2002) examined the effect of incorporating canola to soil depths of up to 20 cm and then planting the area to either winter (autumn sown) or spring (spring sown) wheat, at two sites in the UK. Any emerging canola plants were prevented from contributing further to the seed bank. The site, planting of winter or spring wheat and cultivation depth had no effect on the number of canola seeds present in the soil. Across the two sites, approximately 86% of the canola seed disappeared after the first year and less than 1.25% of incorporated seed was present after 4 years. However, it was not known if the seed was viable because viability was assumed if seed were firm and resistant to gentle pressure.

Despite the lack of Australian data, overseas studies (cited above) suggest that high temperatures and low soil moisture availability experienced after harvest in Australia, may provide conditions to induce secondary dormancy, which may contribute to higher persistence rates than under European or Canadian conditions. It is possible that cool/moist conditions experienced in the Northern Hemisphere after canola harvest is more conducive to germination of canola seed than the hot/dry conditions experienced after harvest in Australia. The persistence of viable canola seed in the soil under Australian field conditions is poorly understood and further research is needed.

4.4.3 Seed banks/persistence

Large seed banks of canola can build up in the soil as a result of high amounts of seed loss before and during harvest. Other mechanisms responsible for adding to the seed bank include ungerminated seed from sowing, seed produced from volunteers and/or seed loss from mature plants due to heavy rainfall, hail, strong winds or lodging.

Loss of seed from plants, particularly in hot and windy conditions, can be up to 70% (Colton & Sykes 1992), which may lead to a substantial number of volunteers appearing in subsequent crops. Data suggest that canola seed density in the soil after harvest can range between 2000 seeds m^{-2} in Canada (Legere et al. 2001) to 10,000 seeds m^{-2} in the U.K. (Lutman 1993). Western Australia surveys have shown harvest losses to be as high as 40 to 150 kg ha^{-1}, with a loss of 20 to 30 kg ha^{-1} considered acceptable (Oilseeds WA 2006). Canadian studies have shown that seedbank density declined ten-fold in the first year, but only slowly declined thereafter. Seasonal variations in seedbank density in Canada occurred as a result of seeds being produced by volunteer plants every spring thereby replenishing the seedbank (Legere et al. 2001). Field surveys conducted across Canada, found volunteer canola still present in

fields 4 to 5 years after a canola crop had been grown, albeit at low densities (Legere et al. 2001; Simard et al. 2002).

Simard et al (2002) sampled 131 fields in Canada (Quebec) and 90% had canola volunteers 1 year after harvest of the canola crop. Plant density at one year post harvest averaged 4.9 plants m^{-2} and decreased to an average of 0.2 plants m^{-2} at 5 years post-harvest. Although this study suggests persistence of the canola at low densities for up to 5 years, the study did not distinguish between persistence of the original crop (ie un-germinated seed at sowing or seed loss during harvest) and seeds from volunteer canola plants, which were able to replenish the seed bank. A similar study in France suggested that *B. napus* seed may persist in the seedbank for up to 8 or 9 years (Pessel et al. 2001), but similar to the above study, the persistence may have been due to seed replenishment from volunteers rather than long term seed bank persistence.

In another Canadian study, which examined persistence of canola seed under conditions that did not allow volunteers to replenish the seedbank, almost no canola seed could be detected after three years (Harker et al. 2006). Canola seed was scattered on the soil surface (to mimic seed loss from harvest) and then subjected to typical crop rotations (with or with out fallow) and farming practices. Canola densities averaged over all locations and treatments were 6.2, 0.7 and 0.0 plants m^{-2} for the three years of the trial, respectively. At the end of the trial a total of 3 viable seeds were detected at two of the seven trial locations across Western Canada, indicating a persistence of 0.0088% (calculations based on data from Harker et al 2006).

The fate of these seeds is either to remain on the soil surface or be buried. Those seeds on the surface may die (due to environmental conditions or via predation), become/remain dormant, or germinate (Lutman 1993). Gulden et al (2004) suggested that in Canada, temperatures near the soil surface of up to 55°C in early summer may contribute to the death of residual canola seed near the surface from the harvest the previous autumn. If this were the case then soil surface temperatures in Australia would likely contribute to the death of seed remaining near the soil surface after harvest (harvest of canola in Australia tends to be in late spring/early summer or early autumn for winter and summer canola crops respectively) and reduce the persistence of canola in subsequent crops. Lutman's (1993) experiments determined little or no dormancy in the freshly harvested seed or seed on the soil surface that had been exposed to elements for 4 weeks; all the seed germinated, except for approximately 0.4% which were found to be rotten.

Due to cultivation, seed can become buried and this seed may also die, remain dormant or germinate (creating seedlings or fail to emerge) (Lutman 1993). In field trials, the effect of burial on seed viability was variable but generally seed survival was low. Some of the seed (1.5%) buried to a depth of 5 cm germinated within 5 or 6 weeks, however, up to 80% of the seed was recovered from the soil as empty seed coats and only 0.6% of seed was recovered as whole seed 7 months after sowing. Seed sown to a depth of 20 cm had similar but somewhat variable results. Most of the seed buried to a depth of 20 cm had survival ranging from 0.07 to 0.5% after up to 12 months of burial, except for one replicate of the trail in the second year that had up to 27.6% survival (Lutman et al. 1993 had no explanation for this deviation).

While there is considerable genotypic variation in the development of secondary dormancy in canola (Pekrun et al. 1997c), generally, low nutrient status and dark conditions are the major contributing factors to the persistence of the seed bank of canola. The vertical distribution of seed in the soil has a significant impact on the persistence of canola seed in the seed bank. Seeds are more likely to persist at deeper soil depths than at shallow depth in the field (Pekrun et al. 1998). A greater proportion of germination occurs at shallow depths due to dormancy-breaking temperature alterations and exposure to light (Pekrun et al. 1998). Persistence will also vary with soil type. Pekrun et al. (1998) found that sandy soils, with low water holding capacity, tended to have a greater proportion of dormant canola seeds in the seed bank than clay soils, which have high water holding capacity.

4.5 Vegetative growth

Canola is an annual crop in Australia, generally completing a lifecycle in at most 7 months (but see Section 4.1). Colton and Sykes (1992) describe the life cycle of the canola plant through seven principal stages (stages 0 to 6). The initial stage is germination and emergence (stage 0), which occurs after the seed absorbs moisture and the root splits the seed coat and the shoot pushes upward through the soil. The cotyledons are pulled upward by the shoot and turn green when exposed to light. After germination, the seedling develops a thin taproot and starts to produce leaves (stage 1). Typically, a canola plant will produce 10 to 15 leaves, but there is no definite number of leaves produced.

As the leaves are developing, the stem begins to extend (stage 2). Internode space is approximately 5 to10 mm and a leaf is attached to the stem at each node. The canola plant produces approximately 15 to 20 internodes.

Flower bud development is stage 3. Initially flower buds remain enclosed in the leaves during early stem elongation. As the stem elongates, the flowers emerge above but are not free from the leaves (green bud stage). The stem continues to elongate until the flowers are free from the leaves and the lowest flower buds assume a flattened shape. Lower buds are the first to become yellow (yellow bud stage) and progressively more buds become yellow as the stem grows. The lowest buds flower last. The flowering period (stage 4) begins with the opening of the first flower on the main stem and finishes when there are no viable buds remaining.

Podding or pod development (stage 5) starts on the lowest one third of the branches on the main stem. This stage is defined by the proportion of pods that have extended to more than 2 cm long. The final principal stage (6) is seed development during which the seeds change from translucent to green and finally black and hard (see Section 4.3). It is during this stage that the canola crop reaches physiological maturity and harvesting occurs (see Section 2.3.3).

SECTION 5 BIOCHEMISTRY

5.1 Toxins

In western countries, traditional rapeseed (ie non-canola quality) was considered unsuitable as a source of food for either humans or animals due to the presence of two

naturally occurring toxicants in the seed, erucic acid and glucosinolates (OECD 1997). The standard for canola oil is that it contains less than 2 % erucic acid (CODEX 2001) and meal contains less than 30 μmoles g^{-1} glucosinolates (Colton & Potter 1999; Oilseeds WA 2006). Breeders have systematically replaced the old rapeseed breeding stock with varieties that meet the canola standards. Australian canola varieties typically contain less than 0.5 % erucic acid and less than 20 μmoles g^{-1}glucosinolates (Colton & Potter 1999).

The presence of erucic acid in rapeseed oil has been associated with fat accumulation in the heart muscle of laboratory rats, resulting in cardiopathogenic effects. A review of feeding studies examining the effect of high erucic acid levels indicates that results have been variable and inconsistent, but may indicate that erucic acid is one of a number of fatty acids that are poorly metabolised by rats (and other laboratory animals) and if fed in large quantities may lead to heart lesions (Kramer et al. 1983). It is not clear how these studies relate to effects on humans, when in contrast, the consumption of early high erucic acid containing rapeseed oils (*B. napus* and *B. campestris*), since ancient times, does not appear to have associated nutritional or health problems (Kramer et al. 1983; Monsalve et al. 2001). In the absence of adequate human data, Food Standards Australia New Zealand (FSANZ) set a no-observable-effects-level (NOEL) for human exposure extrapolated from the NOEL established for pigs. The tolerable level for human exposure is 7.5 mg erucic acid $kg.bw^{-1}$ day^{-1} or approximately 500 mg day^{-1} for the average adult, which is regarded as the provisional tolerable daily intake (PTDI). Based on dietary exposure, the average consumer's intake is 124 mg day or 28 % of the PTDI (FSANZ 2003).

Glucosinolates are located in the seed meal and generally consist of a sugar entity, b-D-thioglucose, bound to an organic aglycone. Upon hydrolysis, glucose and sulfate are cleaved off, releasing free compounds such as isothiocyanate, nitrile, and thiocyanate. These compounds often contribute a bitter, "hot" taste to condiment mustard. Glucosinolates, such as isothiocyanate and thiocyanate, were found to exhibit goitrogenic or antithyroid activity in laboratory animals; whereas nitriles may cause liver and kidney lesions. Thus, the presence of glucosinolates limited the nutritional value of the meal as feed for livestock [see review by Bell (1984)]. This was particularly the case for the older rapeseed varieties that contained up to 10 times the glucosinolate level of modern canola varieties. In addition to previous breeding efforts to select for lower levels, glucosinolate levels in meal have also been reduced during the oil extraction process (Bell & Hickling 2003).

5.2 Allergens

Occupational exposure to *B. napus* pollen (Chardin et al. 2001; OGTR 2002), dust (Suh et al. 1998) and flour (Monsalve et al. 1997; Alvarez et al. 2001) have been implicated in allergic reactions in people and a number of putative allergens have been characterised, including seed storage proteins (Monsalve et al. 1997). It is important to note that *B. napus* seed meal or flour is not considered suitable for human food due to the presence of glucosinolates (ANZFA 2001). Canola oil is the only fraction used for human food. The processing of canola seed is expected to remove all traces of protein from the oil (ANZFA 2001). No allergic reactions to fats (including canola oil) have been reported in people.

Allergic sensitisation to canola can occur via the respiratory tract (through inhaling pollen) or through skin contact (eg during handling). High incidences of hay fever and/or bronchial asthma have been measured in *B. napus* growing areas in Europe during flowering (Bugur & Arner 1978). Yet, other studies conducted in Europe show a low prevalence of allergy to *B. napus* pollen (less than 0.2 %) unless the subjects were occupationally exposed. Those affected were generally already atopic and allergic to other pollens (Fell et al. 1992). Volatile organic compounds given off by growing *B. napus* plants have been shown to play a role in respiratory mucosa and conjunctiva irritation associated with airborne releases from *B. napus* (Butcher et al. 1994). The protein Bra n 2 has been identified as an allergen in *B. napus* pollen (Toriyama et al. 1995).

Data collected on the allergenicity of canola pollen is often confounded by the other flowering plants, particularly grasses, which flower at similar times. Soutar et al (1995) found people who complained of symptoms in relation to the flowering of *B. napus* were rarely allergic to the plant and fewer than half were atopic. Nevertheless, they usually showed increased bronchial reactivity during the season, which may have been due in some cases to other allergens but in others to non-specific irritant effects of the air. There is no evidence of cross-reactivity between *B. napus* and grass pollen (Welch et al. 2000).

Occupational allergies to plants can take the form of either immediate hypersensitivity or delayed hypersensitivity reactions. The latter frequently occur as a consequence of handling plant material and generally manifest as contact dermatitis. However, studies have shown that exposure to *B. napus* flour (ground, dried seed-meal after oil extraction) contained in animal fodder may be a possible cause of occupational asthma in farmers (Alvarez et al. 2001). An allergen, Bra n 1, a seed storage protein from the 2S albumin family, has been identified in *B. napus* (Monsalve et al. 1997). Those at risk from pollen include the rural population in general and farm workers in particular.

5.3 Other undesirable effects of phytochemicals

Sinapine is an alkaloid occurring in mustard seeds, including canola. Sinapine is one of the compounds which gives mustard its hot bitter taste and has been implicated in the fishy egg taint, which limits canola meal feed use for brown egg laying hens (AOF 2007a). It is found only in the seed and upon germination is hydrolysed to form choline and sinapic acid (Tzagoloff 1963).

5.4 Beneficial phytochemicals

Compositional analysis of canola seed

The seed typically has an oil content ranging from 35 to 45% (but can fall outside this range depending upon variety and environmental factors) and a minimum of 35% protein at 13% moisture (AOF 2007a). The hull comprises approximately 16% of the seed weight (approximately 30% of the oil-free seed meal) (Bell 1984). Through plant breeding/selection varieties have less than 7 μmoles of total glucosinolates per g of whole seed, which is approximately 11 μmoles g^{-1} of oil-free meal and well less than the canola standard of 30 μmoles g^{-1} of meal.

Oil composition

Oil is extracted by mechanically crushing the seed and finished using heat and chemical processing steps. Approximately 73% of canola meal in Australia is processed from solvent plants, 25% from expeller and 2% from cold press plants (AOF 2007a). Oil content is generally expressed as a percentage of whole seed at 8.5% moisture and contains: 10 to 12% omega-3 linolenic acid, <0.1% erucic acid, 59 to 62% oleic acid, 18 to 22% linoleic acid and 10 to12% linolenic acid (Mailer 1999). A summary of the composition of canola oil is given in Table 3.

Table 3. Canola oil Composition (per 100 g)*

Calories	884.00
Protein (g)	0.00
Fat Total (g)	100.00
Carbohydrate (g)	0.00
Fiber - Total (g)	0.00
Sugar - Total (g)	0.00
Calcium (mg)	0.00
Iron (mg)	0.00
Magnesium (mg)	0.00
Phosphorus (mg)	0.00
Potassium (mg)	0.00
Sodium (mg)	0.00
Zinc (mg)	0.00
Copper (mg)	0.00
Manganese (mg)	0.00
Selenium (mg)	0.00
Vitamin C (mg)	0.00
Thiamin (mg)	0.00
Riboflavin (mg)	0.00
Niacin (mg)	0.00
Vitamin B6 (mg)	0.00
Folate - Total (mcg)	0.00
Food - Folate (mcg)	0.00
Folate - DFE (mcg_DEF)	0.00
Vitamin B12 (mcg)	0.00
Vitamin A (IU)	0.00
Retinol (mcg)	0.00
Vitamin E (mg)	17.46
Vitamin K (mcg)	71.30
Fat - Saturated (g)	7.37
Fat - Monounsaturated (g)	63.28
Fat - Polyunsaturated (g)	28.14
Cholesterol (mg)	0.00
Stigmasterol (mg)	3.00
Campesterol (mg)	241
Beta-sitosterol (mg)	413

*From USDA National Nutrient Database for Standard Reference (USDA 2007).

Canola oil is high in unsaturated fats (93 %), has no cholesterol or trans fat, and has the lowest saturated fat (7 %) of any common edible oil. Because of this and the fact that it is low in low-density lipoproteins, the US Food and Drug Administration (FDA) now allows manufacturers to claim canola oil's potential health benefits due to reduced risk of coronary disease (Douaud 2006).

Tocopherols

Tocopherols are naturally occurring antioxidants in vegetable oils and have a role in reducing cardiovascular disease (ODS 2007). There are four natural tocopherol isomers (all found in canola) and together with four corresponding tocotrienols, make up the eight vitamers that constitute vitamin E (Chester et al. 2001). The term Vitamin E is used as a generic descriptor for toco and tocotrienol derivatives exhibiting α-tocopherol activity (IUPAC-IUB 1982). Their interaction with polyunsaturated fatty acids is important in preserving the chemical stability of canola oil. Tocopherols content in canola oil ranges from 0.5 to 0.9% (Chester et al. 2001).

Seed meal composition

The composition of seed meal depends on the method of oil extraction (AOF 2007a). Typically, seed meal consists of between 36 to 39 % proteins and an amino acid composition comparable to soybeans[11]; it is slightly lower in lysine but higher in all sulphur-containing amino acids. Fat content ranges from 1.5 to 2% and generally has a richer mineral content than soymeal. Fibre content of canola meal ranges from 11 to 13% (Bell 1984). Glucosinolate levels are typically less than 10 μmoles g^{-1} in the meal from current canola varieties (AOF 2007a).

SECTION 6 ABIOTIC INTERACTIONS

6.1 Abiotic stresses

6.1.1 Nutrient stress

Canola has been successfully grown on soil from pH 5.0 to 8.0 (Colton & Sykes 1992). Soil pH has little effect on canola production except on very acid soils where manganese and aluminium toxicity may affect yield (liming is used on these soils before sowing canola to alleviate the situation) (Potter et al. 1999). Canola will not respond to available nutrients if there is aluminium in the soil and this may result in stunted, single stem plants with roots restricted to the top 5 to 19 cm of topsoil (Colton & Sykes 1992). Canola has a higher requirement for nitrogen, phosphorus and sulphur than cereals and other crops and will not produce high yields unless all three elements are present. Canola needs approximately 40 to 50 kg of nitrogen (30% more than wheat), 8 kg phosphorus and 10 kg sulphur per tonne of grain produced (Colton & Sykes 1992).

6.1.2 Temperature stress

Canola is susceptible to heat stress during grain fill (October-November) that can reduce yields (Potter et al. 1999). High temperatures can also induce both male and female sterility (Polowick & Sawhney 1988). Canola is relatively frost tolerant, however, damage can occur at the cotyledon stage (this is uncommon) and seedlings

[11] Seed meal composition is generally compared to that of soybean meal because of its prevalence as an animal feed source.

affected will blacken and may die. Plants become more frost tolerant as they develop. Lower temperatures during flowering may cause flower abortion, but due to the lengthy flowering season, plants generally recover and compensate for these losses. A late frost, after flowering, can cause major losses (this is relatively infrequent) (Colton & Sykes 1992)

6.1.3 Water stress

Most of Australia is too dry to successfully grow canola. Nonetheless, canola is grown in areas receiving from 325 to 700 mm annual rainfall. These areas typically receive the bulk of their rainfall during the cool, moist winter months, often receiving 65 to 75% of their annual rainfall between May and October. Thus canola is usually sown in the late autumn or early winter (April-June) and harvested in late spring and early summer (November-December). Flowering occurs in August-September and grain fill occurs in October and November. High temperatures during grain fill can result in lower yields and oil content (Potter et al. 1999). Canola is sensitive to waterlogging. Sodic and dispersing soils that surface seal will significantly reduce emergence of canola seedlings. Gypsum is often applied to sodic soils to improve soil structure and alleviate sulphur deficiencies (Potter et al. 1999).

SECTION 7 BIOTIC INTERACTIONS

7.1 Weeds

Broad leaf weeds, particularly weeds from the Brassicaceae family, are the most problematic in canola crops. There are no post-emergent herbicides available to control Brassicaceous weeds in conventional canola once the seeds have germinated and seedlings have emerged. Conventional canola can face many weed problems, (eg in the northern agriculture region of WA, silver grass, wild radish and turnip can devastate early sown crops). Registered herbicides for conventional canola are either grass specific or for limited broadleaf weed control. Consequently, competition from these weeds leads to significant yield losses in the crop. Furthermore, seeds of certain Brassicaceae species can contaminate canola seed, jeopardising the seed quality by increasing levels of erucic acid and glucosinolates. Thus, for better weed control, herbicide tolerant canola (such as TT or IT canola, see Section 2.4.3) are preferred (Carmody & Cox 2001).

7.2 Pests and pathogens

7.2.1 Pests

A number of insects and mites can damage canola crops. Pests such as the redlegged earth mite (*Halotydeus destructor*), blue oat mite (*Penthaleus major*), cutworms (*Agrotis infusa*), aphids (*Brevicorne brassicae* and *Lipaphis erysimi*), Diamond Back moths or cabbage moths (*Plutella xylostella*), heliothis caterpillars (*Helicoverpa punctigera* and *H. armigera*) and Rutherglen bug (*Nysius vinitor*) cause severe and widespread losses in some years. Significant insect damage to canola crops is most likely to occur during establishment and from flowering until maturity (Miles & McDonald 1999).

7.2.2 Diseases

The most important pathogen of canola is blackleg, caused by the fungus *Leptosphaeria maculans*. Blackleg can be carried over from year to year on infected

canola stubble, which can kill seedlings or reduce seed yield in older plants (Howlett et al. 1999). In the early 1970's blackleg wiped out the fledgling canola industry in Australia. Initial resistance to blackleg came from polygenic resistance genes. Then in the 1990s a resistance gene from *B. rapa* spp. *sylvestris* was introduced, but blackleg overcame this resistance by 2003. Losses from blackleg of up to 90% were reported in the Eyre Peninsula (Oilseeds WA 2006).

Sclerotinia stem rot (*Sclerotinia* spp.) is another major disease of canola. It has a wide host range of approximately 400 species of mostly broadleaf crops (eg lupins, field peas, beans) and weeds (eg capeweed). Growing canola after any of these crops or weeds can increase the risk of this disease (Howlett et al. 1999).

Other fungal diseases including club root (*Plasmodiophora brassicae*), downey mildew (*Peronospora parasitica*) and alternaria leaf spot (*Alternaria brassicae*) can cause serious yield loss in wet seasons (Howlett et al. 1999; Oilseeds WA 2006).

A survey conducted in canola crops throughout Western Australia in 1998, revealed the presence of a number of viral diseases including Beet Western Yellow virus (BWYV) and Cauliflower Mosaic Virus (CMV), both spread by aphids. It is not clear of the extent of losses due to BWYV in WA, but losses in Europe are reported to be 10 to 15%. Trials in WA suggest losses could be as high as 37 to 46%, but this would depend on substantial rainfall in the 2 to 3 months prior to canola seeding, which would allow for build up of aphid populations and early infestation of the emerging canola crop (Oilseeds WA 2006). In 1999, BWYV was also detected in NSW (Howlett et al. 1999).

SECTION 8 WEEDINESS

8.1 Weediness status on a global scale

As with all crops cultivated and harvested at the field scale, some seed may escape harvest and remain in the soil until the following season when it germinates either before or following seeding of the succeeding crop. In some instances the volunteers may provide considerable competition to the seeded crop and warrant chemical and/or mechanical control. Volunteers can also be expected away from the planting site (eg along roadsides and around storage facilities) as a result of transportation of seed out of fields (eg in farm equipment) and spillage during transport.

Canola is considered a major weed in agricultural ecosystems in Australia (Groves et al. 2003) and a minor weed in Canada (Canadian Food Inspection Agency 1994) and the USA (Weed Science Society of America 1992). Surveys have shown that canola occurs as a volunteer weed in up to 10% of cereal crops in southern Australia (Lemerle et al. 1996) and similar levels have been reported in Canadian cereal crops (Thomas et al. 1998). Volunteer canola is among the 20 most common weeds in fields in Alberta, Canada, occurring as a residual weed in 11.8 and 10.5 % of all wheat and barley fields surveyed in 1997, respectively (Thomas et al. 1998). Volunteer canola has been recognised as fourth ranked weed in the central cropping region of Manitoba, Canada (Kaminski 2001). Canola also occurs as a weed in cropping regions in the U.S.A (Weed Science Society of America 1992). However, Canola is not considered a significant weed, nor invasive of natural undisturbed habitats in

Australia (Dignam 2001), Canada (Canadian Food Inspection Agency 1994; Warwick et al. 1999; Beckie et al. 2001) or the UK (Crawley et al. 2001a).

8.2 Weediness status in Australia

8.2.1 Cultivated areas

In 2000/2001, a rating system was applied to naturalised, non-invasive species in both natural and agricultural systems based upon information supplied by Australian States and Territories (Groves et al. 2003). As a result, weeds were described as naturalised[12] and were defined as environmental or agricultural weeds[13] depending on how they impact either ecosystem. The weeds were further categorized based on their status within each ecosystem on a scale from 0 (indicating naturalised, but the population no-longer existing or removed) to 5 (indicating naturalised and known to be a major problem at four or more locations within a State or Territory).

Brassica napus is a classified as a category 2 weed in natural ecosystems and category 5 weed in agricultural ecosystems. Category 2 weeds are naturalised and known to be a minor problem warranting control at three or fewer locations within a State or Territory and are described as primarily a weed of agricultural or disturbed areas (Groves et al. 2003). Wheat, which is often grown in rotation with canola, is a category 2 weed in natural ecosystems, a category 3 weed in agricultural ecosystems and is also described as primarily a weed of agricultural or disturbed areas (Groves et al. 2003).

Canola seed can be dispersed to neighbouring non-agricultural areas by mechanisms such as strong winds blowing canola windrows across or off a field or seed may be dispersed with straw and chaff during mechanical harvest. If dispersed canola seed germinated it is unlikely to persist. Seedlings established in adjacent fields would likely be destroyed by normal agricultural practices (herbicide application, cultivation). Seedlings established in non-agricultural areas would not likely spread and persist, as canola are poor competitors and do not establish well in unmanaged areas (Oram et al. 2005; CFIA 2005). Unless the habitat is regularly disturbed, or seed replenished from outside, canola will be displaced by other plants (Salisbury 2002c).

8.2.2 Non-cropped disturbed habitats

Although canola is mainly a plant of disturbed habitats (ie agricultural fields), it will take advantage of disturbed land (Salisbury 2002c). Canola seed can be disseminated to neighbouring non-agricultural areas by a variety of mechanisms (see above). However, as noted above, canola is a poor competitor and is not regarded as an environmentally hazardous colonising species. Unless the habitat is regularly disturbed, or seed replenished from outside, canola will be displaced by other plants (Salisbury 2002c).

[12] Naturalised non-native species may be defined as those that have been introduced, become established and that now reproduce naturally in the wild, without human intervention (Groves et al. 2003)

[13] Environmental weeds are naturalised non-native species that have invaded non-agricultural areas of natural vegetation and are presumed to impact negatively on native species diversity or ecosystem function. Environmental weeds are distinguished from agricultural weeds by the ecosystem they impact.

Populations of canola can be found on roadside verges, in field margins and along railway lines in all countries where it is grown. An Australian survey encompassing a total of 4000 km of road and 400 observations recorded incidences of canola plants growing within 5 m of the roadside (Agrisearch 2001). The incidence of canola in the major canola growing districts was as follows: southern NSW (31 %), Victoria (13 %), SA (9 %), WA (20 %) and Tasmania (14 %). The occurrence of predominantly isolated plants suggested they had not originated from seed derived from plants grown the previous season, but from individual seeds dropped during transportation. Dignam (2001) surveyed 103 local councils across Australia and evidence of canola was present in 30 % of the councils surveyed. In the survey, the density of roadside canola populations was mostly low. Only 5 % of councils and 4 % of road and rail authorities surveyed, indicated canola was present in large numbers.

A four year study in France examined the factors involved in the persistence of feral *B. napus* populations (Pivard et al. 2007). This study determined that 35 to 40 % of roadside populations were due to dispersal from adjacent fields – mainly seed dispersed at harvest with a much smaller portion dispersed at sowing. Approximately 15 % of roadside populations were due to dispersal from farm equipment or during transport. The remaining 50% was due to persistence, with up to 10 % attributed to local recruitment (ie seed from the feral population replenishing the seed bank) and 40 % due to the existing seedbank.

8.2.3 Undisturbed natural habitats

Canola is classified as a category 2 weed in natural ecosystems. Category 2 weeds are naturalised and known to be a minor problem warranting control at three or fewer locations within a State or Territory (Groves et al. 2003). Canola is not considered a significant weed, nor invasive of natural undisturbed habitats in Australia (Dignam 2001) or Canada (Canadian Food Inspection Agency 1994; Warwick et al. 1999; Beckie et al. 2001). Crawley et al (2001b) determined that over a 10 year period, canola was not invasive or persistent in undisturbed non-arable habitats in the U.K. Due to selective breeding, crop plants function optimally under managed agricultural conditions, such as high soil fertility or low plant competition. These conditions rarely occur in natural habitats, resulting in poor fitness of canola plants (ie reduced recruitment, low survivorship, poor competitive ability, low seed production). In the absence of disturbance, canola is unable to compete with other weeds and does not persist (Hall et al. 2005).

8.3 Control measures

Canola is usually grown in rotation with wheat as the follow on crop. Volunteer canola (non-herbicide tolerant canola) can be controlled in the post-emergent wheat crop using a variety of chemicals. For example, herbicides containing chemicals such as flumetsulam, MCPA (2-methyl-4-chlorophenoxyacetic acid), sulfosulfuron, or metosulam may be used at the early post-emergent stage, whereas MCPA can also be used at the late post-emergent stage (Brooke et al. 2007). Broad-spectrum herbicides such as glyphosate, broad-leaf herbicides such as 2,4-D, or mechanical means can also be used to control volunteer canola plants.

SECTION 9 POTENTIAL FOR VERTICAL GENE TRANSFER

9.1 Intraspecific crossing

Intraspecific crossing refers to fertilisation between *B. napus* plants including adjacent plants, interplot and field scale crossing. The transfer of genes between canola populations is well documented (Paul et al. 1995; Scheffler et al. 1995; Downey 1999). Research on interspecific crossing has been summarised by Beckie et al (2003) and more recently by Husken and Dietz-Pfeilstetter (2007).

The summary by Beckie et al (2003) focused on interplant, interplot, and field scale trials, which utilised markers such as petal colour, erucic acid, isozymes, chlorophyll deficient mutants, antibiotic or herbicide resistance and emasculated or male sterile recipient plants. Based on the seven references cited in Beckie et al (2003) average outcrossing between adjacent plants would be approximately 30%, but rates up to 55% have been recorded. A few of the cited studies reported maximum distances of up to 1.5 km for wind-mediated pollen dispersal when pollen was measured directly (Timmons et al. 1995) and between 400 m and 4 km when pollination was detected using male sterile or emasculated bait plants (Timmons et al. 1995; Thompson et al. 1999; Norris et al. 1999). Studies using male sterile or emasculated plants only represent the potential for gene flow and would overestimate gene flow levels due to lack of pollen competition (Baker & Preston 2004). Furthermore, it should be noted that while such measurements give an indication of the potential for outcrossing they do not indicate the likelihood of outcrossing actually occurring (Salisbury 2002b).

In contrast, Husken and Dietz-Pfeilstetter (2007) focused on interspecific crossing from herbicide tolerant (HT) to non HT *B. napus* border rows or fields and considered the effect of the size of the pollen donor and recipient fields as well as the design of the trial. Mean values of cross fertilisation for two classes of field design and over various distances is given in Table 4 (below). This study indicates that the bulk of cross fertilisation occurs with in the first 10 m of the recipient field. While there are limitations to the studies examining outcrossing in canola, such as size, shape and orientation of the pollen source and recipient; isolation distance and pollen barriers between pollen source and recipient; climatic conditions (wind direction and speed, temperature, humidity); local topography; the number of bees and/or other insects;

Table 4. **Mean values of cross fertilisation (%) between HT and non-HT *B. napus* (from Husken and Dietz-Pfeilstetter, 2007).**

Distance [a] (m)	Continuous design [b]			Discontinuous design [c]			Mean over both designs		
	Mean	Std [d]	n [e]	Mean	Std	n	Mean	Std	n
0-10	1.78	2.48	26	0.94	0.51	10	1.54	2.15	36
10-20	0.33	0.45	7	0.40	0.47	8	0.37	0.45	15
20-50	0.05	0.05	10	0.14	0.11	11	0.10	0.09	21
50-100	0.04	0.04	3	0.11	0.11	11	0.09	0.1	14
>200	n.d.[f]	n.d.	n.d.	0.05	0.05	6	n.d.	n.d.	n.d.

[a] Distance from pollen source; [b] Continuous design = HT trial surrounded by non HT border area; [c] discontinuous design = from HT trial to non HT field patches; [d] Standard deviation; [e] Number of data points; [f] Not enough data available

genotype and flowering synchrony (Husken & Dietz-Pfeilstetter 2007) (Scheffler et al. 1993), the data indicate that interspecific crossing decreases as the distance from the pollen source increases.

There are limited data on outcrossing rates under Australian conditions (Rieger et al. 2002). Outcrossing rates between commercial fields of non-GM herbicide tolerant canola and conventional canola at distances from 0 to 2.6 km were variable, ranging between 0 and 0.15 %. The maximum outcrossing rate of 0.197 % was measured at 1.5 km. Outcrossing was less than 0.01 % at 2.6 km from the pollen source. Outcrossing was not detected at sites from 3 to 6 km from the pollen source. When averaged across the individual paddocks where outcrossing had occurred, outcrossing did not exceed 0.07 %. Outcrossing occurred in 63 % of the fields, but only a few had outcrossing rates greater than 0.03 %.

Results from the above overseas studies contrast with this Australian study, which did not show a decline in outcrossing over distance. A major difference between the Rieger et al (2002) study and the overseas studies was that the former utilized large 25- to 100-hectare pollen source fields, whereas the latter used relatively small pollen sources. It is possible that dispersal of pollen from smaller sources may be influenced more by insect pollen vectors, such as bees, whereas dispersal of pollen from larger sources may be more influenced by wind. Outcrossing rates between plots appear to depend on spatial configuration making them difficult to compare among experiments (Baker & Preston 2003; Klein et al. 2006). The large edge to area ratios of small plots may increase the impact of pollen exchange at the interface (Baker & Preston 2003) although there did not appear to be an edge effect reported by Reiger et al (2002).

Although the probability of outcrossing appears to be low, the large number of canola flowers and the many small seeds produced per plant ensures a substantial quantity of outcrossed seed can still be produced. Some seed may shatter onto the ground before or at harvest and germinate the following season with the succeeding crop. Although many of these seedlings may be killed by frost, disease, insect attack, early herbicide treatments and/or tillage, a proportion of seedlings may either survive or emerge later in the season, to compete with the succeeding crop, warranting further chemical or mechanical control.

The probability of gene flow is a function of the spatial scale of the introduction. Limited field experiments may not accurately predict outcrossing in large scale plantings. Estimates of low levels of gene flow from moderate-sized plots to larger planted areas [eg (Scheffler et al. 1993)] are more representative of gene flow from a feral population to a nearby crop, than the reverse (Squire et al. 1999). At larger spatial scales there is a greater possibility for contact with sensitive species or habitats or for landscape-level changes because at larger scales, more ecosystems could be altered.

Canola could also outcross to other *B. napus* groups or subspecies including vegetables such as Swedes, rutabaga, and kale provided they are in close proximity and there is synchrony of flowering. *B. napus* vegetables are not recognised as weeds in agricultural environments and they are generally harvested prior to flowering, unless they are grown for seed production, when precautions would usually be taken

to maintain seed purity. Thus, hybrids between canola and vegetable crops of *B. napus* are unlikely to occur (Salisbury 2002a).

9.2 Interspecific crossing

There are a number of factors that affect pollen-mediated gene flow between plants and include sexual compatibility of the recipient species (whether the same or different species), flowering time, size of pollen source and sink, pollination vectors, pollen viability and environmental factors. Interspecific gene flow data between *B. napus* and other Brassicaceae species has been reviewed by a number of authors (Salisbury 1991; Scheffler & Dale 1994a; Salisbury & Wratten 1997; Rieger et al. 1999; FitzJohn et al. 2007) and is summarised below in Table 5 (Salisbury 2002b).

Species have been divided into groups [14] , with Group I species having the highest and Group IV the lowest sexual compatibility with *B. napus*. Sexual compatibility was based on the following:

- glasshouse rescued hybrids – hybrids resulting from hand pollination under controlled conditions (usually glasshouse), followed by embryo rescue
- glasshouse hand hybrids – hybrids resulting from hand pollination under controlled conditions (usually glasshouse), embryo rescue not needed
- field hybrids – hybrids produced under field conditions (unassisted by man)
- gene introgression – transfer of gene(s) from *B. napus* genome into the genome of the other species involved in hybridisation

Hybridisation has occurred with species in Groups III and IV following hand pollination and the use of embryo rescue methods. However, the hybridisation data from these sophisticated experiments gives no measure of the likelihood of successful hybridisation in the field (Scheffler & Dale 1994a). No field hybrids have been reported for any Brassicaceae species from Group III to VI, nor have any glasshouse-hand or rescued hybrids been reported for weedy species that are not members of the Tribe Brassiceae (Group VI) and as such, regarded as extremely unlikely to be hybridise naturally with canola.

Group I species (*B. rapa* and *B. juncea*) have the highest sexual compatibility with *B. napus*, with a high probability of hybridisation and gene introgression occurring between these species and *B. napus*. Hybrids between *B. napus* and Group II species (eg *Raphanus raphanistrum*) have been recorded in the field, although successful gene introgression has not been measured. Interspecific crosses involving *B. napus* are discussed below, whereas intergeneric crosses are discussed in the following section.

[14] Salisbury used the term 'category', but 'group' is used here to avoid confusion with weed categories discussed earlier.

Table 5. Potential gene flow between canola (*B. napus*) & Australian Brassicaceae species (modified from Salisbury, 2002)

	Group I	Group II	Group III	Group IV	Group V	Group VI
Tribe	Brassicaceae	Brassicaceae	Brassicaceae	Brassicaceae	Brassicaceae	Other[6]
Glasshouse 'rescued' hybrids	Yes	Yes	Yes	Yes	No	No
Glasshouse hand hybrids	Yes	Yes	Yes	No	No	No
Field hybrids	Yes	Yes[2]	Not reported	Not reported		
Gene introgression	Yes/Likely[1]	Not reported				
Weeds	*Brassica rapa* *Brassica juncea*[1]	*Raphanus raphanistrum* *Hirschfeldia incana* *Sinapis arvensis*	*Brassica fruticulosa* *Brassica nigra* *Brassica tournefortii* *Diplotaxis muralis* *Diplotaxis tenuifolia* *Rapistrum rugosum*	*Brassica oxyrrhina* *Diplotaxis tenuisiliqua*	*Conringia orientalis* *Carrichtera annua* *Cakile maritima*	*Capsella bursapastoris* *Cardaria draba* *Lepidium sp.* *Myagrum perfoliatum* *Sisymbrium orientale* *Sisymbrium irio* *Sisymbrium erysimoides* *Sisymbrium officinale*
Condiment, fodder & vegetable species	Forage *B. napus*[1] *B. napus* vegetables[1] *B. rapa* vegetables[1] Condiment *B. juncea*[1]		*Brassica alboglabra*[3] *Brassica chinensis*[4] *Brassica nigra* *Brassica oleracea* *Brassica pekinensis*[4] *Raphanus sativus*[5] *Sinapis alba*			

→ DECREASING SEXUAL COMPATIBILITY →

[1] Considered likely to happen over a period of time **if** the species are in physical proximity and have flowering synchrony.
[2] Frequency of interspecific hybrids approx. 10^{-4} to 10^{-8}. Likelihood of subsequent introgression or formation of fertile amphidiploids significantly less again.
[3] This species is sometimes considered to be a subspecies of *B. oleracea*.
[4] These species have sometimes been considered to be subspecies of *B. rapa*.
[5] Hybridisation has occurred between *R. sativus* and male sterile *B. napus* in glasshouse using bees for pollination, hybrids had reduced pollen fertility (Ammitzboll and Jorgensen 2006). There are no reports of *R. sativus* in Australian Virtual Herbarium database as of 16/02/2007
[6] Salisbury's table also included the following native, but non-weedy species for which there have been no reports of hybridisation with *B. napus*: *Arabidella* (6 *sp.*), *Balbaretinia* (1 *sp.*), *Barbarea* (2 *sp.*), *Blennodia* (25 *sp.*), *Cardomine* (5 *sp.*), *Carinavalva* (1 *sp.*), *Cheesemania* (1 *sp.*), *Cuphonotus* (2 *sp.*), *Geococcus* (1 *sp.*), *Harmsiodoxa* (3 *sp.*), *Irenepharsus* (3 *sp.*), *Lepidium* (35 *sp.*), *Menkea* (6 *sp.*), *Microlepidium* (*2 sp.*), *Pachymitus* (*1 sp.*), *Phlegmatospermum* (*4 sp.*), *Rorippa* (*4 sp.*), *Scambopus* (*1 sp.*), *Stenopetalum* (*9 sp.*).

9.2.1 Brassica rapa

Brassica rapa ssp. *sylvestris* (= *B. campestris* L.) is a diploid species, which has a similar life history to canola, but with a shorter growing season. Varieties of *B. rapa* were grown on a limited scale in Australia in the 1970s and early 1980s. By mid 1980s, breeding programs on *B. rapa* ceased and it was superseded by the release of superior quality canola varieties (Colton & Potter 1999). *Brassica rapa* is still grown as oilseed rape in Europe in both spring and winter forms.

Brassica rapa ssp. *sylvestris* is distributed throughout Queensland, New South Wales, Victoria, Tasmania and South Australia and sometimes occurs as a weed of disturbed and cultivated land (Auld & Medd 1987). While not regarded as a problem in South Australia, it is a minor problem in Queensland, New South Wales and Victoria and a major problem in cereals and vegetables in Tasmania (Hyde-Wyatt & Morris 1989); (Holm et al. 1997; Groves et al. 2000). Four subspecies of *B. rapa* are recognised in Australia (spp. *chinensis*, *oleifolia*, *rapa* and *sylvestris)* that range in classification from category 1 to 4[15] (spp. *sylvestris*) weeds in natural ecosystems and category 1 to 5[16] (spp. *sylvestris*) weeds in agricultural ecosystems (Groves et al. 2003).

Brassica napus (AACC) and *B. rapa* (AA) share a common set of chromosomes, making interspecific outcrossing common [eg (Bing et al. 1991; Jorgensen & Andersen 1994; Scheffler & Dale 1994b; Bing et al. 1996; Brown & Brown 1996; Mikkelsen et al. 1996a; Mikkelsen et al. 1996b; Salisbury & Wratten 1997; Jørgensen 1999; Snow & Jorgensen 1999)]. Viable hybrids can be produced in the field when canola is crossed with *B. rapa* (Jorgensen & Andersen 1994; Bing et al. 1996; Brown & Brown 1996; Mikkelsen et al. 1996b; Metz et al. 1997) in either direction. However, the frequency of hybrids depends on parental genotypes, experimental design, and population size (Scott & Wilkinson 1998; Palmer 1962; Bing et al. 1991; Jorgensen & Andersen 1994; Jorgensen et al. 1996; Landbo et al. 1996; Hauser et al. 1997; Jorgensen et al. 1998; Jørgensen 1999).

The estimation of hybridisation frequencies between *B. rapa* and *B. napus* varies significantly depending on the experimental design. Gene flow measurements by Scott and Wilkinson (1998) from canola to *B. rapa* populations growing outside field boundaries suggested hybridisation frequencies of 0.4 to 1.5 % and hybrid seedling survivorship of less than 2 %. Danish studies have shown that the proportion of hybrid seed from *B. rapa* can be as high as 93% when *B. rapa* occurs as a single plant within a canola crop. In contrast, the proportion of hybrid seed was 13% (*B. rapa* as the female) or 9% (*B. napus* as the female) when the two species were planted in a 1:1 ratio (Jorgensen et al. 1996). Canadian studies reported similar hybridisation rates, 13% for *B. rapa* populations within canola fields and 7% between adjacent fields of *B. rapa* and canola (Warwick et al. 2003). *B. rapa* is an obligate out-crossing species and consequently more hybrids are found when *B. rapa* is the female (Jorgensen & Andersen 1994; Jorgensen et al. 1996; Hauser et al. 1997; Jorgensen et al. 1998).

[15] Category 4 indicates plants that are naturalised and known to be a major problem at three or fewer locations within a State or Territory.
[16] Category 5 indicates plants that are naturalised and known to be a major problem at four or more locations within a State or Territory.

In other North American studies, high density (600:1) of canola: *B. rapa* resulted in hybridization frequencies ranging from 4 to 22% (average 10%), whereas a low density (180:1) had a frequency of approximately 2%. Hybridization was highest if *B. rapa* occurred within the canola (37.2%) compared with plot margins (5.2%). In field experiments with a ratio of 5-15 plants of *B. rapa* to 1 transgenic *B. napus x B. rapa* hybrid, 50% of the resulting seed was transgenic if the hybrid was the maternal parent, whereas 0.074% were transgenic if *B. rapa* was the maternal parent (Halfhill et al. 2004).

Where natural interspecific hybrids occur, they have reduced fertility and low seed set (average 2 to 5 seeds per pod) compared with the parents (Jorgensen & Andersen 1994). Reduced dormancy of *B. rapa* x canola hybrids relative to the persistent wild *B. rapa* (Jorgensen et al. 1999), coupled with the reduced fertility of the interspecific hybrid (Jorgensen et al. 1999) makes it very unlikely that populations of these hybrids would persist.

The fitness of these hybrids has been examined, with contrasting results [see Warwick et al. (2007) for further discussion]. *Brassica napus*, *B. rapa* and *B. napus x B. rapa* hybrids were subjected to simulated herbivory and interspecific competition trials in England (Sutherland et al. 2006). Several vegetative and reproductive performance measures were used to determine the effect of herbivory and plant competition. The under performance of hybrid plants compared to the parent species suggests that the hybrid is less fit than either parent. These results may explain the relative rare occurrence of the hybrid in nature given the potential for hybridisation discussed above. Of particular interest is that the hybrids exhibited no competitive advantage over *B. rapa*. Analysis of F1 seeds found 15% to be normal seeds, 73% were aborted seeds and 12% exhibited precocious germination – which may also explain why hybrid numbers are low in the wild.

Hauser et al (1998b) determined that weedy *B. rapa x B. napus* F1 hybrid[17] hybrids had an intermediate fitness compared to the parent species and were significantly more fit than *B. rapa*. In the subsequent generation, F2 hybrids had reduced fitness (Hauser et al. 1998a) indicating a further bottle neck for gene flow from *B. napus* (Sutherland et al. 2006).

Further studies examined productivity and competitiveness among *B. napus*, *B. rapa*, *B. napus x B. rapa* F1 hybrid, BC1F1[18] and BC2F2[19] generations (Halfhill et al. 2005). Average vegetative growth and nitrogen use were lower and plants were less competitive for hybrid generations than for *B. rapa*. This result contrasts the results of Hauser et al (1998a,b) above, in which the F1 hybrid showed a competitive advantage over the *B. rapa* parent. It has been suggested the contrasting results of the two studies may be due to the use of biotypes of the *B. rapa* parent that have different degrees of weediness (Halfhill et al. 2005).

[17] F1 hybrid – F1 stands for Filial 1, the first generation resulting from crossing between distinctly different parental types. Self-pollination or crossing among F1 plants would result in the F2 generation.

[18] BC1F1 – refers to the offspring of a single backcross between an F1 hybrid and one of the parental types (recurrent parent). Backcrossing the BC1F1 to the recurrent parent results in the BC2F1 generation.

[19] BC2F2 – refers to the offspring of self-pollination or crossing among the BC2F1 generation (see footnote above).

Although the above results suggest contrasting fitness levels for the F1 hybrid and subsequent backcross generations; spread, persistence and introgression of a gene from canola to *B. rapa* has been demonstrated under field conditions (Warwick et al. 2007). Hybrids between HT GM canola and *B. rapa* formed under field conditions, and subsequent backcrossed generations (also occurring under field conditions) were observed over a six year period. After this period, individual plants resulting from backcrosses of the F1 hybrid to *B. rapa* were examined and found to be herbicide tolerant, diploid - molecular markers and flow cytometry suggested they were almost entirely composed of the *B. rapa* genome, and had high male fertility (approximately 90%). The above results indicate that genes from *B. napus* can be introgressed in to the *B. rapa* genome under field conditions.

Canola can also outcross to the vegetable forms of *B. rapa* (including turnip, Chinese cabbage and pak choi) if they are in close proximity and there is synchrony of flowering. *B. rapa* vegetables are not recognised as weeds in agricultural environments and they are generally harvested prior to flowering, unless they are grown for seed production, when precautions would usually be taken to maintain seed purity (Salisbury 2002a). Thus, hybrids between canola and vegetable crops of *B. rapa* are unlikely to occur.

9.2.2 Brassica juncea

Several thousand hectares of conventional Indian mustard (*Brassica juncea*) have been grown annually in south eastern Australia for approximately 25 years as part of a small industry. *B. juncea* occurs in Queensland, New South Wales, Victoria, South Australia and Western Australia (Groves et al. 2000). Based on the weed classification system discussed above, *B. juncea* is a category 3 weed in natural ecosystems, a category 5 weed in agricultural systems and described as primarily a weed in agricultural or disturbed areas. A category 3 weed is naturalised and known to be a minor problem warranting control at 4 or more locations within a State or Territory. For comparison, barley (*Hordeum vulgare*) falls in the same categories for natural and agricultural weeds and is also primarily a weed in agricultural or disturbed areas (Groves et al. 2003).

Similar to canola, Indian mustard occurs in disturbed habitats along roadsides, railway lines and in field margins in regions where it has been cultivated (Oram et al. 2005). In Australia, feral populations of canola rarely persist (Salisbury 2002a; Salisbury 2002c; Brooks et al. 2003; Baker & Preston 2004) and observations suggest that Indian mustard is less likely than canola to volunteer in subsequent crops or to persist as a feral population (Oram et al. 2005).

Canola (AACC) and *B. juncea* (AABB) have a common set of chromosomes, enhancing the likelihood of interspecific hybridisation and gene flow (Salisbury 2002a). Spontaneous occurrence of interspecific hybrids in the field has been reported in several countries (Bing et al. 1991; Frello et al. 1995; Bing et al. 1996; Jorgensen et al. 1998; Bielikova & Rakousky 2001). Under natural conditions outcrossing can occur between canola and Indian mustard, with recorded rates ranging from 3 to 4.7 % when canola is the male parent and plants are in close proximity (Bing et al. 1991; Jorgensen et al. 1996). The reciprocal cross was less successful (Jorgensen et al. 1996; Jorgensen et al. 1998). *B. napus x B. juncea* hybrids have reduced pollen fertility (ranging from 0 to 28 %) but have been shown to produce

viable seed and survive to the next generation (Bing et al. 1991; Jorgensen et al. 1996).

9.2.3 Brassica oleracea

Hybrids between *B. oleracea* vegetables (eg cauliflower, broccoli, Brussels sprouts, kohlrabi, etc) and *B. napus* have been reported under artificial but not under natural conditions (Salisbury 2002a). These vegetable crops are also harvested prior to flowering (Salisbury 2002a; Salisbury 2006), thus hybridisation is unlikely to occur.

9.3 Intergeneric crossing

Many Brassicaceae species occur as weeds of disturbed habitats in Australia, particularly agricultural habitats.

9.3.1 Tribe Brassiceae species

Gene movement between canola and other members of the Brassicaceae family occurs at extremely low levels in nature. Large sources of pollen may have a considerable effect on a small population of compatible plants (Ellstrand et al. 1989; Klinger et al. 1991). The flowering periods of many weedy Brassicaceae species overlap with canola. Depending on the season and region, the synchrony of flowering between species can also influence the rate of outcrossing in the field. Generally, commercially grown canola flowers from September to January, while many weedy Brassicaceae species begin flowering around August. However this will vary with environmental conditions and under ideal growing conditions, some weedy species may flower at any time during the year (Rieger et al. 1999).

Hybrids occurring naturally in the field between canola and Brassicae species have been reported for three economically important weed species in Australia: *Raphanus raphanistrum* (wild radish), *Hirschfeldia incana* (Buchan weed) and *Sinapis arvensis* (charlock) (see Table 5). Natural hybrids between *B. napus* and other weed species in the *Brassiceae* tribe have not been reported, although a few hybrids have been generated through controlled hand pollinations and embryo rescue. There have been no reports of hybrids, either naturally occurring or through controlled hand pollinations and embryo rescue, between *B. napus* and other weed species in tribes other than *Brassiceae* (Salisbury 2002a; Salisbury 2006).

The potential for outcrossing and gene introgression from *B. napus* into *R. raphanistrum* (wild radish), *H. incana* (Buchan weed) and *S. arvensis* (charlock) the species are discussed in more detail below.

Raphanus raphanistrum (wild radish)

Raphanus raphanistrum (wild radish) occurs in Queensland, New South Wales, Victoria, Tasmania, South Australia and Western Australia (Groves et al. 2000). It is a major weed of cropping regions, particularly in southern Australia and is classified as a category 5 weed in agricultural ecosystems (Groves et al. 2003). Large numbers of wild radish can also occur along roads and railway lines in and around canola growing areas in Australia (Agrisearch 2001; Dignam 2001). Although Dignam (2001) indicated that it has never been reported as a weed in National Parks, it is a weed of some natural areas and has been classified as a category 5 weed in natural ecosystems of Australia (Groves et al. 2003).
Wild radish has been shown to hybridise with *B. napus* under both laboratory and field conditions (Rieger et al. 2001; Eber et al. 1994; Mikkelsen et al. 1996a; Chevre

et al. 1997; Darmency et al. 1998; Rieger et al. 1999). Differences in the frequency of outcrossing between the two species depend on the direction of pollination. Studies suggest that hybridisation into canola (canola pollinated by wild radish) is more likely to occur. However, under Australian field conditions, the outcrossing rate from wild radish to canola (ie canola pollinated by wild radish) was still very low, with only two hybrids located in 5.2×10^7 canola seedlings (an outcrossing rate of 1 in 2.6×10^7, or 3.8×10^{-8}) (Rieger et al. 2001; Rieger et al. 1999). Studies examining hybridisation frequencies between *B. napus* and *R. raphanistrum* under field conditions are summarised below in Table 6.

Table 6. Frequency of hybridisation between *B. napus* and *R. raphanistrum* under field conditions.

Country	Frequency *B. napus* as female	Frequency *R. raphanistrum* as female	References
Australia	3.8×10^{-8}	$< 1.6 \times 10^{-5}$	(Rieger et al. 2001).
France	2.1×10^{-5} to 5.1×10^{-4}	3×10^{-5} to 1×10^{-7}	(Chevre et al. 2000)
Canada	Not reported	3.1×10^{-5}	(Warwick et al. 2003).
USA	$< 5.2 \times 10^{-5}$	Not reported	(Halfhill et al. 2004)

Outcrossing rates between wild radish and canola can vary depending on the source of the *R. raphanistrum* population. Ammitzboll and Jorgensen (2006) examined gene flow between wild radish and male sterile canola using bees as pollinators in glasshouse conditions. Hybridisation frequencies varied significantly among the populations depending on the pollen source. Observed frequencies were 2, 53 and 100 % for the Danish, Swiss and French populations of *R. raphanistrum,* respectively. These differences may be due to reported variation in prezygotic barriers which limit hybridisation of *R. raphanistrum* with *B. napus* pollen. Variation ranged from approximately 40 % of wild radish plants' pistils allowed no or few pollen tubes to grow and few fertilised ovaries to a few plants which showed no distinction between wild radish *and B. napus* pollen (Gueritaine & Darmency 2001).

F1 hybrids were reported to have <1% pollen viability, making self-compatibility difficult to evaluate (Warwick et al. 2003) and were almost invariably sterile (Pinder 1999), but fertility (if present) improved in subsequent backcross generations with wild radish (Chevre et al. 1997; Chevre et al. 1998). Backcrossing F1 hybrids (male) to *R. raphanistrum* (female) resulted in plants (BC1F1) with low male fertility (8.7%) and improved female fertility over the F1 hybrids. The level of sterility in the F1 hybrids was such that it was approximately 100 times more difficult to obtain seeds from the F1 hybrid than from the original interspecific cross (Chevre et al. 1998). Chevre (2003) noted that over 4 generations of backcrossing of the F1 hybrid to *R. raphanistrum*, both male and female fertility increased equivalent to that of wild radish.

Concurrent with the reported low fertility, was a reduced seed set in the hybrid plants. Chevre (1998) reported that F1 hybrid seed production was low, with only 0.78 seeds produced per plant. In the BC1F1 generation male fertility was 8.7% and seed production increased to 11 seeds per plant produced. In the subsequent backcross generation fertility increased again with 229 seeds produced per plant.

Vegetative development of the backcrossed generations (hybrid generations pollinated by wild radish) was retarded due to chlorophyll deficiency. However, reciprocal crosses (wild radish pollinated by a hybrid) were dark green with good vigour, indicating an incompatibility between the radish nucleus and the canola chloroplasts (Chevre et al. 2003). The F1 hybrid was shown have significantly lower rates of seedling survival, rosette diameter and dry matter production compared to either parent under field conditions, suggesting it was less likely to emerge and survive to reproduction under agronomic or natural conditions (Gueritaine et al. 2003).

French studies examining the fate of the F1 hybrid seed indicated that hybrid seeds are rare in proportion to the total soil seedbank (no more than 1 seed per 10 m^2) and can survive for similar periods as canola seeds in cultivated soils, decreasing to ≤1 % survival after 3 years (Chadoeuf et al. 1998).

French studies also examined genomic structure of the F1 hybrids and subsequent generations (Chevre et al. 1999; Chevre et al. 2000; Chevre et al. 2003). F1 hybrids between HT canola (AACC, 2n=38) and wild radish (RrRr, 2n = 18) are expected to have the genomic structure of 2n=28(ACRr) (Chevre et al. 2003). Among the 23 HT hybrids examined, 18 had the expected genomic structure of 2n=28(ACRr), one resulted from an unreduced gamete of wild radish (2n=37, ACRrRr) and 4 hybrids had a genomic structure of 2n=56 (AACCRrRr). The morphology of all the hybrids was similar to the canola, except for the 2n=37 hybrid, which was intermediate between the two parental species.

The HT ACRr (2n=28) hybrids were backcrossed with the *R. raphanistrum* parent over 4 generations, with HT tolerant offspring backcrossed in each subsequent generation. The chromosome number decreased over the generations such that after the 4th backcross, 91% of the hybrids had fewer than 23 chromosomes but none were herbicide tolerant. These results show that the HT gene from canola had not been introgressed through recombination into the wild radish genome (Chevre et al. 2003). Further studies indicate that generation and HT tolerant line are two factors that influence the rate of decrease in chromosome number (Chevre et al. 2007). Transfer of introduced genes by recombination between chromosomes of different genomic origin is thought to be extremely rare, as demonstrated by studies in hexaploid wheat (Hedge & Waines 2004). This is likely due to the spatial separation of chromosomes from different genomes during the cell cycle as observed in hexaploid wheat, which contains 3 genomes (Avivi et al. 1982) and the F1 hybrid generated by crossing barley and wild rye (Leitch et al. 1991).

Gene transfer from canola to wild radish will only occur as a result of hybridisation and introgression of genes into the wild population. Introgression involves the transfer of segments of a genome and genes between hybrid backcrossing with the wild radish population. While hybridisation between canola and wild radish has been documented at very low rates (see Table 6), reduced fertility and seed set, retarded vegetative development, and low F1 hybrid seed survival in soil would limit the opportunity for back crossing to wild radish. Additionally, differences in genomic structure, reduced chromosome numbers in each successive back cross generation and documented lack of successful introgression of genes from canola into wild radish, suggests that introgression is unlikely to occur.

Hirschfeldia incana (Buchan weed)

Hirschfeldia incana (Buchan weed) occurs in Queensland, New South Wales, Victoria, Tasmania, South Australia and Western Australia. It is a minor problem in agricultural areas of Queensland and New South Wales (Groves et al. 2000). Buchan weed will invade disturbed native vegetation and can also occur in large numbers along railways and roadsides in canola growing regions in Australia (Dignam 2001). *H. incana* is classified as a category 4 weed in natural ecosystems and category 5 weed in agricultural ecosystems in Australia (Groves et al. 2003).
Spontaneous hybridisation with canola is known to occur in this species, although where Buchan weed is fertilised by canola pollen, the frequency of hybridisation is low. Studies done in France have demonstrated that when canola was interspersed with Buchan weed at a density of 625: 1 (canola: Buchan weed), 1.5 % of the Buchan weed seeds were hybrids (Lefol et al. 1996b). When male sterile canola was interspersed with Buchan weed (1:1 ratio), the hybridisation frequency increased and 70 % of seeds were hybrids (Lefol et al. 1996b).

Lefol et al (1996b) determined that hand pollination and embryo rescue of canola (male) x Buchan weed (female) crosses gave 2.5 hybrid seed per 100 flowers, but that reciprocal crosses did not yield any hybrid seed. Male sterile canola and Buchan weed (1:1 or 1:4 ratio) in cages with honeybees for pollination resulted in hybrid seed (up to 26 hybrids per plant), whereas Buchan weed and male **fertile** canola (1:30) in cages with honeybees gave no hybrids out of 24,800 seedlings.

This last result contrasts field results where Buchan weed and male fertile canola (1:625) yielded 16 hybrids from 853 seedlings or 1 hybrid per plant. The authors suggested that some of the Buchan weed in the caged study (above) may have been self-compatible (normally they are self-incompatible). The work of Darmency and Fleury (2000) indicated that *H. incana* is variable for self-incompatibility and that crossing among such plants can yield high numbers of pods and seeds per plant. It is also possible that the higher ratio of canola to Buchan weed under field conditions virtually eliminated the possibility of cross-pollination among Buchan weed, resulting in higher rates of hybridisation (similar to canola x *B. rapa* hybrid formation, see (Halfhill et al. 2004).

Further field studies in France with male sterile canola and Buchan weed in a 1:1 ratio yielded 24,600 hybrids from 68,000 plants or 0.36 hybrid per male sterile canola plant (Lefol et al. 1996b). Similar results (also in France) determined hybridisation between HT male sterile canola and *H. incana* at a frequency of 0.6 hybrids/plant over a 3 year field study (Darmency & Fleury 2000).

Fertility, seed set and seed viability from F1 hybrids is greatly reduced. Anthers of the hybrids produced almost no pollen grains and the few grains produced were aborted. The hybrid plants produced fewer flowers, pods, seeds per pod, and overall 2 x 10^3 fewer seeds per plant than Buchan weed. The total seed from 168 hybrids was 32 and only 5 of these seeds germinated. Three of these BC1F1 hybrids were sterile and the other 2 plants only generated 62 seeds when backcrossed with Buchan weed (Lefol et al. 1996b).

Survival of hybrid seed in soil was intermediate between the parents. In undisturbed soil, buried *B. napus* seed had a 1% germination rate after one year and completely

disappeared from the soil after 3 years, while seed of *H. incana* remained at 50% viability throughout the 3 study. Seed of the F1 hybrid was intermediate to the parent species with a 15% survival in the first year and complete extinction by 41 months (Chadoeuf et al. 1998).

While hybridisation between canola and Buchan weed has been documented, gene transfer from canola to *H. incana* is extremely unlikely due to low rates of hybridisation under field conditions; little or no fertility in the F1 hybrid; and poor seed set, viability and persistence of seed from the F1 hybrid, which would limit backcrossing between the F1 hybrid and Buchan weed. BC1F1 hybrids had high rates of sterility and poor seed set. Additionally, there have are no documented occurrences of introgression of genes from canola into the Buchan weed population. Backcrossing over 5 generations determined that a HT gene from canola was not successfully integrated into the Buchan weed genome (Darmency & Fleury 2000).

Sinapis arvensis (charlock)

Sinapis arvensis (charlock) occurs in Queensland, New South Wales, Victoria, Tasmania, South Australia and Western Australia. Charlock is a problem in agricultural areas and is particularly serious weed in cropping regions of New South Wales (Groves et al. 2000). It can also occur in disturbed sites along roadsides and railways in canola growing regions in Australia (Dignam 2001). Charlock is classified as a category 5 weed in both agricultural and natural ecosystems in Australia (Groves et al. 2003).

Charlock and canola are generally not considered to be sexually compatible. The majority of studies to date are from France and have determined that hand pollination followed by embryo rescue or ovule culture are the only methods for successfully effecting hybridisation. Reciprocal crosses yielded hybridisation frequencies of 1.2% (*B. napus* as female) and 0.1% (*S. arvensis* as female) (Lefol et al. 1996a). Similar results were reported by Chevre et al (1996) with frequencies of 3.7% (*B. napus* as female) and 0 % (*S. arvensis* as female).

When male sterile oilseed rape and charlock were grown in insect-proof cages with bees present for pollination (Lefol et al. 1996a) or isolated to prevent pollination from other rapeseed plants (Chevre et al. 1996), the frequency of hybrid seed production ranged from 0.012 to 0.18%. However, when charlock and male fertile oilseed rape were grown in similar cages or open field conditions, there were no hybrids identified out of 2.9 million viable seeds harvested from the charlock, suggesting a hybridization frequency of $< 3.4 \times 10^{-7}$. Lefol et al (1996a) estimated that the probability of a single flower of either of these species generating an interspecific hybrid was less than 10^{-10}.

Warwick (2003) collected seed from 79 *S. arvensis* populations from fields or field margins in Canada where HT *B. napus* populations were grown 1 or 2 years previously. None of the 43,000 seedlings screened were HT, suggesting a hybridisation rate of less than 2×10^{-5}.

Chevre et al (1996) examined 12 F1 hybrids and reported low male fertility based on stained pollen grain. Ten of the 12 hybrids had 0% and 1 plant each had 1 to 10% or 11 to 30% stained pollen grain. All the interspecific hybrids were vigorous and had a morphology intermediate between the two parents.

Hand pollinated of 7224 F1 hybrid flowers with charlock pollen resulted in 2 seeds that did not germinate (Lefol et al. 1996a). Similarly, Chevre et al (1996) hand pollinated 881 F1 hybrid flowers with charlock pollen and were able to rescue 3 embryos, though the fate of these embryos was not reported. Open pollination between the mostly male sterile F1 hybrids and charlock had a hybridisation frequency of 0.12 % (Chevre et al. 1996).

The reciprocal cross of 808 F1 hybrid flowers hand pollinated with oilseed rape pollen generated 9 seeds. One of these seeds produced a BC1F1 plant which looked like oilseed rape but could only be successfully backcrossed again with oilseed rape. The BC1F1 x oilseed rape backcross produced 13 seeds from 65 pollinated flowers (Lefol et al. 1996a).

Although hybridisation between canola and charlock has been reported at very low frequencies under conditions with restricted pollen competition (ie from hand pollinated or using male sterile canola), under open pollinated conditions no interspecific hybrids have been generated. Studies of F1 hybrids indicate extremely low male fertility and little or no viable seed resulting from backcrosses to charlock. Backcrosses to canola were only successfully backcrossed again to canola and produced few seeds. The above results suggest that there is no clear path for introgression of genes from *B. napus* into *S. arvensis* and that introgression is highly unlikely to occur under field conditions.

9.3.2 Other plant species

Another mechanism by which canola can transfer genetic material to sexually non-compatible plants is through 'bridging'. Bridging is defined as 'a mating made between two incompatible species'. Such a possibility of the 'bridging' phenomenon may occur with *B. juncea* acting as the intermediate species. The occurrence of hybrids between canola and *B. juncea* is rare. Under natural conditions outcrossing can occur between canola and Indian mustard, with recorded rates ranging from 3 to 4.7% when canola is the male parent and parent plants are in close proximity (Bing et al. 1991; Jorgensen et al. 1996). The reciprocal cross was less successful (Jorgensen et al. 1996; Jorgensen et al. 1998). The hybrids do not persist long enough in the environment to serve as a bridge due to poor fertility, poor germination, and high seedling mortality. However, even though *B. napus x B. juncea* hybrids have reduced pollen fertility, ranging from 0 to 28%, they have been shown to produce viable seed and survive to the next generation (Bing et al. 1991; Jorgensen et al. 1996). Introgression of canola genes into *B. juncea* would be more likely to occur if the genes are carried on the shared genome (AA) (Salisbury 2006).

If genes from canola were introgressed into the *B. juncea* genome, then *B. juncea* may act as 'bridge' to transfer the canola genes into *B. nigra*. Outcrossing between *B. juncea* (AABB) and *B. nigra* (BB) may be possible because they share a common genome (BB). However, *B. juncea* and *B. nigra* are not fully compatible. Hybrids between these two species have only been produced under artificial conditions and backcrossing to *B. nigra* has never been successful (Salisbury 2006). Thus, it follows that crosses between *B. napus x B. juncea* hybrids and *B. nigra* would be even less compatible. Another genetic barrier for gene transfer is that it has to occur by

chromosomal crossing over in the canola and *B. juncea* hybrid to be stably introduced into *B. nigra* (Scheffler & Dale 1994b).

Brassica rapa may also act as an intermediate species or bridging species between canola and incompatible species. Canola and *B. rapa* can produce hybrids and introgress into successive generations (see Section 9.2.1). Gene transfer between canola x *B. rapa* hybrids and species unrelated to canola, may occur therefore between those species related to *B. rapa*. However, the stability of gene introgression into *B. rapa* has not been measured. Furthermore, the persistence of canola x *B. rapa* hybrids is considerably less than *B. rapa* due to lowered fertility and reduced dormancy (Bing et al. 1991; Jorgensen et al. 1999). Additionally, subsequent backcross generations had further reductions in productivity and competitiveness (Hauser et al. 1998a; Halfhill et al. 2005), indicating a further bottleneck for gene flow from *B. napus* (Sutherland et al. 2006). However, spread, persistence and introgression of a gene from canola to *B. rapa* has been demonstrated under field conditions (Warwick et al. 2007). Based on the above data, the opportunity for *B. rapa* to acting as a bridging species in limited.

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