Australian Government
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Office of the Gene Technology Regulator

The Biology of *Brassica napus* L. (canola) and *Brassica juncea* (L.) Czern. & Coss. (Indian mustard)

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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 of canola courtesy of Brian Weir.

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TABLE OF CONTENTS

ABBREVIATIONS USED IN THIS DOCUMENT

ACT	Australian Capital Territory
AFLP	Amplified Fragment Length Polymorphism
AOF	Australian Oilseed Federation
APVMA	Australian Pesticides and Veterinary Medicines Authority
ASA	Australian Seeds Authority
BWYV	*Beet western yellows virus*
CaMV	*Cauliflower mosaic virus*
Canola	Canadian oil, low acid
CMS	Cytoplasmic male sterility
DNA	Deoxyribonucleic acid
DPI	Department of Primary Industry
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GRDC	Grains Research & Development Corporation
ha	Hectare
HOLL	High Oleic, Low Linolenic
IT	Imidazolinone tolerant
ITC	Isothiocyanates
ITSA	International Seed Testing Association
Mbp	Megabase pair
Mya	Million years ago
n	Haploid number of chromosomes
NGS	Next Generation Sequencing
NSW	New South Wales
NT	Northern Territory
OECD	Organisation for Economic Co-operation and Development
PTDI	Provisional Tolerable Daily Intake
QLD	Queensland
QTL	Quantitative Trait Locus
RFLP	Restriction Fragment Length Polymorphisms
RNA	Ribonucleic acid
SA	South Australia
SNP	Single Nucleotide Polymorphism
spp.	Species
SRAP	Sequence Related Amplified Polymorphism
SSR	Simple Sequence Repeat
TAS	Tasmania
TILLING	Target Induced Local Lesions in Genomes
TT	Triazine Tolerant
TuMV	*Turnip mosaic virus*
TuYV	*Turnip yellows virus*
VIC	Victoria
WA	Western Australia

PREAMBLE

This document describes the biology of *Brassica napus* L. and *B. juncea* (L.) Czern. & Coss., with particular reference to the Australian environment, cultivation and use. Information included relates to the taxonomy and origins of cultivated *B. napus* and *B. juncea*, general descriptions of their 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 organisms for use in risk assessments of genetically modified *B. napus* and *B. juncea* that may be released into the Australian environment.

The term 'canola' is derived from ***Can****adian* ***o****il*, ***l****ow* ***a****cid*, proposed by the Western Canadian Oilseed Crushers' Association in 1978 to refer to varieties[1] of *B. napus* with low erucic acid and glucosinolate content. In 1980, the trademark was transferred to the Canola Council of Canada (Eskin 2013). Canola now refers to three *Brassica* species that meet these compositional criteria: *B. napus* (also known as Argentine canola); *B. rapa* (also known as Polish canola); and *B. juncea* (also known as Indian mustard). For the purpose of this document, *B. napus* canola and *B. juncea* canola will be used to refer respectively to oilseed varieties of *B. napus* and *B. juncea* that meet internationally agreed compositional criteria. Canola will be used as a generic term to designate both species. Varieties not meeting agreed compositional criteria will be referred to as rapeseed and/or Indian mustard.

Canola is grown primarily as an oilseed, from which oil is extracted. The oil is used for cooking and in food products such as margarine. Canola seeds yield 35-45% oil. A by-product of the oil extraction process is the generation of a high-protein meal that may be used as animal feed. Worldwide, canola is the third most important edible vegetable oil crop after soybean and palm oil and the third most important oil meal crop after soybean and cotton (Snowdon et al. 2007).

The highest annual canola production occurs in the European Union, China, Canada, and Australia. Initial trials in Australia of *B. napus* and *B. rapa* began in the early 1960s, with the two crops first grown commercially in 1969. It was another decade before canola varieties became available. Today, in Australia commercial *B. napus* canola production occurs mainly in New South Wales, Victoria, South Australia and Western Australia, with an area of over 2.7 million hectares planted in 2013-2014 (ABARES 2015). The distribution of *B. napus* canola production coincides with the wheat belt, with *B. napus* often grown as a break crop between cereal rotations.

B. juncea, commonly known as Indian mustard (or rai) (OECD 2012) is cultivated worldwide as a condiment (mustard), oilseed or vegetable crop with the greatest commercial production occurring in India and Canada. In Australia, commercial production occurs on a relatively small scale with several thousand hectares planted annually in western Victoria, central New South Wales and/or South Australia.

[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).

SECTION 1 TAXONOMY

1.1 Brassicaceae family

The Brassicaceae family consists of approximately 372 genera and over 4000 species worldwide (The Plant List 2013). Some members of the Brassicaceae family are agriculturally important crops. In addition to the commercially valuable species, many wild species of Brassicaceae grow as weeds, particularly in regions of North America, South America and Australia (Couvreur et al. 2010). The model plant *Arabidopsis thaliana* is also a member of this family, its genome the first plant genome sequenced. For these reasons, the biology, genetics and phylogeny of the Brassicaceae have been widely studied.

Approximately 58 genera and 200 species of native/introduced Brassicaceae are present in Australia (ANBG 2015). Species used as food crops are introduced and belong to the genus *Brassica*. Other introduced Brassicaceae include plants that are classified as weeds, the most important being:

- *Lepidium draba* (hoary cress or white weed)
- *Diplotaxis tenuifolia* (sand rocket, sand mustard or Lincoln weed)
- *Hirschfeldia incana* (Buchan weed)
- *Myagrum perfoliatum* (musk weed)
- *Raphanus raphanistrum* (wild radish)
- *Rapistrum rugosum* (turnip weed) (Parsons & Cuthbertson 2001)

Other introduced species of Brassicaceae are used as ornamental plants in Australia, such as *Arabis albida* (rock cress), *Cheiranthus cheiri* (wallflower) or *Iberis amara* (candytuft) (Parsons & Cuthbertson 2001). Native Australian Brassicaceae are in a number of genera, including *Arabidella*, *Blennodia*, *Cardamine*, *Lepidium* and *Stenopetalum* (ANBG 2015).

1.2 *Brassica* genus

The *Brassica* genus consists of approximately 100 species worldwide (Gomez-Campo & Prakash 1999; Purty et al. 2008). Many *Brassica* plants are common crops, from oilseeds to vegetables and condiments. Such crops include canola, mustard, cabbage, cauliflower, broccoli, Brussels sprouts and turnip. The most important *Brassica* oilseed crops worldwide are *B. napus*, *B. rapa* and *B. juncea*. The cultivation of *B. napus* and *B. rapa* is of major importance in North America and Europe. *B. juncea* is the predominant oilseed crop in India, Nepal and Bangladesh (Purty et al. 2008). *B. napus* is the main *Brassica* crop grown in Australia, with *B. juncea* representing only a minor part of oilseed production.

The genetic relationship between the *Brassica* oilseed species was largely established as a result of cytogenetic and breeding studies carried out in the 1930s (Figure 1) (Morinaga 1934; U.N. 1935). It was proposed that *B. juncea* (2n=36), *B. napus* (2n=38) and *B. carinata* (2n=34) were natural amphidiploid[2] hybrids derived from combinations of the diploid species *B. nigra* (2n=16), *B. oleracea* (2n=18) and *B. rapa* (syn. *campestris*) (2n=20). *B. napus* is polyphyletic, deriving from multiple

[2] Amphidiploids are tetraploids containing the diploid chromosome set of both parents.

hybridisation events, with *B. oleracea* one of several maternal ancestors (Allender & King 2010; Chalhoub et al. 2014).

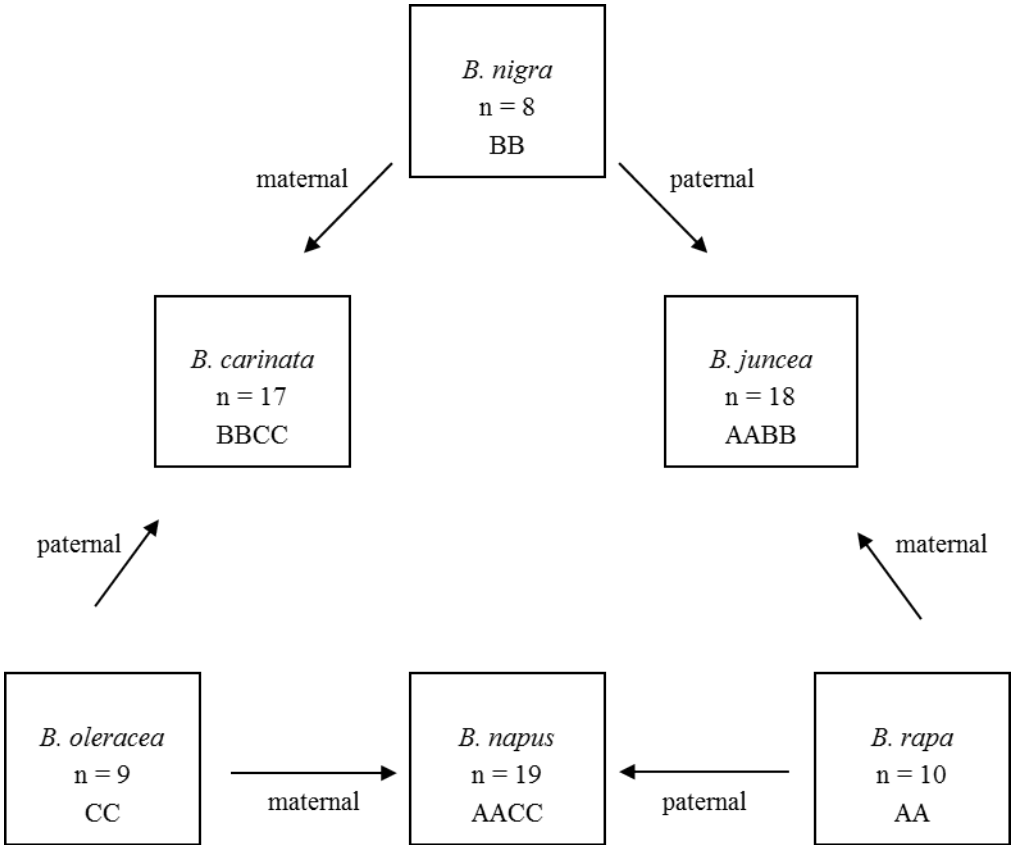

Figure 1. Genomic relationships between the main cultivated *Brassica* species, also known as U's triangle. (n refers to the haploid number of chromosomes). According to Morinaga (1934) and U (1935). Adapted from Purty et al. (2008).

The genomic constitution of the species is described with the letters 'A', 'B', and 'C', each letter representing a haploid genome. Interspecific hybridization for *Brassica* spp. has been described as being unidirectional when happening naturally (Purty et al. 2008).

Cytogenetic relationships between the *Brassica* species have since been supported by studies of nuclear DNA contents, the artificial synthesis of amphidiploids, and the use of genome-specific chromosome markers. Flow cytometry experiments demonstrated that the B and C genomes contain 27% and 44% more DNA, respectively, than the A genome (Sabharwal & Dolezel 1993). As the nuclear genome content of a cell is proportional to the number of haploid chromosomes, this method has been used to identify ploidy level and genomic constitution of hybrid *Brassica* plants. Studies by Bennett & Leitch (2011) and Johnston et al. (2005) have determined the haploid DNA contents of the main oilseed species as:

- 527 Mbp for *B. rapa*
- 1,129-1,443 Mbp for *B. napus*
- 1,068 Mbp for *B. juncea*

For comparison, the haploid genome sizes of *Arabidopsis thaliana*, ecotype Columbia (family Brassicaceae) and *Oryza sativa* subsp. *japonica* are estimated to be 157 Mbp and 577 Mbp, respectively (Bennett & Leitch 2011).

Linkage group identification studies have shown that *Brassica* spp. have a hexaploid ancestor, derived from a whole genome triplication. Phylogenetic studies have shown that genome triplication happened after the split between the two genera *Arabidopsis* and *Brassica* (Lysak et al. 2005; Wang & Fristensky 2001). This triplication event has been supported by identification of syntenic genes[3] between *B. rapa* and other *Brassica* species (Cheng et al. 2012).

Genome triplication was followed by a series of chromosome fusions, as shown by the presence of telomere-related sequences within *B. nigra* linkage groups (Johnston et al. 2005; Lagercrantz 1998). Phylogenetic trees based on Restriction Fragment Length Polymorphisms (RFLPs) (Song et al. 1990) or on chloroplast sequence analysis (Lysak et al. 2005) revealed two separate *Nigra* and *Rapa/Oleracea* lineages. These two lineages are estimated to have diverged quite recently, about 7.9 Mya.

Genome rearrangements (chromosome fusion, inversions, non-reciprocal translocations) have been widely described in artificial (re-synthetised) amphidiploid *Brassica* (Allender & King 2010; Parkin et al. 1995; Song et al. 1990). Interestingly, Panajbi et al. (2008) have shown that natural allopolyploid *Brassica* spp. have gone through few large scale genomic rearrangements.

SECTION 2 ORIGIN AND CULTIVATION

2.1 Centre of origin, diversity and domestication

The earliest traces of *Brassica* spp. date back 7000 years: *B. napa* and *B. juncea* were found in excavations of a Neolithic village from the Shanxi province, China (OECD 2012; Wu et al. 2009). *B. juncea* is described as one of the earliest domesticated plants, with records of its use in Indian agriculture dating back to 2300 BC. As a polyphyletic species, its centres of origin have been widely discussed (Chen et al. 2013; Edwards et al. 2007; Gomez-Campo & Prakash 1999). Afghanistan (and adjoining regions) is currently described as a primary centre of origin for oilseed forms (Chen et al. 2013). China, where the largest diversity of subspecies is observed, is considered as a probable primary centre for vegetable types (OECD 2012; Wu et al. 2009). India/Pakistan and Asia Minor have been described as secondary centres. Using Simple Sequence Repeat (SSR), Amplified Fragment Length Polymorphism (AFLP) and Sequence Related Amplified Polymorphism (SRAP), it was demonstrated that oilseed varieties cultivated in China, India, Europe, Australia, Japan and Canada could be divided in two genetically distinct groups (Chen et al. 2013; Srivastava et al. 2004; Wu et al. 2009). One group consists of varieties from Central/Western India and Eastern China, the other consists of varieties from Northern/Eastern India, Central/Western China, Europe, Australia, Japan and Canada.

B. napus is of relatively recent origin and thought to have first emerged in the Mediterranean coastal region, where both its progenitor species are found. No reference to *B. napus* is in the ancient literature, unlike *B. rapa* and *B. juncea*. The first record of cultivation of oilseed rape in Europe dates back to the Middle Ages but it is not clear if the species grown was *B. napus* or *B. rapa* (Appelqvist & Ohlson 1972). Seeds were grown mainly for lamp oil and soap-making, as their bitter taste made them an unsuitable source of human food or animal feed (Appelqvist & Ohlson 1972; Daun et al. 2015).

[3] Syntenic genes are conserved blocks of genes within sets of chromosomes that are being compared.

Two main components of *Brassica* plant material, erucic acid and glucosinolates, have been shown to cause undesirable effects in humans and animals. *Brassica* ssp. seeds naturally contain up to 40% of erucic acid, a 22-carbon monounsaturated fatty acid, thought to cause growth retardation and heart diseases (Stefansson & Hougen 1964). Glucosinolates are allelochemicals[4] naturally found at concentrations higher than 60 micromoles per gram in the seed of *Brassica* plants (Pessel et al. 2001). These compounds are responsible for the hot and pungent flavours of the *Brassica* vegetables. They can be either toxic, anti-nutritional or beneficial to health, depending on their structure and concentration (EFSA 2008). See Section 5 for more details.

B. napus and *B. rapa* forage and vegetable varieties were introduced to North and South America in the 18th century. The oilseed form was only introduced in Canada in 1936 and in Australia in the early 1960s (OECD 2012). Because of health concerns, Canadian breeders produced a series of new cultivars with low erucic acid concentrations. The first very low erucic acid *B. napus* variety was produced in 1961, followed in 1968 by an "extremely low" erucic acid variety. Then, in 1974, in order to make seed meals more suitable for animal feed, a "double-low" cultivar was released with both extremely low erucic acid and very low glucosinolate levels. From 1978 onwards, this and subsequent cultivars have been referred to as canola, for **Can**adian **o**il, **l**ow **a**cid (Eskin 2013; Fleury 2013).

B. juncea canola varieties are much more recent, dating back to 2002. *B. juncea* canola cultivars show good growing characteristics, less pod shattering and are more drought tolerant than *B. napus* cultivars. However, the first *B. juncea* canola varieties available have shown a lower yield than *B. napus* (Fleury 2013).

2.2 Production and commercial uses

An estimate of the world oil crop production for the 2014/2015 growing season was 547.4 million tonnes, with soybean, rapeseed[5], cotton, sunflower and palm the major producing plants (Table 1) (FAO 2015).

Table 1. World production of major oilseed crops. Adapted from FAO (2015).

	2013/2014 (million tonnes)	2014/2015* (million tonnes)	2015/2016# (million tonnes)
Soybean	283.4	319.7	218.2
Rapeseed	71.9	71.4	64.3
Palm	54.4	63.2	65.1
Cottonseed	44.7	44.9	40.9
Sunflower	42.4	40.9	39.9

* estimated production (October 2015); # forecast production (October 2015)

The four major production areas for rapeseed are: China, India, Canada and the European Union, each producing approximatively 7 million hectares per year. Australia currently has 2.7 million hectares under cultivation (ABARES 2015; Carré & Pouzet 2014). Rapeseed represents 14% of oil production worldwide and is the second largest oil producing crop after soybean (which represents 55% of total oil

[4] Allelochemicals are secondary metabolites, which are not required for plant metabolism. They are often involved in plant defence against herbivores.

[5] Rapeseed is used here instead of canola, as some old, non-canola quality varieties might still be used in some areas.

production). It ranks third in edible oils after soybean and palm. Production has increased by a factor of 2.4 in the last 20 years to a record 71.9 million tonnes in 2014 (Table 1) (Carré & Pouzet 2014). In 2015/2016, production is forecast to drop due to adverse weather conditions in Europe and Canada, and to lower plantings in Australia and China (FAO 2015).

Both the oil and meal are used in food, feed and/or industry. Canola oil (*B. napus* and *B. juncea*) is mainly used in Europe, North America, Australia and Japan for cooking and in food products such as spreads, dressings and shortening or processed food (Daun et al. 2015). *B. juncea* is a particularly important crop in India where it represents 90% of rapeseed production and one third of total oil production (Kumar et al. 2009). Oil is used for cooking, while whole seeds/leaves are used as condiments.

Canola/rapeseed oil is also produced for cosmetics and oleochemical industries. Historically, rapeseed oil has been used as a marine engine lubricant, before being replaced by petrol-based oils. Industry currently considers ultra-high oleic acid varieties as a new class of "green" lubricants, with better characteristics than petrol-based oils (Lowell et al. 2010). High erucic acid varieties are also grown for industry purposes, with the purified erucic acid used to produce slip agents, emollients, food emulsifiers or lubricants (Daun et al. 2015). Canola oil is also considered a suitable biofuel but the current industry estimates point towards a decrease of biofuel production for 2015/2016 (FAO 2015). *B. juncea* has been described as a potential tool for phytostabilization for metal-contaminated soils (Perez-Esteban et al. 2014). See Section 6.1.2 for more details.

Canola meal is the second major oilseed meal produced worldwide, with 33.6 million tonnes. It is widely used as animal feed, with preferential use for dairy cattle, pig and poultry (Daun et al. 2015). It is also considered as a potential substitute to meal for fish farms (Enami 2011). Industry standards require canola meal to be low in glucosinolates (max 30 micromoles per gram of seed) and erucic acid (less than 2%) to be suitable for animal feed (AOF 2007; CODEX 2009). Canola meal is also used as a fermenting substrate for the production of industrial enzymes, such as phytases or xylanases (involved in food, paper or biofuel production) (Daun et al. 2015).

In case of drought or late frosts, canola can be cut and sold as hay or silage, as a way to mitigate the risks associated with taking the crop to grain (McCormick 2007). Canola hay is seen as a suitable feed source for dairy cows and other livestock (GRDC 2010a).

2.3 Cultivation in Australia

Canola is the major broadleaf crop in Australian temperate cereal rotations and the 3rd largest broad acre[6] crop in Australia after wheat and barley, representing 77% of Australia's oilseed production (ABARES 2015). Western Australia (WA), New South Wales (NSW), Victoria (VIC) and South Australia (SA) produce over 99% of Australia's total canola production, with sporadic plantings in Queensland (QLD) and Tasmania (TAS) (ABARES 2015). Australian canola production was at a record high of 4.14 million tonnes in 2012/2013 (ABARES 2014). The major domestic demand is for oil, with meal being a by-product. In 2013/2014, 969,000 tonnes of canola seed were required for domestic oil consumption, and three quarters of the Australian

[6] Broad acre is a term used, mainly in Australia, to describe farms or industries engaged in the production of grains, oilseeds and other crops, or the grazing of livestock for meat or wool, on a large scale.

production was exported (ABARES 2015; GRDC 2009). Australia's main export markets are Japan, China, Pakistan, Europe and Bangladesh.

Approximately 40,000 ha of *B. juncea* were grown in 2015, representing 1.5-2% of the *Brassica* oilseed crop grown in Australia. *B. juncea* has mainly been grown in New South Wales (N. Goddard[7], personal communication, 2015).

2.3.1 Commercial propagation

B. napus and *B. juncea* reproduction is through seed production. Modern cultivars are mostly F_1 hybrids but the Australian industry started mainly with open-pollinated genotypes (Lemerle et al. 2014). Open-pollinated cultivars still dominate, representing more than 75% of canola grown in Australia (Zhang et al. 2016). Farmers are used to sowing retained seeds from open-pollinated crops as a way to reduce costs (Potter 2013). Up to 40% of total canola seeds are currently retained by farmers, mainly for conventional, open pollinated cultivars (N. Goddard, personal communication, 2015). However, retaining seeds can have major effects on subsequent crop production, due to potential genetic drift and reduced seed viability (Marcroft et al. 1999). Comparison of certified and farm-saved seeds has shown that the use of retained seeds from both open pollinated genotypes (Marcroft et al. 1999) and hybrid genotypes (Potter 2013) has negative impacts on germination, early vigour, resistance to pathogens, yield and/or oil quality. The financial impact linked to the use of farm-saved seeds was calculated, with an average financial loss of 12.7% compared to certified seeds (Potter 2013). The recommendation from the canola industry is to use only certified seeds for planting (Marcroft et al. 1999).

B. napus and *B. juncea* seed production for commercial sale follows a seed certification scheme based on the rules and directives of the Organisation for Economic Co-operation and Development (OECD) Seed Schemes and International Seed Testing Association (ITSA) (OECD 2013). Australia has also its own seed certification scheme, following the same rules as those for the OECD Seed Scheme. The Australian Seeds Authority (ASA) administers the OECD and Australian Seed Certification Schemes (accessed on 28 April 2016).

Seed certification is based on a four step process. Breeders' seed is sown to produce pre-basic seed, which will be used to produce basic seed. Basic seed is the basis of all seed certification programs and is intended for the production of certified seed. Certified seed is used for sowing crops and pastures, not for further seed multiplication. Basic and certified seeds are the two most important categories of the certification process.

Certification rules are defined for every crop. For *Brassica* sp., the paddock used to produce seeds must not have grown another *Brassica* spp. crop for three (to produce certified seed) to five (to produce basic seed) years. Plants grown for seed certification have to be isolated from any source of contaminating pollen, originating from crop or weed species. Isolation distances for *Brassica* spp. basic and certified seed production are of 200 m and 100 m, respectively (Seed Services Australia 2013). According to the ASA national seed quality standards, certified canola seed must be at least 99% pure (by mass), have a minimum germination of 85% and have less than 20 contaminating seeds per kilo (ASA 2011; Seed Services Australia 2013).

[7] Nick Goddard is the Executive Director of the Australian Oilseeds Federation.

2.3.2 Scale of cultivation

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 (Table 2, Figure 2). However, the development of early maturing varieties is expanding growing areas into the low rainfall areas of the wheat belt. Canola is often used in crop rotation with cereals and pulses (DPI Vic 2012). Canola production is described by Lemerle et al. (2014) as an opportunity for Australian farmers to improve integrated weed and pathogen management at low cost. Trials run in northern NSW have shown that both *B. napus* and *B. juncea* are the most effective winter crops for reducing crown rot infection levels in a subsequent wheat crop (GRDC 2011). Canola is also an important tool in the management of herbicide resistance in weeds (Matthews et al. 2015).

Table 2. 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* 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

* Temperature and rainfall from Bureau of Meteorology (accessed on 28 April 2016)

Canola production grew significantly in Australia from 146,000 ha in 1990 to an estimated total area of 1,400,000 ha in 2000 (Colton & Potter 1999). In 2013/2014, approximately 3,464,000 tonnes was produced on over 2,721,000 ha (ABARES 2015), for an average yield of 1.28 t/ha. As with many agricultural crops, the area planted and seed production can fluctuate greatly from year to year. Further, for any year, national figures can hide wide variations in each State.

The five year average to 2014/2015 was 3,445,000 tonnes over 2,648,000 ha, with approximately:

- 39% of the production in WA
- 30.3% in NSW
- 19.4% in VIC
- 10.7% in SA

In the year 2014/2015, canola represented about 12.1% of the total area of Australia planted with winter crops[8] (see Table 3a for details) (ABARES 2015).

Table 3. Australian canola production for 2014/2015. Adapted from AOF (2015) and N. Goddard, personal communication (2015).

(a)

	Harvested area (kha)	Production (kt)	% of national production
Western Australia	1247	1635	47.65
New South Wales	575	835	24.34
Victoria	483	647	18.86
South Australia	302	314	9.15
Total	2607	3431	/

(b)

Variety	% of national production
TT*	60
Clearfield	15
GM	20
Conventional*	5
	100

* Up to 2/3 of these seeds can come from farmer retained stocks in a typical year, i.e. around 40% of total seeds as a national average (N. Goddard, personal communication, 2015).

Each State has an appropriate government agency (e.g. Department of Primary Industry, DPI), which tests and recommends varieties suitable to the canola growing regions of the State. For example, an information guide published by the NSW DPI lists 52 varieties of *B. napus* and one of *B. juncea* available in 2015, 12 being newly released varieties (Matthews et al. 2015).

These varieties are classified as:

- conventional (non-GM, not tolerant to any major herbicide used with canola)
- triazine tolerant (non-GM, TT: tolerant to group C herbicides, i.e. inhibitors of photosystem II)
- imidazolinone tolerant (non-GM, IT or Clearfield®: tolerant to group B herbicides, i.e. inhibitors of acetolactate synthase)
- glyphosate tolerant (GM, Roundup Ready®: tolerant to group M herbicides, i.e. inhibitors of EPSP synthase)

[8] Winter crops include barley, canola, chickpeas, faba beans, field peas, lentils, linseed, lupins, oats, safflower, triticale and wheat (ABARES 2015).

- glufosinolate tolerant (GM, InVigor®: tolerant to group N herbicides, i.e. inhibitors of glutamine synthetase) (see Table 3b).

Information guides published by seed companies and DPI provide data for canola on characteristics including mean seed yields or pest resistance, as well as most suitable rainfall regimes. Information on new *B. napus* and *B. juncea* varieties being trialled in Australia can be found at the National Variety Trial Online (accessed on 13 April 2016).

2.3.3 Potential for expansion of B. napus *and* B. juncea *growing regions*

Cultivation of B. napus *canola in northern NSW and southern QLD*

Canola production in northern NSW and southern QLD first started in the late 1980s- early 1990s but the crop suffered from frost damage and a series of drought years. The north-eastern wheatbelt area is characterised by high rainfall variability, yield-damaging frost in spring and high temperature during grain filling. Yield and oil content were highly variable and often disappointing for farmers. For example, yield for 1990 was 1.4 t/ha but only 0.7 t/ha in 1991 (compared to 1.17 t/ha nationwide) (Holland et al. 2001). Growers' perception was that available cultivars were poorly adapted to these environmental conditions. Furthermore, growing *Brassica* spp. has a negative impact on arbuscular mycorrhizal (AM) fungi[9]. Low colonization by AM fungi is linked to phosphorus and zinc deficiency in subsequent crops. This disadvantages commonly grown summer crops, such as cotton, sorghum, maize or sunflower which are highly dependent on AM. Wheat, barley and oat production is less impacted (Holland et al. 2001; Ryan 2001).

Canola production was resumed in 1999, due to a surge of interest in the benefits of crop rotation on weed and pathogen control. Increased grower experience and production of early-maturing cultivars led to a strong growth in canola production in the region, in particular in north-western NSW (Robertson & Holland 2004).

Two main limitations have been identified for the north-eastern wheatbelt regarding canola production. First, new harvesting methods are needed to avoid harvest losses due to the use of lower-yielding, more rapidly-maturing varieties. Second, due to transport costs, distance from current delivery points is described by farmers as the biggest limiting factor for the expansion of canola production (Holland et al. 2001).

Further expansion is considered: new short season varieties were field tested in Central QLD in 2011/2012 by the GRDC. A Grow Note for canola in the northern region was released in 2015, focusing on conditions and soil types in northern NSW and QLD (GRDC 2015). This demonstrates an increased interest in canola production in these areas. Most of the expansion effort so far has focused on low and medium rainfall zones (Robertson & Holland 2004) but the high rainfall zone is now also considered a possible expansion area. Lilley et al. (2015) proposed canola as a dual-purpose, long growing season crop, for grazing and grain.

Cultivation of B. napus *canola in Western Australia*

In WA, canola has traditionally been grown in areas of at least 450 mm rainfall, but experience has shown that canola can also be grown profitably in the lower rainfall areas (approximately 325 mm) of the northern grain belt (Carmody & Cox 2001).

[9] Arbuscular mycorrhizal (AM) fungi are beneficial soil fungi. There are obligate symbionts colonising the roots of most crop and pasture species grown in Australia. The fungi provide nutrients (mainly phosphorus and zinc) to the plants in return for photosyntates. *Brassica* sp do not host AM fungi.

Profitability depends upon a number of interrelated factors; the most limiting being the timing of opening rainfall and high temperature during pod fill. Other factors include weed competition, soil acidity, fertiliser timing, blackleg disease, insect pests and harvest management. Managing these factors is the key to profitable canola production in the northern grain belt of WA (Carmody & Cox 2001).

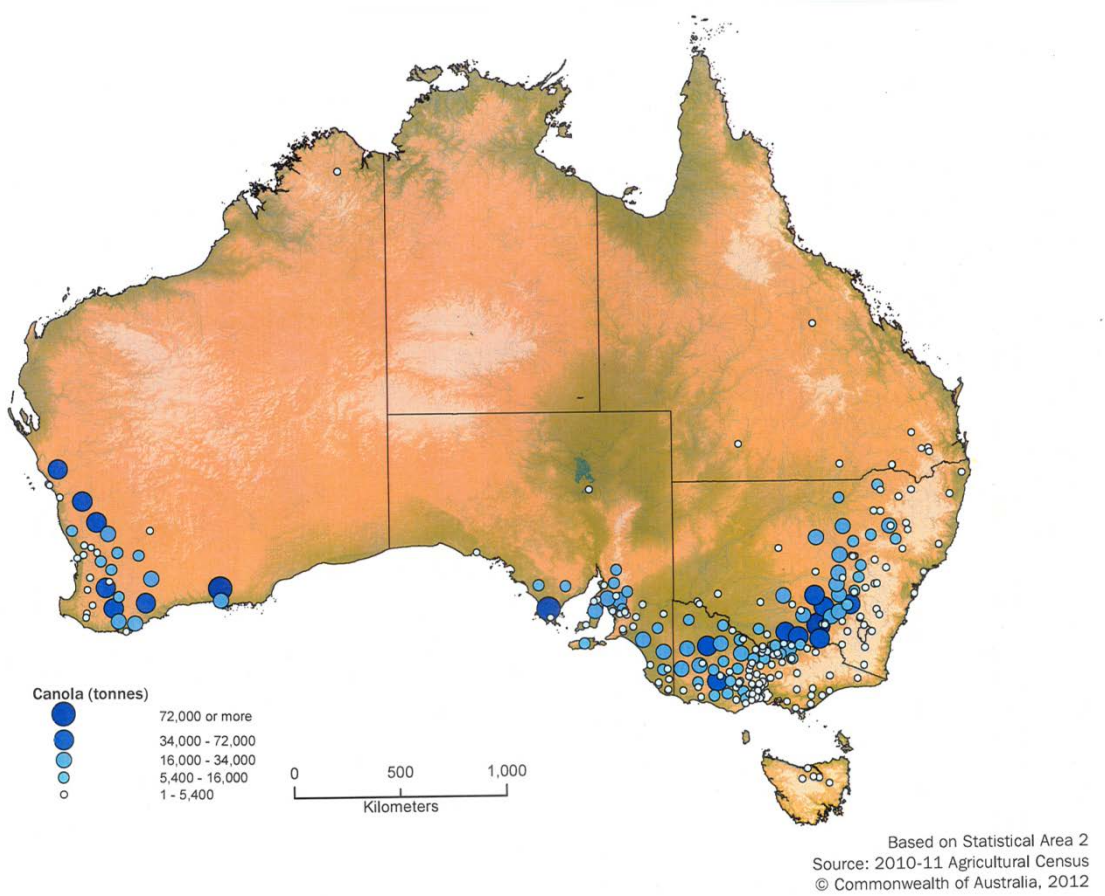

Figure 2. Canola production in Australia as recorded in the 2010/2011 agricultural census. According to the Australian Bureau of Statistics (accessed on 13 April 2016)

B. juncea *canola*

B. juncea has been studied for the last 25 years as a potential alternative oilseed crop to *B. napus* (Potter 2011). Given its drought-tolerant, disease-resistant and pod shattering-resistant phenotype, *B. juncea* has been envisaged as a more suitable oilseed crop than *B. napus* in semi-arid regions of Australia (Burton et al. 1999). The oil from *B. juncea* canola can replace *B. napus*, or the two products can be blended (GRDC 2009).

The first Australian *B. juncea* canola variety was released in 2007, to be grown in low rainfall zones. However, due to lower oil content, farmers were recommended to grow this *B. juncea* canola only where long-term average *B. napus* yields are less than 1.2 t/ha. Using cropping system models, these regions have been identified as extending west of Wee Waa in northern NSW through Warren and Ungarie, the southern Mallee of VIC, and parts of the south-east, mid-north and central Eyre Peninsula of SA (Hunt & Norton 2011). The boundary between *B. napus* and

B. juncea areas essentially follows that of the 100 mm winter rainfall isohyet[10] (Figure 3).

Figure 3. Proposed growing areas for *B. juncea* cultivation in the south-eastern region in Australia (Hunt & Norton 2011). Median simulated *Brassica* grain yields are given below each location. Those inland of the dotted line are less than 1.5 t/ha. The 100 mm average winter rainfall isohyet (1961-1991) is indicated by a dashed line. The seaward boundary of the region suggested as ideal for *B. juncea* by other authors (Haskins et al. 2009; Norton et al. 2009) is indicated by the solid line.

Another limitation to the use of the first developed *B. juncea* canola is linked to seed size. *B. juncea* seed is smaller than *B. napus* under good growing conditions. In case of drought, *B. juncea* seed can become even smaller and lighter. In 2007 and 2008, this led to harvest losses, as seeds were blown out of the harvester (Haskins et al. 2009).

Growing *B. juncea* canola varieties could be of economic importance for Australia: if *B. juncea* was grown on 10% of the low rainfall cereal growing area, the production area would be approximately 600,000 ha (Norton et al. 2005). Potter (2011) suggested that new herbicide-tolerant, high-yield cultivars would be needed, in order to compete with *B. napus* cultivars. Breeding of novel *B. juncea* canola varieties has continued and the first herbicide- and drought-tolerant hybrid *B. juncea* canola variety was released in 2013. This cultivar is described as having a similar oil content, profile and quality to *B. napus* canola (Matthews et al. 2015).

2.3.4 Cultivation practices

What are the reasons for growing canola?

Canola is considered the most profitable break crop available to grain growers in southern Australia. According to the GRDC, a recommended crop rotation sequence

[10] An isohyet is a line on a map connecting points having the same amount of rainfall in a given period.

is as follows: legume pasture (clover or lucerne) / canola / cereal / pulse (lupin or field pea) / cereal / cereal (GRDC 2009).

Canola is usually grown in rotation with wheat as the follow-on crop, and provides an important disease and weed break. Studies have shown an average yield increase of 20% when wheat is grown after canola compared to wheat monoculture. Benefits from growing canola can flow on to following crops for up to three years (GRDC 2009). The canola root system has a positive impact on soil structure and moisture, resulting in higher yield and protein level in the following cereal crop.

Growing canola in a rotation cropping system reduces the incidence of wheat pathogens such as take-all (*Gaeumannomyces graminis* var. *tritici*), crown rot (*Fusarium pseudograminearum*) or common root rot (*Bipolaris sorokiniana*) fungi. Canola acts as a grass weed competitor, minimising the pool of grass hosts available for fungal spore survival (Lemerle et al. 2014). Furthermore, growing and decaying *Brassica* roots release isothiocyanates (ITC) into the soil. These molecules are derived from glucosinolate degradation (Angus et al. 2015). ITC were first described as actively suppressing fungal inoculum present in the soil, by a mechanism called biofumigation[11] (Smith et al. 1999). However, more recent studies have shown that the levels of ITC released into the soil are likely too low to directly impact pathogens. Watt et al. (2006) suggest that ITC released in the soil could have an indirect impact on pathogenic fungi, by influencing the composition of the rhizosphere's microbial communities. Increased populations of plant symbiotic fungi (such as *Trichoderma* sp., an antagonist of *Fusarium pseudograminearum*) following a canola rotation have been described as a possible explanation for the decline of pathogenic inoculum (Watt et al. 2006).

B. napus is likely to remain the dominant canola species grown in Australia. In conditions of adequate rainfall, *B. napus* usually outperforms available *B. juncea* varieties, providing greater yields and profit (Gunasekera et al. 2009). However, *B. juncea* canola varieties are seen by breeders as a suitable alternative in low rainfall environments, or as a spring crop in higher rainfall regions. *B. juncea* canola is described as drought and heat tolerant, blackleg resistant and suitable for direct harvest, whereas *B. napus* frequently requires windrowing (Pritchard et al. 2008). Furthermore, as *B. juncea* is generally quite vigorous in its early stages of growth, it has the capacity to easily cover ground, reducing water loss and weed competition. It is also described as early-flowering, which could make it a viable crop in drought-struck areas (Potter 2011).

How to grow canola?

Canola is mostly grown as a winter annual in winter-dominant rainfall environments between 30ºS and 38ºS (Norton et al. 1999). Yields for broad acre production average 1 to 2 t/ha but range up to approximately 5 t/ha in areas with a long, cool growing season and adequate moisture (Walton et al. 1999). Spring type canola varieties are the main varieties 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

[11] Biofumigation was first defined as the pest suppressive action of decomposing *Brassica* spp. tissues. It was later expanded to include other animal and plant residues.

12 months in Europe, due to vernalisation requirement and 4 months in Canada, due to day length and warm temperatures (Walton et al. 1999). Canola has also been grown in Australia over long growing seasons for dual-purpose (grazing and grain) production in the medium rainfall zone and is predicted to be of interest for farmers in high rainfall zones (GRDC 2009; Lilley et al. 2015).

Small areas of canola are sown in late spring or early summer in more temperate regions. These crops are located in areas with 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 recommended sowing rate for *B. napus* is 3 to 4 kg/ha. The trend towards hybrids with superior early vigour allows experienced growers to reduce seedling rate to as low as 1.5 to 2 kg/ha (GRDC 2009). These sowing rates are used to achieve a planting density of approximately 60 to 80 plants/m^2 (Walton et al. 1999). It was recently recommended, under WA conditions, to lower sowing rates to an average 50 plants/m^2 when using hybrid cultivars (French et al. 2016).

Because of its small size, canola seed takes longer to establish than cereal seeds. Emergence depends on temperature, soil moisture and seeding depth (see Section 4.4 for more details).

Under optimal soil moisture for germination, canola seed is sown at 2 to 4 cm depth, which leads to rapid emergence (shoots will emerge within 4 to 5 days). When soil moisture is low 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 patchy emergence, poor growth and reduced yield. When sufficient moisture is not available at 5 cm, a common practice is to dry sow: seeds are sown at a shallow depth, and left to wait for rain (Oilseeds WA 2006). Dry sowing has disadvantages, even for *B. juncea* canola: subsequent low rainfall may induce split germination and uneven growth of the crop. It also prevents any pre-sowing eradication of weeds (Haskins et al. 2009; McCaffery et al. 2009a).

The optimum time to sow depends on a range of environmental factors but also on the relative time to maturity of a variety. Mid and late-maturing varieties should be sown early in the recommended sowing window, while early-maturing varieties should be sown late. Sowing time is a compromise. Sowing too early increases the risk of frost damage and lodging. Australian canola varieties are relatively frost tolerant and seedling loss is not a major concern. The main damage is due to late frosts after flowering, resulting in aborted seeds and reduced yields (Walton et al. 1999). Late sowing into cold soils reduces plant growth and makes seedlings more vulnerable to pests and diseases (GRDC 2009; Kirkegaard et al. 2016). It also increases the risk of pods developing in hot and dry weather. Canola is most susceptible to drought stress from flowering to early and middle phases of seed filling, with water deprivation leading to seed abortion and reduced oil content (GRDC 2009). Soil moisture is usually exhausted by crop maturity (this phenomenon is referred to as terminal drought) and, for each week sowing is delayed beyond the optimum period, average yields drop by about 5-10% (GRDC 2009; Gunasekera et al. 2009; Kirkegaard et al. 2016). Impact of early/late sowing is also linked to seed management practices: the use of certified or farmer-retained seeds has a strong influence on early vigour, growth and yield (see Section 2.3.1 for more details).

Both *B. napus* and *B. juncea* have a higher requirement for nitrogen, phosphorus, sulphur and potassium than cereals and other crops and will not produce high yields unless all these elements are adequately supplied. Fertilizer requirement depends on yield expectation and needs to be assessed against environmental variations. *Brassica* crops remove from the soil on average (per tonne per ha, according to Colton & Sykes (1992)):

- 40 kg nitrogen
- 7 kg phosphorus
- 9 kg potassium
- 10 kg sulphur

Nitrogen fertiliser rates vary depending on paddock fertility and expected yield (see GRDC (2009) for details regarding calculations of nitrogen fertiliser rates).

Both *B. napus* and *B. juncea* conventional varieties are very sensitive to Group B herbicides (inhibitors of acetolactate synthase, such as chlorsulfuron or triasulfuron) and Group C herbicides (inhibitors of photosystem II, such as atrazine and simazine). Cultivation should avoid residues of these herbicides as they damage canola (Agriculture Victoria; accessed on 22 April 2016).

Canola is harvested in early summer when the seeds have reached their maximum dry weight and the crop can be swathed (windrowed) or direct-harvested (GRDC 2010b). A canola crop is ready when the majority of pods are dry and rattle when shaken. *B. napus* crops are swathed: the crop is cut and placed in rows to dry. Swathing is undertaken 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). The windrow lies in horizontal bundles, supported by the cut stems 10 – 20 cm off the ground, and remains in the paddock for 8 to 19 days prior to harvest. When most of the seed has matured and the moisture content is 9% or less, the windrow is picked up by the harvester (GRDC 2010b; Pritchard & Bluett 2008). 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). The swathing process hastens drying of the crop, reduces the possibility of seed losses due to pod shattering, and ensures even ripening.

As an alternative to swathing, canola can be direct harvested. Direct harvest is increasingly seen as a viable option with the release of new *B. napus* and *B. juncea* varieties that are less prone to shattering. Direct harvesting reduces harvesting costs and is a cost-effective option for:

- crops with a yield potential of approximately 1 t/ha or less
- crops which are short
- plants with a low stand, where the stems are unable to keep the windrow off the ground

Direct harvest can also occur after application of chemical desiccants or pod sealants. 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 (Carmody & Cox 2001; GRDC 2010b). However, the use of chemical desiccants can prove a financial burden for growers.

2.4 Crop Improvement

Australian canola has mainly been improved through recurrent selection in a closed population. This has led to inbreeding and genetic drift, with a loss of potentially valuable alleles (Cowling 2007). One of the major current challenges for Australian breeders is to introgress new genetic diversity, a key for adaptation to changing environments, while retaining the traits that were enhanced over the past 30 years. Germplasm from outside of Australia may provide valuable alleles for improvement. However, these imported germplasms need to be introgressed gradually, as they will most likely not be adapted to Australian conditions (Cowling 2007).

In 2006, the Australian Oilseed Federation (AOF) and the Grain Research and Development Corporation (GRDC) identified a series of agronomic and quality traits needed for canola germplasm development. They established the National Brassica Germplasm Improvement Program (NBGIP), defining five key priorities for improvement:

- improved/alternative sources of blackleg resistance
- increased water use efficiency/drought tolerance
- reduced pod shatter
- increased frost tolerance during seed development and
- increased oil content stability and increased protein content (GRDC 2013; Salisbury et al. 2007).

Some more traits for germplasm enhancement, defined by NBGIP as preliminary and future traits are:

- increased resistance to sclerotina, viruses and pests
- improved early vigour
- salt tolerance and
- modified fatty acid composition for industrial uses (Amjad & Cowling 2007; Salisbury et al. 2007).

NBGIP proposes to use a set of methodologies, including marker assisted selection, interspecific hybridization, Target Induced Local Lesions in Genomes (TILLING) and incorporation of GM traits (Figure 4).

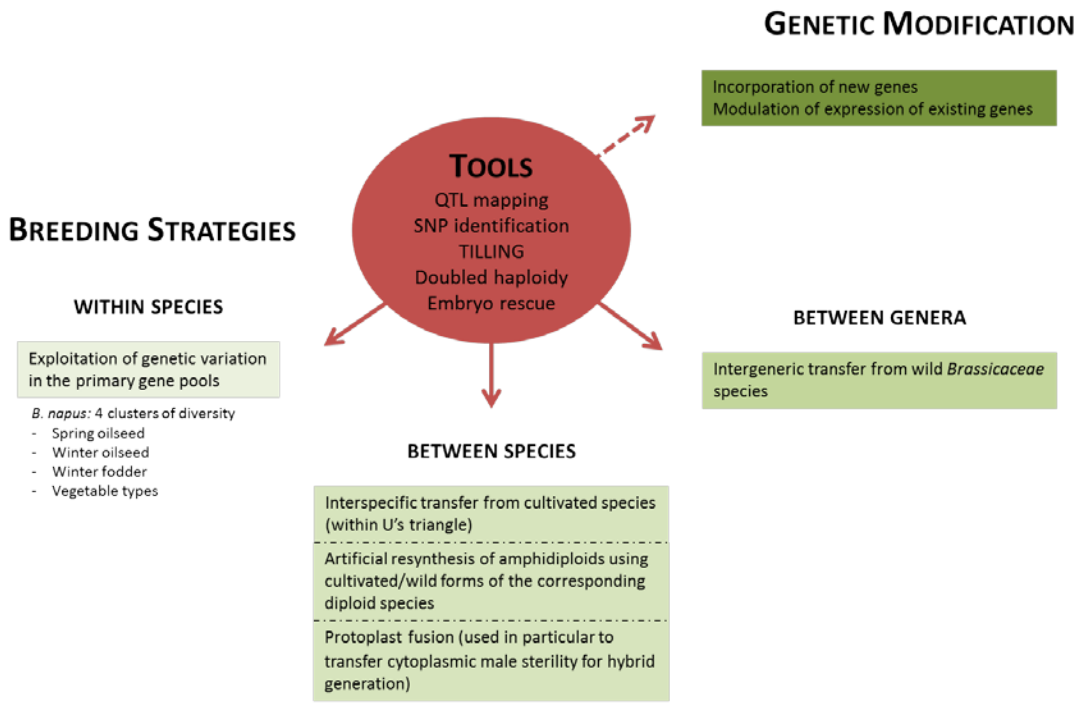

Figure 4: main strategies and tools available for canola improvement. Adapted from Redden et al. (2007) and Rahman (2013). QTL: Quantitative Trait Loci; SNP: Single Nucleotide Polymorphism; TILLING: Target Induced Local Lesions in Genomes. See text for details.

2.4.1 Breeding in Australia

Canola has moved in less than forty years from being a minor crop to one of the major oilseeds for food and feed industries in Australia and overseas (Wan et al. 2009). Australian public breeding programs started in 1970, in VIC, followed by NSW and WA (Buzza 2007; Salisbury & Wratten 1999). Private breeding began in 1980, a major focus being the development of hybrids (Salisbury & Wratten 1999). The first *B. napus* canola cultivars adapted to Australia growing conditions, Marnoo and Wesroona, were released in 1980 (Buzza 2007). The first canola-quality *B. juncea* variety for Australia, Dune, was released in 2007 (Burton et al. 2007).

See Potter et al (2016) and Salisbury et al (2016) for an extensive review and perspective of breeding progress in Australia since 1978.

Improved agronomic traits

Early canola varieties introduced into Australia from Canada 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 to delay the onset of flowering until after a satisfactory leaf canopy had developed (Walton et al. 1999) (Buzza 2007). Early and very early-maturing varieties, better adapted to drier environments, have been developed by breeding programs (Salisbury & Wratten 1999). The recent identification of Quantitative Trait Loci[12] (QTL) involved in canola flowering response to photoperiod and temperature has been described as a promising avenue to adapt varieties to changing climates (Nelson et al. 2014). Raman et al

[12] Quantitative Trait Loci are loci that correlate with variation in a given phenotype.

(2016) recently reported the identification of QTL associated with yield and flowering time.

Breeders also recognised that growth and yield of canola would almost always be limited by water availability, particularly during seed set and maturation. Thus, improving water use efficiency and drought tolerance have been a major focus in canola breeding (GRDC 2007b; Wan et al. 2009). Because of its tolerance to drought and high temperatures, *B. juncea* has been used as an alternative to *B. napus* in low rainfall zones in a series of breeding programs (Oram et al. 1999).

Resistance to lodging and shattering are other sought-after traits (Hossain et al. 2012; Salisbury & Wratten 1999). Reduced plant height decreases the risk of lodging, while shattering resistance facilitates direct harvesting of canola (Salisbury & Wratten 1999). Improvements in these agronomic traits have increased yield, as considerable seed loss can occur due to lodging, shattering and the extra handling during windrowing.

Cleistogamy[13] has also been described by some as a desirable agronomic trait in order to limit cross-pollination (Gruber et al. 2012). Cleistogamy does not exist naturally among the genetic resources of *B. napus* and *B. juncea*. However, lines of cleistogamous *B. napus* have been obtained using chemical induced mutagenesis strategies (Fargue et al. 2006; Leflon et al. 2010). The cleistogamous trait obtained has been described as imperfect: up to 72-89% of flowers were observed to be totally closed (Leflon et al. 2010). Pollen emission in cleistogamous plants was quantified as low as 10% of what is observed for open flowers (Fargue et al. 2006).

Resistance to blackleg

Blackleg disease, caused by *Leptosphaeria maculans*, is one of the most devastating diseases of canola worldwide. In Australia, isolates of *L. maculans* have the ability to cause losses of up to 90% yield and it is predicted that, without management of the disease, the canola industry would disappear from Australia (Raman et al. 2012; Van de Wouw et al. 2014). The most severe epidemic observed in Australia occurred in 1972, causing a widespread collapse of the emerging canola industry (Buzza 2007; Li et al. 2007b). The varieties used were spring varieties from Canada, grown as winter crops and had not been selected for blackleg resistance (Buzza 2007). Since the late 1980s, Australian breeders have released a number of resistant lines, turning canola into a viable industry in the early 1990s (Li et al. 2007b). By the late-1990s, Australian mid-season varieties had the highest levels of blackleg resistance of any spring canola varieties in the world. These varieties were based on single dominant gene-derived resistance from *B. rapa* ssp. *sylvestris* (Li et al. 2007b). In 2003, the resistance was overcome, initially in WA and in other parts of southern Australia (Li et al. 2007b), threatening the industry. New sources of resistances are currently studied, using winter germplasm and polygenic resistance (Salisbury et al. 2007). The development of new resistances is to be associated with modified cropping practices, as detailed in the GRDC blackleg management guide (GRDC 2012). Another proposed strategy to minimise disease in crops is to use canola multilines[14] cultivars or mixtures that have different resistance genes (Van de Wouw et al. 2014). Available

[13] Cleistogamy describes the trait of certain plants to propagate by using non-opening, self-pollinating flowers.

[14] Multilines are mixtures of lines differing in a specific disease or pest resistance and bred for phenotypic uniformity of agronomic traits.

lines with similar maturity time and herbicide resistance could be grown as a single crop, as this is done for wheat, barley and rice.

Non-GM herbicide tolerance

Canola is highly susceptible to weed competition during the early stages of growth, potentially leading to major yield losses. Excessive weed presence at harvest can also lower grain quality, thus potentially leading to more losses (GRDC 2009). Weed pressure from species, such as wild radish (*Raphanus raphanistrum*), wild turnip (*Brassica tournefortii*), Indian hedge mustard (*Sisymbrium orientale*) or Patterson's curse (*Echium plantagineum*) was the main constraint to canola production in medium rainfall zones of southern Australia prior to the introduction of herbicide-tolerant varieties (Sutherland 2010).

The first non-GM herbicide-tolerant *B. napus* cultivar in Australia was a Triazine Tolerant (TT) canola, Siren, released in the mid-1990s. The first TT varieties released had a reduced radiation-use efficiency compared to non-TT lines, resulting in lower yields and lower oil content. Average yield penalty was about 15% (Pritchard 2014). This was compensated for by better weed control and TT varieties quickly captured the majority of the canola seed market. Current TT varieties have on average now closed the yield gap (Pritchard 2014). The first imidazolinone tolerant (IT, also known as Smart canola or Clearfield®) was released in Australia in 2000. IT varieties do not carry a yield penalty and have been widely adopted (Agriculture Victoria; accessed on 22 April 2016).

TT and IT canola varieties are both non-GM. The TT trait is derived from natural mutations observed in a wild biotype of *B. rapa*, transferred to *B. napus* through hybridization (Beversdorf & Kott 1987; Beversdorf et al. 1980). Tolerance is due to a single base pair change in the sequence of the chloroplast *psbA* gene encoding the D1 (QB) protein involved in electron transport of photosystem II (Reith & Straus 1987). IT was developed through chemical mutagenesis. The observed tolerance phenotype is due to mutations in the enzyme acetohydroxyacid synthase (AHAS), involved in the biosynthesis of branched-chain amino-acids (Swanson et al. 1989; Tan et al. 2005). IT varieties have been released for canola and also for corn (where the tolerance was first discovered), rice, wheat, sunflower and barley (Tan et al. 2005).

Fewer options are currently available for herbicide tolerance in commercial varieties of *B. juncea*. The first *B. juncea* canola IT varieties, OasisCL and SaharaCL, were released in 2008 (Potter et al. 2008). The first IT hybrid cultivar was released in 2013 (see Section 2.3.3 for more details). TT *B. juncea* varieties are currently being trialled in SA (EPARF 2015).

Non-GM herbicide tolerant varieties represent the vast majority of Australian *B. napus* canola-quality production, with 60% of TT cultivars and 15% IT (N. Goddard, personal communication, 2015). Very little detail is available regarding *B. juncea*. Details of currently available herbicide tolerant varieties can be obtained by consultation of various state government publications and the NVT website (accessed on 22 April 2016).

Improved oil and protein quality/quantity

As described above, one of the first aims of breeding in Australia was to produce canola-grade cultivars. Since then, the oleic acid content of mainstream Australian canola varieties has remained relatively constant at approximately 60%. However, further improvements and production of specialty varieties have been undertaken. One

objective has been to further enhance oleic acid levels and reduce linolenic acid, to increase oil stability for specific applications such as deep-frying (Salisbury & Wratten 1999). HOLL (for High Oleic, Low Linolenic) *B. napus* cultivars have been developed, with up to 70% oleic acid content and less than 3.5% linolenic acid (Gororo 2007). Burton, 2009 suggested that *B. juncea* HOLL varieties could also be of interest for farmers. Other specialty cultivars for health products, such as omega-3 canola oil, are being developed, both in Australia and overseas, using conventional breeding and genetic modification (see below) (Potter et al. 2007).

Variety improvement has also focused on meal quality and digestibility, aiming at higher protein content and less fibre. These meals are low in glucosinolates, making them a suitable feed for poultry, pigs and cattle (AOF 2007).

Breeding has also focused on non-food, industrial applications. Specialty high erucic acid varieties have been developed, for use in the manufacture of paints, inks, nylon and plastic films (NSW Department of Primary Industries 2014). Canola-quality plants, particularly *B. juncea* canola could be used for biodiesel production (Haskins et al. 2009; McCaffery et al. 2009a). See Section 2.2 for more details.

Breeding and selection for oil with improved melting point, pour point and chemical stability has been proposed as a future target (NSW Department of Primary Industries 2014).

Hybrids as a breeding method

Overcoming genetic bottlenecks is critical for improvement of agronomic traits (such as shatter resistance or flowering time) but also for protecting the crop from diseases and pests (Osborn et al. 2007; Rahman 2013; Raman et al. 2014a; Redden et al. 2007). Intraspecific, interspecific and intergeneric crosses have been used by breeders to improve both oilseed and vegetable *Brassica* spp. crops. Hybrids are also widely used in breeding seeds for commercial planting due to heterosis[15], leading to increased yield performance and early vigour.

B. napus and *B. juncea* are largely self-pollinating (see Section 4.2 for more details) and the main constraint to commercial exploitation of hybrids has been the availability of an effective pollen control and fertility restoration system. The most efficient and widely used system is cytoplasmic male sterility (CMS). This system is based on genetic miscommunication between mitochondrial and nuclear genes, leading to abnormal anther and/or pollen development. There are three components to the system:

- an A line carrying the mitochondrial genome leading to male sterility,
- a B line, fully fertile, used to maintain the A line (A and B are sister lines[16]),
- a R line, with a nuclear gene restoring fertility. The R line should be highly heterotic to the A line.

The first non-GM *B. napus* hybrids based on a CMS system were released in Australia in 1988. These did not out-perform conventional varieties sufficiently to justify the higher seed cost. However, a number of hybrid *B. napus* varieties with improved yields have since become available to growers (McCaffery et al. 2006; Potter et al.

[15] Heterosis refers to the phenomenon that progeny of diverse varieties of a species or crosses between species exhibit greater biomass, speed of development, and fertility than both parents.
[16] A and B are genetically identical except that B possesses normal cytoplasm and is therefore male-fertile.

2007). CMS lines have also been developed for *B. juncea*, through wide hybridisation (Malik et al. 1999). Gene technology has also been used to develop hybrid production systems.

Interspecific and intergeneric crosses are an important source of gene diversity (Figure 4). Such crosses are often difficult, due to limited chromosome homology, abnormal meiosis or low recombination rates (Feng et al. 2009; Navabi et al. 2011). Several techniques have been developed to increase the breadth of germplasms available for crossing. Embryo rescue is routinely used to overcome species barriers in difficult crosses. Immature embryos are excised from the ovaries and grown on artificial media, to avoid abortion by the plant (Navabi et al. 2011). To avoid sexual incompatibility barriers, nuclear and cytoplasmic genomes can also be combined by protoplast fusion (also known as somatic hybridization). Protoplast fusion has been used to alter fatty acid composition in seeds or increase resistance to blackleg (Hu et al. 2002). So far, most somatic hybrids have shown a high degree of sterility and/or have exhibited morphological abnormalities. These hybrids are mostly used as bridges to transfer specific desirable traits.

Haploids and doubled haploids are also used to generate hybrids. Haploid cells from pollen or egg cells are isolated and cultured *in vitro* and chromosome doubling is chemically induced (often using colchicine). Doubled haploid lines are used more often than haploid ones, for they are more stable and fertile. Doubled haploids are completely homozygous and can be used in interspecific crosses, especially when these crosses involve parents with different levels of ploidy (Mason et al. 2015; Rahman 2013). Doubled haploids have been considered as an option to create new hexaploid species (Mason et al. 2015). Natural polyploidy in *Brassica* is confined to the occurrence of tetraploid plants. There are no hexaploid or higher polyploid *Brassica* species. Combining the three A, B and C genomes could produce varieties with increased tolerance to abiotic stresses such as drought or salinity and diseases (Pradhan et al. 2007; Pradhan et al. 2010). So far, breeding of hexaploid lines has been limited by high chromosomal instability and infertility (Chen et al. 2011).

Use of molecular techniques in breeding

Marker assisted selection and chromosome mapping started in the 1980s for canola, with the development of RFLP, AFLP and other genetic markers. These markers were used to produce the first linkage maps for *B. rapa* and *B. napus* in the early 1990s (OECD 2012). Other, more powerful genetic tools have since been developed, leading to the construction of high-resolution genetic maps.

Genetic markers such as RFLP, AFLP or SSR are used routinely to identify QTL. These identified QTL can then be used for breeding, to improve agronomic qualities such as flowering time and photoperiod responsiveness (Nelson et al. 2014), concentration of glucosinolates (Harper et al. 2012) or resistance to diseases (Hayward et al. 2012). Two high density QTL maps have recently been constructed for *B. juncea*, using crosses of eastern European and Indian varieties. These maps showed that yield-related QTLs in *B. juncea* were originating from the A genome rather than from the B genome (Ramchiary et al. 2007; Yadava et al. 2012).

Complete, annotated reference genome sequences for *B. rapa* (Wang et al. 2011b), *B. napus* (Chalhoub et al. 2014) and *B. oleracea* (Liu et al. 2014) are now publicly available. Such tools are predicted to help gene discovery and breeding of *Brassicas* (Wang & Freeling 2013). Computational methods have been used to analyse the

structure of the *B. rapa* genome and compare it with *Arabidopsis* (Tang & Lyons 2012).

Recent advances in molecular techniques, such as Next Generation Sequencing (NGS), have made the characterisation of candidate resistance genes easier. By using whole-genome shotgun reads of the parents of a population segregating for resistance to blackleg, it has been possible to identify two candidate genes in a major resistance locus, Rlm4 (Tollenaere et al. 2012).

NGS has led to Single Nucleotide Polymorphism (SNP) being widely used for QTL mapping and comparative genomics. In particular, deep transcriptome RNA sequencing (RNA seq) has reduced costs as SNP detection can focus on coding regions only (Devisetty et al. 2014a). *B. napus*, *B. juncea* and *B. rapa* genomes have recently been investigated using SNP-based fine mapping methods (Devisetty et al. 2014b; Raman et al. 2014a). Distribution and frequency of SNP are important data for their use as genetic markers. SNP rate among *B. rapa* cultivars is of about 1 in 150-200bp, while it is of about 1 in 1.6kb between two cultivars of *B. napus* (Devisetty et al. 2014a). SNP frequency observed in *Brassica* spp. is within the range of those reported for other plant species.

TILLING is a direct, cost-efficient reverse genetics technique for point mutation or SNP screening. It is used in natural or mutagenized populations (following treatment with a chemical mutagen such as ethyl methanesulfonate). Combining TILLING and NGS helps identifying mutants in polyploid species and will be of interest for breeders (Gilchrist et al. 2013).

2.4.2 Genetic modification

Genetic transformation of canola started in the late 1980s and early 1990s, with the first commercial release in 1994 in the US. Both biolistics and *Agrobacterium tumefasciens*-based nuclear transformation techniques are used routinely, with methods used for *Arabidopsis* adapted for *B. napus* and then *B. juncea* (Chhikara et al. 2012; Dutta et al. 2008; Wang et al. 2003). Hypocotyls, cotyledons, stems, leaf discs, microspores or protoplasts can be used to regenerate GM plants (see (Dutta et al. 2008) for details). As for *Arabidopsis*, *Agrobacterium*-mediated transformation of *B. napus* and *B. juncea* can be done by floral dip, by vacuum-infiltrating immature floral buds (Chhikara et al. 2012; Wang et al. 2003). Floral dip transformation efficiency is quite low: about 0.8% of seeds analysed by Chhikara et al. (2012) were found positive by Southern blot. Floral dip is routinely used as no tissue culture is required, thus reducing time and cost associated with transformation.

A protocol for chloroplast transformation of *B. napus* has been described recently (Cheng et al. 2010). Chloroplast transformation offers several advantages compared to nuclear transformation. The method is based on homologous recombination, making it a high-precision engineering technique. Chloroplasts are prokaryotic and multiple transgenes can be stacked, if linked together as operons. Furthermore, there is no epigenetic control or gene silencing mechanisms in chloroplasts. Thus the risk of transgene non-expression is reduced compared to nuclear transformation (Clarke & Daniell 2011).

GM canola varieties commercially released so far worldwide have been genetically modified for herbicide tolerance, high oleic acid content and/or a hybrid breeding system. Current laboratory work and field work in Australia and overseas mainly

focus on pathogen resistance (Zhang et al. 2015), abiotic stress tolerance (Chakraborty et al. 2012), oil quality (Tan et al. 2011) or yield (Kant et al. 2015).

SECTION 3 MORPHOLOGY

3.1 Plant morphology

The morphology of *B. napus* is very similar to that of *B. juncea*, with few distinctive characteristics. They are annual (spring cultivars) or biennial (winter cultivars) plants, between 70-170 cm and 120-210 cm in height, respectively. In Australia, they are winter-growing crops, sown in autumn and maturing in spring, with a growing season of 5-6 months (Edwards & Hertel 2011).

A well-developed plant produces between 10 and 15 leaves (Colton & Sykes 1992). The oldest leaves at the base are the largest, forming a rosette which is up to 50 cm wide. They are lobed, bristly, dark bluish green waxy leaves with a rounded tip, about 100-300 mm long and 50-150 mm wide. Lobes are often completely separated towards the petiole. The terminal lobe is usually the largest one. The middle and upper leaves are smaller (up to 100 mm long), spear-shaped and smooth, sessile (no petiole) and not lobed (Bailey 1976; Kershaw 1998). Two main differences exist between *B. napus* and *B. juncea* leaves: *B. napus* upper leaves clasp the stem while *B. juncea*'s do not. The leaves of *B. juncea* are also a lighter green and have indented vein patterns (Edwards & Hertel 2011).

Leaves are attached to the stem at a node. Plants have one main supporting stem, with about 15-30 nodes at a spacing of 5-10 cm. Secondary stems (branches) bud from the axil of the leaves. Branches will support 1-4 leaves. Stems are polygonal in cross-section, with longitudinal striations often present on upper parts of the stem. Stems are important for photosynthesis during pod and seed growth, as the leaves are entering senescence.

Both species have a taproot system to a maximum depth of about 120 cm (Duke 1983).

3.2 Reproductive morphology

B. napus and *B. juncea* flowers are bisexual and develop in indeterminate simple inflorescences (or racemes). The flowers are regular with 4 sepals and 4 petals (see Figure 4 below), and are 6-25 mm wide. The diagonally opposite petals form a cross, which is where the original family name, Cruciferae (now Brassicaceae) stems from (OECD 2012). Petals are usually 8-15 mm long, white to pale yellow for *B. napus*, bright yellow for *B. juncea*. Petal colour variation from white to dark yellow or even pink has been recorded in different cultivars (Downey & Rakow 1987). Each flower contains 6 stamens and a pistil of 2 carpels. Nectaries are found at the base of the stamens.

Seeds develop in 2-celled, elongated capsules called siliques (or pods). Pods are 6-9 cm long and 5 mm wide, with a beak 1-2 cm long. They are smooth, almost cylindrical, with a prominent mid-vein and normally contain 15-25 seeds (Bailey 1976; Edwards & Hertel 2011). In *B. juncea*, pods are held more upright than in *B. napus*.

Seeds are spherical and about 1 - 2 mm wide. *B. juncea* seeds are generally smaller than *B. napus* seeds (2.0-3.0 g/1000 seeds for *B. juncea* compared to 3.0-4.0 g/1000

seeds for *B. napus*). Seed colour varies from light yellow to brown and black. The seed coat is sometimes slightly pitted (Edwards & Hertel 2011).

Figure 5. Flowering raceme of *B. napus* canola. Photo courtesy of Brian Weir.

SECTION 4 DEVELOPMENT

4.1 Reproduction

Both *B. napus* and *B. juncea* reproduce through seeds. There are no reports of vegetative reproduction under field conditions (*in vitro* asexual reproduction is possible, see Section 2.4.2 for more details).

4.2 Pollination and pollen dispersal

B. napus and *B. juncea* have bisexual and entomophilous flowers (i.e. they can be pollinated by insects). The two species are largely self-compatible[17] and mainly self-pollinating, with a self- to cross-pollination ratio of about 70:30 (Downey &

[17] Self-incompatibility is the ability of a fertile hermaphrodite plant to recognize and reject its own pollen, preventing self-fertilization (Hiroi et al. 2013). 50 out of 57 of *Brassica* species (including *B. rapa* or *B. oleracea*) are self-incompatible. For these species, self-incompatibility causes the inhibition of pollen tube growth. Self-recognition mechanisms have been heavily studied in *B. rapa*. *B. napus* and *B. juncea* are mainly self-compatible, with the exception of some lines (Cui et al. 1999; Stone et al. 2003). Some authors have suggested that self-incompatible lines could be used for hybrid breeding. See OECD (2012) for review.

Rakow 1987; Treu & Emberlin 2000). The importance of cross-pollination varies depending on variety and on prevailing environmental conditions (namely weather conditions – wind and temperature - and presence of pollinators) (see Section 9 for more details).

Brassica pollen grains are heavy and slightly sticky (Treu & Emberlin 2000). They are produced in large quantities, with more than 9 kilos emitted per ha per day over a period of 4-5 weeks (Damgaard & Kjellsson 2005; Westcott & Nelson 2001). Pollen can be dispersed by physical contact between neighbouring plants. Hoyle et al. (2007) suggested neighbour-to-neighbour plant contact is an important mechanism of pollination in commercial fields, where plant densities are very high.

Because of their small size (30-40 µm wide), canola pollen grains can become air-borne and be transported by wind. Timmons et al. (1995) described *Brassica* pollen as moving rapidly from the source and not remaining airborne for significant periods of time. Pollination can also be mediated by insects, with a positive impact on canola seed weight and oil quality (Bommarco et al. 2012; Gavloski 2012; Steffan-Dewenter 2003). *B. napus* and *B. juncea* flowers produce nectar with relatively high concentrations of sugars which makes them particularly attractive to feral and managed honey bees (*Apis mellifera*) (Husken & Dietz-Pfeilstetter 2007). Australian native bees are thought to play only a minor role in canola pollination. Native stingless bees are the only native bees used for crop pollination in Australia. As they are only found in tropical and subtropical areas, they are unsuitable for canola pollination (Cunningham et al. 2002). Hoverflies have been described as alternative pollinators but their impact on canola pollination also appears to be quite low compared to honey bees (Jauker & Wolters 2008). Bumblebees (*Bombus* spp.) play a major pollination role in Europe (Cresswell 1999). However, since bumblebees only occur in Tasmania and are geographically discrete, these insects play a minor role in the pollination of *B. napus* and *B. juncea* crops in Australia.

Brassica pollen has been described as being viable for up to 5 days under natural conditions, with a viability rate of 20% measured 72 hours after emission (Bots & Mariani 2005; Ranito-Lehtimäki 1995). Pollen viability varies with environmental conditions, particularly temperature and humidity. *B. napus* pollen longevity and germinability is reduced in case of high temperature stress (Young et al. 2004). Under controlled conditions, pollen sterility can be induced at flowering by a temperature regime of 32°C/26°C day/night, with plants grown throughout their life cycle at 27°C/17°C found to be almost totally sterile (Edwards & Hertel 2011). *B. juncea* pollen is still able to germinate after up to 4 hours at 60ºC (Rao et al. 1992).

See Section 9 for more details regarding pollen flow.

4.3 Fruit/seed development and seed dispersal

4.3.1 Fruit and seed development

Each *B. napus* or *B. juncea* plant produces hundreds of small (1-2 mm diameter), spherical, light brown to black seeds (Buzza 1991), with approximately 280,000-300,000 mature seeds per kg (Colton & Sykes 1992).

Fertilisation is usually completed within the first 24 hours following pollination (Downey & Rakow 1987). The pods begin to develop immediately after each flower is fertilised and will reach maturity in about 80 days. Pods and stems are the major photosynthetic organs after flowering, as pod development coincides with a reduction in the number of leaves. Pods are less efficient than leaves in terms of photosynthetic

capacity, because they have fewer stomata per area. The number of seeds in a pod depends on the amount of solar radiation received, with an average of 15-25 seeds in a mature pod (from 30 ovules per pod at flowering) (Edwards & Hertel 2011).

Seed development happens as follows: seed expansion begins about 15 days after fertilization and lasts for 12 days. The seed coat expands to its full size (the seeds are translucent and watery) and the embryo grows to full size. Twenty days after flowering, seed filling begins in the cotyledons. The accumulation of oil and protein lasts for 35-55 days. By 42 days post-flowering, seed development is complete. Seeds then dehydrate and change from green and soft to black (for *B. napus*) or black to yellow (for *B. juncea*) and hard (Edwards & Hertel 2011). Seeds reach their maximum dry weight about 70 days post-flowering (Colton & Sykes 1992; Edwards & Hertel 2011).

Abiotic stress can impact seed development. Water stress or heat stress at flowering reduces the number of pods per plant. Heat stress also reduces individual seed weight and fatty acid composition. These stresses have cumulative effects on the crop. Developing seeds are also sensitive to frost, while mature, dry seeds are resistant, due to their low moisture content. Biotic stresses, such as aphids present in high density or pathogens, can also lead to impaired seed development or even seed death (Edwards & Hertel 2011).

4.3.2 Seed dispersal

Individual *B. napus* and *B. juncea* seeds are released as siliques dry out and shatter. Pod shattering is an undesirable trait in agriculture as it is linked to seed loss. Harvest seed loss can represent 1.5-8.5% of the average canola yield, 675-3,825 seeds/m^2 for an average yield of 1.5 t/ha (Salisbury 2002c). The domestication of many common crop plants has involved the loss of natural shattering (Sang 2009). However, in the case of cultivated *B. napus*, shattering of siliques remains a problem. In efforts to breed shattering resistance into commercial varieties, a number of studies have investigated natural variation in this trait amongst accessions of *B. napus*. A large number of QTL have been identified (Hossain et al. 2012; Raman et al. 2014b; Raman et al. 2011; Rameeh 2013). Compared to *B. napus*, shattering resistance is greater in *B. juncea*, and research has also been conducted to move this trait into *B. napus* (Hossain et al. 2012).

B. napus and *B. juncea* seeds lack an adaptation to dispersal but, due to their large number and small size, they can be transported by different vectors (Garnier et al. 2008). The main means of dispersal are discussed below.

Wind and water have been observed as vectors for dispersal (Lutman 1993; Mallory-Smith & Zapiola 2008). However, no data is available to quantify their relative importance. Windrows of canola plant material including seed may be blown into adjacent fields by high winds. The dispersal distance will depend on the wind strength, the amount of trash on the ground and the moisture content of the seeds.

Seeds may be transported as bed load sediment in rivers and creeks. Alternatively, heavy rains or flooding could transport residual canola seed remaining on the soil surface after harvest.

Because of their small size and large numbers, *B. napus* and *B. juncea* seeds can be dispersed by animals, e.g. ants, birds and grazing mammals. Birds can shred or remove pods during development and at maturity (Stanley & Marcroft 1999). Mice can climb plants and feed on pods or eat non-germinated seeds sown close to the

surface. Seed survival studies have been performed in Australia, both on mammals and birds. Sheep were placed on a diet containing 10% of whole canola seed for ten days (Stanton et al. 2003). Less than 2% of ingested seed was excreted whole. Germination rates of the excreted seed were highest (approximately 40%) on first day after feeding of canola seed began, but then dropped by an order of magnitude. The percentage of viable seed excreted daily was therefore in the order of 1% of daily intake. The authors recommended a 7-10 days holding period before moving livestock to ensure all viable seeds had been passed (Stanton et al. 2003).

Australian doves, ducks, finches and cockatoos, as well as house sparrows have been placed on a diet containing whole *B. napus* seeds (Twigg et al. 2009; Twigg et al. 2008; Woodgate et al. 2011). Viable seeds were only found in faeces from wood ducks, representing less than 0.01% of ingested seeds. Cockatoos did not readily eat canola seeds. Moreover, husks were recovered from food bowls for cockatoos and sparrows. Woodgate et al. (2011) deemed unlikely that dehusked seeds would survive passage through the gut.

Human activity, and in particular vehicle movement, has been implicated as a main source of canola seed long distance transport (Munier et al. 2012; von der Lippe & Kowarik 2007). Surveys done in North Dakota, US's biggest canola producing area, have shown that feral populations of *B. napus* are found in high densities along major highways but not along smaller roads (Schafer et al. 2011). In Japan, where *B. napus* is mainly imported from Canada, the frequency of *B. napus* feral populations was high along the outbound roads from the harbours to the oil factories. Feral population frequency was low along the inbound roads to the harbours (Kawata et al. 2009). Garnier et al. (2008) described wind turbulence behind vehicles as the main mean for seed projection. The authors showed that seed dispersal was unidirectional and correlated with traffic: roads with less traffic saw little to no dispersal. The maximum dispersal distance observed was 21.5 m, which is comparable to other species with a similar seed weight (Bullock & Clarke 2000; Garnier et al. 2008). *B. napus* populations from seed spillages have also been detected in WA on a 3500m roadside transect from the delivery site (Busi & Powles 2016). Plants were counted on road margins and/or in the median strip.

4.4 Seed germination and seed dormancy

Very little information is available regarding *B. juncea* seed germination and dormancy. *B. juncea* seed is described as being able to germinate in drier conditions than *B. napus* and as being more frost resistant (Oram et al. 2005).

Mature, dry *Brassica* seeds may remain viable for years or decades in controlled conditions: seeds stored in manila envelopes at -20ºC have maintained high germination ability after 32 years (OECD 2012). Seeds buried 20 cm deep in pots persist for up to 16 years in undisturbed soil (Madsen 1962). However, the germination rate decreased over time, with a maximum rate of 1% observed after eleven years.

B. napus seed can germinate under a variety of conditions (Pekrun et al. 1998). However, germination rates are reduced at low temperatures (Nykiforuk & Johnson-Flanagan 1999). Germination rate of 50% was reached one and four days post imbibition (dpi) for seeds kept at 22ºC and 10ºC respectively. At 6 ºC, only 10% of seeds at had germinated 8 dpi (Nykiforuk & Johnson-Flanagan 1999). The effect of low temperatures on germination ranges from thermal effects (frost injuries) to

developmental delays due to the loss of physiological coordination (Nykiforuk & Johnson-Flanagan 1999).

Because of the importance of harvest losses (see Section 4.3.2 for more details), seed viability under field conditions is an important factor to predict the presence/amount of volunteers[18] in subsequent crops.

Seeds lost at harvest can enter the soil seedbank[19] when they are buried by tillage (Gruber et al. 2009; Gulden & Shirtliffe 2009). Most seeds present in the seedbank will die, decompose or be eaten by predators (beetles, rodents and birds) before germination (Gulden & Shirtliffe 2009). Seed predation is greatest when seeds are buried at shallow depths. Attacks by pathogens such as bacteria and fungi are most frequent when seeds are buried deeper. Other mechanisms involved in seed mortality in the seedbank are lethal germination (when seedlings exhaust their reserves before reaching the soil surface) and desiccation. Dry seeds can remain viable for very long periods of time but desiccation tolerance is lost when seeds are subjected to frequent wetting/drying conditions prior to germination (Gulden & Shirtliffe 2009).

Overseas studies

B. napus seeds showed a sharp decline in seed number when incorporated to the seedbank of arable fields in the UK (Lutman et al. 2002). The authors calculated an annual decline rate of 85.7% in disturbed soil, with an overall persistence estimated to be less than 1% after one year. A subsequent study confirmed the importance of soil disturbance for speedy decline of *B. napus* seedbank: up to 1.8% of seeds survived in undisturbed soil for 11 years (Lutman et al. 2003). The observed seed persistence was highly variable between plots. One limitation of this study is that the seeds' ability to germinate was not measured: viability was only assessed by checking the firmness of the seeds. Lutman et al. (2005) provided a regression model showing that 95% decline in seedbank population would take up to 9 years.

Seed persistence in the seedbank is linked to dormancy (Lutman et al. 2003). The initial persistence of seeds depends on the number of seeds incorporated into the seedbank and their ability to become dormant[20]. Longer-term persistence depends on the decline rates of the dormant seeds (Lutman et al. 2003). Seeds can exhibit primary dormancy, i.e. they are dispersed from the parent in a dormant state, or they can develop secondary dormancy after harvest if environmental conditions do not favour germination (Bewley 1997; Schatzki et al. 2013). *B. napus* and *B. juncea* seeds have virtually no primary dormancy (Lutman et al. 2003). This can lead to pre-harvest sprouting[21] in regions characterized by high humidity during harvest season. Pre-harvest sprouting has mainly been observed in commercial F1 hybrids (Feng et al. 2009; Schatzki et al. 2013). Seeds on the ground can become secondary dormant in unfavourable conditions. Pekrun et al. (1998) describe *B. napus* seeds as having a high potential to build up secondary dormancy. Darkness, sub-optimal oxygen supply

[18] Volunteers are unwanted plants in succeeding crops emerging from the soil seedbank.
[19] A seedbank is defined by Gulden & Shirtliffe (2009) as a place where seeds remain until germination.
[20] A dormant seed does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors that are otherwise favourable for its germination (Finch-Savage & Leubner-Metzger 2006).
[21] Pre-harvest sprouting is the phenomenon of seeds germinating on the mother plant following rain during maturation and before harvest (Feng et al. 2009).

and water stress have been described as key drivers to induce secondary dormancy in *B. napus* seeds (Lutman et al. 2003; Pekrun et al. 1998).

Darkness/burial seems crucial for the development of secondary dormancy: seeds left on the soil surface for four weeks have a much lower potential to persist than seeds that were immediately incorporated into the soil (Pekrun et al. 1998). Burial depth also had an impact on seed persistence: most of the dormant seeds were found buried deeper than 10 cm. Seeds at a shallow depth were shown as less likely to remain dormant (Pekrun et al. 1998). The authors suggest that persistence of dormant seeds is linked to situations in which seeds can develop light sensitivity by modifying the balance between phytochrome red and far red forms (Pekrun et al. 1998). Dormant seeds are highly reactive to very short light flashes: germination of dormant seeds kept in the dark can be triggered by a 1/430 of a second long flash of light (Pekrun et al. 1997). Secondary dormancy can also be lifted by low temperatures (2-4^{o}C) (Gulden et al. 2000) or by alternating warm and cold temperatures (Pekrun et al. 1998).

Secondary dormancy in *B. napus* has a genetic component: cultivars can be classified as low, medium or high dormancy types (Gruber et al. 2009; Gulden et al. 2000). QTL have recently been identified for both primary and secondary dormancy phenotypes in *B. napus* (Gruber et al. 2012; Schatzki et al. 2013). However, genetic background is not the only component involved in developing secondary dormancy: environmental conditions such as temperature or water supply can also be involved in the predisposition for secondary dormancy (Gruber et al. 2009; Gulden et al. 2000).

Regression models calculated that it would take up to 9 years for a 95% decline in seedbank population (Lutman et al. 2005). Considering an average harvest seed loss of 3575 seeds/m^2 and a 95% decline over time, up to 200 seeds/m^2 would still be present in the seedbank after nine years. The likelihood of the presence of more than two volunteer plants per m^2 is therefore considered as high by the authors. Another study reported a density of 0.01 GM volunteer plant per m^2 ten years after a trial of GM herbicide-tolerant *B. napus* (D'Hertefeldt et al. 2008). Munier et al. (2012) found up to 1 volunteer plant per m^2 four years after a GM trial. However, data presented were obtained from a very small area (0.4ha) and lacked precision.

Cultivation practices play an important role in controlling soil seedbanks. Minimising seed loss at harvest is considered a crucial point to avoid seedbank build up (Salisbury 2002c). Leaving the stubble untouched after harvest or delaying post-harvest cultivation for four weeks has been described as a means of reducing the future seedbank (Lutman et al. 2003; Pekrun et al. 1998). Fields should not be ploughed immediately after harvest as inappropriate post-harvest cultivations combined with dry weather can lead to a persistent soil seedbank (Lutman et al. 2003).

Australian studies

In Australia, *B. napus* does not appear to persist in the seedbank for as long as in Europe. The majority of volunteers germinated in the first year following winter sown *B. napus*, with no volunteers reported for 82.5% of the sites after three years (Salisbury 2002c). Incorporation into the soil seedbank was more common for late spring/summer sown trials, with the main volunteer germination event observed after two years in 54% of the sites (Salisbury 2002c). The rapid decline of *B. napus* seed in the seedbank was confirmed in SA with a maximum of 4 seeds per m^2 recovered after 3.5 years, resulting in an average density of 0.16 volunteer per m^2. Germination rate

was very low, with only 4% of recovered seeds germinating (Baker & Preston 2008). Cultivation practices such as no tillage or a non-aggressive, minimum tillage system (as adopted by most Australian farmers) could explain this rapid decline (Baker & Preston 2008; D'Emden et al. 2008). Furthermore, in SA, fields are rarely cultivated in the months after harvest (Baker & Preston 2008). Seeds will remain on the soil surface after harvest in November/December, until sowing in April/May. Predation by insects and birds, as well as exposure to the sun will result in the loss of a large number of viable seeds, with the remaining seeds less prone to secondary dormancy (Baker & Preston 2008).

Fewer volunteers of *B. juncea* than of *B. napus* have been reported in subsequent crops during field trials in the ACT (Oram et al. 2005).

4.5 Vegetative growth

B. napus and *B. juncea* are annual crops in Australia, generally completing a lifecycle in 7 months. Colton and Sykes (1992) describe the life cycle of the canola plant through seven principal, overlapping stages (Figure 6):

- stage 0: germination and emergence
- stage 1: leaf production
- stage 2: stem extension
- stage 3: flower bud development
- stage 4: flowering
- stage 5: pod development
- stage 6: seed development

Growth and development are complex processes. The time it takes to complete each growth stage depends on temperature, moisture, day length, nutrition and cultivar. Temperature and moisture are the two most important environmental factors regulating *B. napus* and *B. juncea* development (Edwards & Hertel 2011).

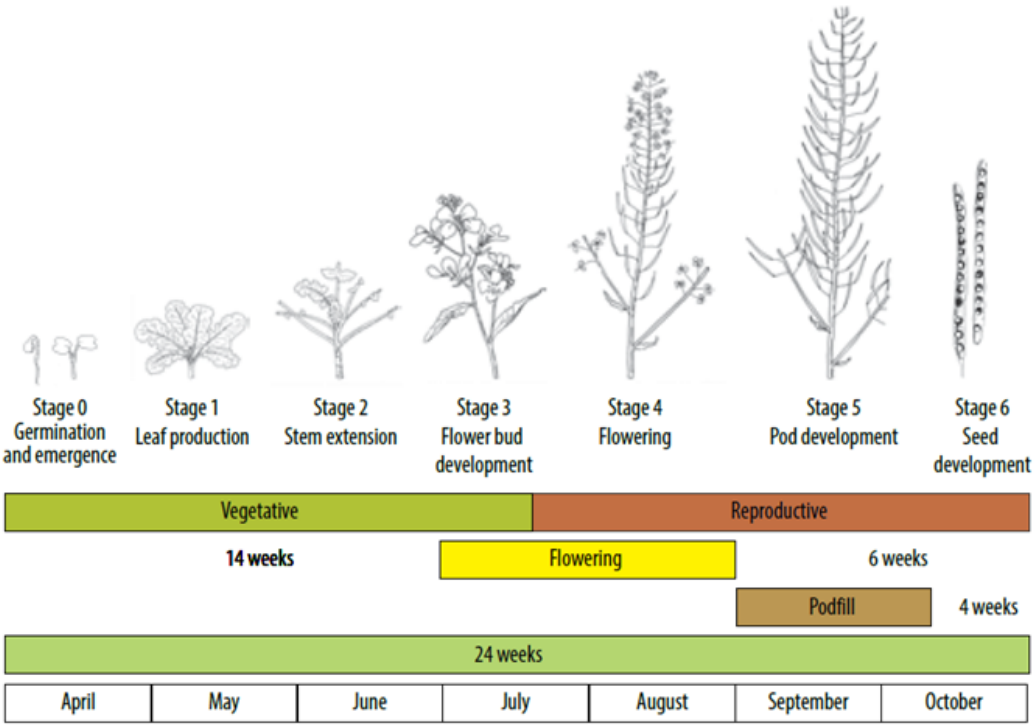

Figure 6. Growth stages of *B. napus*. Source: NSW DPI (Edwards & Hertel 2011). See text for more details.

The initial stage (stage 0, germination and emergence) is from dry seed to fully expanded, green cotyledons. After imbibition, the radicle (root) ruptures the seed coat. The hypocotyl (the shoot) then pushes upwards through the soil, pulling the cotyledons and shedding the seed coat. Once emerged and exposed to light, the cotyledons expand and become green. This marks transition to stage 1. A well-grown *B. napus* or *B. juncea* plant produces 10-15 leaves. There is no definitive number of leaves produced. Early leaves may die and drop from the base of the stem before leaf production is complete (GRDC 2009).

While the leaves are developing, the stem starts to extend (stage 2). Progression within stage is defined according to how many detectable internodes are found on the stem. A well-grown plant produces approximately 15-20 internodes, each with a minimum 5-10 mm in length (GRDC 2009).

Flower bud development is stage 3. During early stem elongation the flower buds remain enclosed in the leaves. As the stem elongates, the flowers emerge but are not free from the leaves. The stem continues to elongate until the flowers are free from the leaves and the lowest flower buds become flattened. Lower buds are the first to become yellow and progressively more buds become yellow as the stem grows.

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. Flowering is indeterminate, beginning at the lowest part of the main inflorescence and continuing upwards (OECD 2012). Flowering of the secondary stems is delayed compared to the main stem.

Silique development (stage 5) starts on the lowest third of the branches on the main stem. This stage is defined by the proportion of siliques that have extended to more than 2 cm long. The final principal stage (stage 6) is seed development during which

the seeds change from translucent to green and finally brown or black and hard (see Section 4.3 for more details). It is during this stage that the canola crop reaches physiological maturity and harvesting occurs (see Section 2.3.3 for more details).

SECTION 5 BIOCHEMISTRY

5.1 Toxins

Erucic acid and glucosinolates have been described as potentially toxic for humans and animals. The gene pool of *B. napus* (and to a lesser extend the gene pool of *B. juncea*) has been subjected to strong selection for low erucic acid and low seed glucosinolate content (see Section 2.2 for more details). By definition, canola quality *Brassica* has been bred to contain less than 2% erucic acid and less than 30 micromoles of glucosinolates per gram of seed solids (CODEX 2009). Modern Australian canola quality *B. napus* typically contain less than 0.5% erucic acid and less than 20 micromoles of glucosinolates per gram in the seed (Colton & Potter 1999).

Erucic acid and glucosinolate contents in most *B. juncea* varieties cultivated in India are above international standards, with cultivars containing an average 40% erucic acid, and 75 micromoles of glucosinolates per gram of defatted seed (Chauhan & Kumar 2011). Breeding programs in India have focused on reducing the levels of erucic acid and glucosinolates and some varieties fulfilling these criteria have been developed and registered for cultivation (Kumar et al. 2010). This breeding has involved germplasm that originated in Australia (Chauhan et al. 2011).

5.1.1 Erucic acid

Erucic acid is a 22-carbon monounsaturated fatty acid, with a single double bond at the omega 9 position. Erucic acid constitutes about 30-60% of the total fatty acids of rapeseed and mustard. It is synthetised in the cytosol by elongation of oleic acid, which is produced in plastids (Bao et al. 1998). Studies demonstrating a possible correlation between exposure to dietary erucic acid and number and severity of heart lesions in rats have led to human health concerns (Sauer & Kramer 1983). Myocardial lipidosis has also been described in pigs and monkeys following erucic acid consumption, indicating that this fatty acid is poorly metabolised (Gopalan et al. 1974; Shenolikar & Tilak 1980). Interestingly, clinical signs such as weight loss were typically absent and no long-term effect was observed. Furthermore, there is no evidence that dietary erucic acid can be correlated to these effects in humans. The consumption of high erucic acid-containing rapeseed oils (*B. napus*, *B. juncea* and *B. rapa*) since ancient times does not appear to have been associated with nutritional or health problems (Monsalve et al. 2001; Sauer & Kramer 1983).

Because of physiological differences with humans, rats are not considered an appropriate model to study the effect of erucic acid (FSANZ 2003). It has been suggested that the incidence and severity of heart lesions in rats can be influenced by feeding of marine/vegetable oils but may not be specifically related to the erucic acid content of the oil (FSANZ 2003). Because of this and in the absence of adequate human data, FSANZ has set a no-observable effect level (NOEL) of 750 mg/kg bw/day, based on results obtained for nursling pigs. A provisional tolerable daily intake (PTDI)[22] was derived from it, using a safety risk factor of 100 (10 for

[22] The Provisional Tolerable Daily Intake (PTDI) is a permissible human daily exposure to contaminants associated with the consumption of otherwise wholesome and nutritious food (FSANZ

extrapolating data from pigs to humans and 10 for variations within humans). The tolerable level for human exposure is thus 7.5 mg/kg bw/day (about 500 mg erucic acid per day for an average adult) (FSANZ 2003). For the average consumer, the dietary intake of erucic acid is 124 mg/day or 28% of the PTDI.

5.1.2 Glucosinolates

Glucosinolates are plant secondary metabolites synthetised by members of the Brassicaceae family. All glucosinolates have the same basic structure, consisting of a β-D-thioglucose group, a sulphonated oxime group and a side chain (Ishida et al. 2014). They are designated as aliphatic, aromatic and indole glucosinolates depending on whether their side chain originates from aliphatic amino acids, aromatic amino acids or tryptophan, respectively (Hasan et al. 2008). Glucosinolates accumulate in vacuoles and have little biological activity (OECD 2012). They contribute to the hot taste and pungent odour of condiment mustard and Brassicaceae vegetables (Ishida et al. 2014). Typically, levels of glucosinolates vary in the organs of any given *Brassica* species, with higher concentrations observed in flower buds and seeds (Bellostas et al. 2007; Bellostas et al. 2004; Clossais-Besnard & Larher 1991; OECD 2012).

When plant tissue is damaged, glucosinolates are hydrolysed by thioglucosidases (alternative name: myrosinase; Enzyme Commission number: EC3.2.1.147). This produces a range of molecules, namely isothiocyanates, thiocyanates, nitriles, goitrin and/or epithionitriles depending on pH and other conditions (Ishida et al. 2014). These breakdown products are associated with a range of biological effects, with roles in plant defence against herbivores and pathogens. These compounds can have both a positive or negative impact on human and animal nutrition. Glucosinolates have been linked to the anti-carcinogenic properties of *Brassica* vegetables (Mithen et al. 2000; Velasco et al. 2008; Wang et al. 2011a). Conversely, isothiocyanates and thiocyanates exhibit goitrogenic or antithyroid activity in laboratory animals, whereas nitriles may cause liver and kidney lesions (Bell 1984). In some livestock, damage to both the liver and thyroid gland has been reported, and fertility is impaired (EFSA 2008). Thus, the presence of glucosinolates limits the nutritional value of the meal as feed for livestock. 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 can also be reduced during the oil extraction process (Canola Council of Canada 2015). Moisture content of the seed during processing should be between 6 and 10%. Above 10% moisture, glucosinolate hydrolysis will proceed rapidly, and below 6% moisture, the thioglucosidase enzyme is only slowly inactivated by heat. At the start of the seed cooking phase, temperature must be raised to 80-90ºC as rapidly as possible. Thioglucosidase-catalyzed hydrolysis of glucosinolates will proceed with increasing temperature until the enzyme is deactivated (Canola Council of Canada 2015).

Glucosinolates have been described as having allelopathic[23] effects that could be used for plant management. Seed meals from *B. juncea* and other members of the Brassicaceae family have been shown to have herbicidal activity against major weeds, while meals from *B. napus* and *B. juncea* reduce the impact of the pathogen

2003). The tolerable intake is referred to as "provisional" as there is often a lack of data on the consequences of human exposure at low levels and new data may result in changes to the tolerable intake.

[23] Allelopathy is a biological phenomenon by which an organism produces one or more molecules that influence the growth, survival and reproduction of other organisms.

Rhizoctonia solani AG8 on wheat production (Handiseni et al. 2011; Handiseni et al. 2013). Overexpression of cassava glucosinolates in *Arabidopsis thaliana* has led to enhanced disease resistance (Brader et al. 2006). However, the manipulation of glucosinolate content in Brassicaceae could impair the microbial communities living in vicinity, and thus impacting the soil ecosystem as a whole (Bressan et al. 2009).

5.2 Allergens

Oil is the only canola product used in the human diet. Processing of canola seed is expected to remove all traces of protein in the oil (ANZFA 2001). No allergic reactions to fats (including canola oil) have been reported in the literature.

However, some cases of food allergy to *B. napus* have been reported (Poikonen et al. 2006; Poikonen et al. 2008; Puumalainen et al. 2006). Eleven percent of atopic Finnish children with suspected food allergies showed sensitivity to crushed seed extracts from *B. rapa* and/or *B. napus* (Poikonen et al. 2006). The authors considered that even small quantities of protein residues in refined or cold-pressed canola oils might be sufficient to produce sensitisation. Mustard allergy has also been reported in France, and has also been investigated in Spain. Mustard is currently included in the list of 14 allergenic foods that must be declared on food labels of pre-packaged foods in the EU (EFSA 2013).

Occupational exposure to *B. napus* pollen, dust and flour has also been implicated in allergic reactions in people (Alvarez et al. 2001; Chardin et al. 2001; Monsalve et al. 1997; Suh et al. 1998). Allergic sensitisation to canola can occur *via* the respiratory tract or through skin contact, e.g. during handling. Occupational allergies to plants can take the form of either immediate hypersensitivity or delayed hypersensitivity reactions. The latter frequently occurs as a consequence of handling plant material and generally manifests as contact dermatitis.

A number of pollen allergens have been reported from *B. napus* (Chardin et al. 2003; Chardin et al. 2001; Focke et al. 2003; Okada et al. 1999; Toriyama et al. 1995). Proteins belonging to the 2S albumin class of seed storage proteins (napins[24]), characterized as allergens in other plant species, have been identified in the seeds of both *B. napus* and *B. juncea* (Monsalve et al. 1997; Monsalve et al. 2001; Puumalainen et al. 2006). BnIII napin, which accounts for 30% of all napins in *B. napus* was identified as its major allergen (Monsalve et al. 1997). Five napins were isolated from *B. juncea*, with Bra j IE being the most abundant (Gonzalez de la Pena et al. 1991; Monsalve et al. 1993). However, there is poor evidence that *B. napus* or *B. juncea* pollen actively sensitise: only 0.2% of patients with respiratory allergies displayed a monovalent sensitisation to *B. napus* pollen (Hemmer 1998; Hemmer et al. 1997). Hemmer et al. (1998) speculated that cross reactivity between *B. napus* or *B. juncea* and other allergens is the main explanation for the observed allergic symptoms. Hypersensitivy to *B. napus* has mainly been observed in patients with atopic dermatitis and a history of pollen allergy (Chardin et al. 2001; Moneret-Vautrin et al. 2012; Poikonen et al. 2008). Monsalve et al. (1997) demonstrated cross reactivity between BnIII napin (from *B. napus*) and Sin a1, the major allergen in *B. alba* seeds, which are used in the production of yellow mustard.

Soutar et al. (1995) found that people who thought their allergic symptoms occurred in relation to the flowering of *B. napus* were rarely allergic to extracts of the plant and

[24] Napins consist of a small and large protein chain linked by disulphide bonds and are extremely resistant to pepsin digestion or temperature/pH denaturation.

fewer than half were atopic. Nevertheless, they usually showed increased bronchial reactivity during flowering season, which may have been due to other allergens and/or to non-specific airborne irritants. Volatile organic compounds given off by growing *B. napus* plants have been shown to play a role in respiratory mucosa and conjunctiva irritation (Butcher et al. 1994).

5.3 Other undesirable phytochemicals

Sinapine is an alkaloid occurring in the seeds of many Brassicaceae, including *B. napus*, *B. juncea* and *Arabidopsis* (Milkowski & Strack 2010). It is found only in the seed and is hydrolysed upon germination to form choline and sinapic acid (Tzagoloff 1963). Sinapine is one of the compounds which give mustard its hot bitter taste. It has been implicated in producing a fishy egg taint when brown egg laying hens are fed too much canola meal (AOF 2007).

5.4 Beneficial phytochemicals

Compositional analysis of canola seed

A summary of the composition of canola seed is given in Table 4a.

At 6% moisture, the seed typically has an oil content ranging from 35-45%. However, the seed oil content can fall outside this range depending on variety and environmental factors. Average oil content in Australian canola has fluctuated from 41-44% between 1998 and 2008 (GRDC 2009). The average protein content of Australian canola has varied from 35.5-41% (in oil-free meal at 10% moisture) over the same 10 year period (GRDC 2009). The hull comprises approximately 16% of the seed weight and accounts for approximately 30% of the oil-free seed meal (Bell 1984).

Oil composition

A summary of the composition of canola oil is given in Table 4b. A comparison of the main seed quality characteristics of *B. napus* and *B. juncea* is provided in Table 5.

Oil content is expressed as a percentage of whole seed at 6 or 8.5% moisture (GRDC 2009; Mailer 1999). Canola oil (both from *B. napus* and *B. juncea*) is high in unsaturated fats (92.1%), has no cholesterol or trans-fat, and has the lowest saturated fat (7.9%) 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 potential health benefits for canola oil due to reduced risk of coronary disease (Douaud 2006).

Table 4. Canola quality parameters, oil content and composition.
(a) Average quality parameters of canola. Adapted from GRDC (2009), USDA (1999).

Quality parameter	Mean
Oil content (% in whole seed, 6% moisture)	41.5
Protein content (% in oil-free meal, 10% moisture)	39.2
Total glucosinolates (µml/g of meal, 6% moisture)	20.0
Calories (per 100g of oil)	884
Saturated fats (% in oil)	7.9
Monounsaturated fats (% in oil)	63.7
Polyunsaturated fats (% in oil)	28.2
Erucic acid (% in oil)	0.1
Vitamin E (mg/100 g of oil)	17.46
Vitamin K (mg/100 g of oil)	71.3

(b) Average fatty acid profile of canola oil. Adapted from GRDC (2009).

Fatty acid	Trivial name	Percentage
14:0	Myristic	0.1
16:0	Palmitic	4.7
16:1	Pamitoleic	0.4
18:0	Stearic	2.4
18:1	Oleic	62.2
18:2	Linoleic	19.7
18:3	Linolenic	8.5
20:0	Arachidic	0.5
20:1	Gadoleic	1.0
22:0	Behenic	0.2
22:1	Erucic	0.1
24:0	Lignoceric	0.1
24:1	Nervonic	0.1

Table 5. *B. napus* and *B. juncea* seed characteristics. Adapted from Edwards & Hertel (2011).

	B. napus canola	*B. juncea* canola	*B. juncea* condiment mustard
Oil (%)	36-42	34-40	34-40
Oleic acid (%)	57-63	57-63	variable
Linoleic acid (%)	18-25	18-25	variable
Linolenic acid (%)	8-13	8-13	variable
Erucic acid (%)	<1	<1	1-20
Glucosinolate in meal (µmoles/g, 10% moisture)	<30	<30	110-160

The oil of non-canola quality *B. juncea* is described as having a distinct nutty flavour. The erucic acid content is considered sufficiently low to make it suitable for human consumption (see Table 5 for details) (Edwards & Hertel 2011).

Tocopherols

Tocopherols are naturally occurring antioxidants in vegetable oils and have a role in reducing cardiovascular diseases (ODS 2016). There are four natural tocopherol isomers (all found in canola) that, together with four corresponding tocotrienols, make up the eight vitamers that constitute vitamin E (Chester et al. 2001). Tocopherol content in canola oil ranges from 0.5-0.9%, depending on growing conditions (Chester et al. 2001). Tocopherol composition between canola varieties is relatively consistent, with 63-74% γ-tocopherol and 26-35% α-tocopherol; δ-tocopherol and β-tocopherol are present in trace amounts (Chester et al. 2001).

The term Vitamin E is used as a generic descriptor for tocopherol and tocotrienol derivatives with α-tocopherol activity (IUPAC-IUB 1982). Their interaction with polyunsaturated fatty acids is important in preserving the chemical stability of canola oil.

Seed meal composition

The composition of seed meal depends on the method of oil extraction (AOF 2007). Typically, seed meal protein concentration is of 36-39% with an amino acid composition comparable to soybeans[25]; it is slightly lower in lysine but higher in all sulphur-containing amino acids. Fat content ranges from 1.5-2% and the meal generally has a richer mineral content than soymeal. The fibre content of canola meal ranges from 11-13% (Bell 1984).

The glucosinolate content varies with growing conditions, and increases with water stress. The meal from canola-quality *B. juncea* varieties is considered safe for stockfeed whereas meal from traditional *B. juncea* varieties, with high levels of erucic acid and glucosinolates, is deemed not suitable (AOF 2013).

SECTION 6 ABIOTIC INTERACTIONS

6.1 Abiotic stresses

6.1.1 Nutrient stress

Canola has been successfully grown on soils ranging from pH 5.0-8.0 (Colton & Sykes 1992). Soil pH has little effect on canola production, except on very acid soils where manganese and aluminium toxicity may result in stunted and single stem plants, affecting yield (Colton & Sykes 1992; Potter et al. 1999). This situation can be alleviated by liming soils before sowing.

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 (per tonne per hectare) 40 kg of nitrogen, 7 kg phosphorus and 10 kg sulphur (Colton & Sykes 1992). Gypsum is often applied to sodic soils to improve soil structure and alleviate sulphur deficiencies (Potter et al. 1999).

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

6.1.2 Heavy metals

Brassicaceae are known to be accumulators of heavy metals. *B. juncea* is one of the most promising candidates for the removal of metals or radioactive elements such as cadmium, caesium, copper, nickel, lead, uranium or zinc (Prasad & de Oliveira Freitas 2003). In areas where arsenic contamination of soils is a problem, such as regions of India and Bangladesh, *B. juncea* could be used to remediate metals from the environment (Rahman et al. 2012).

6.1.3 Temperature, water and salinity stress

Most of Australia is too dry and/or hot to successfully grow *B. napus* or *B. juncea*. Temperature and water stress are linked: a plant will suffer heat stress at a lower temperature if it is also under drought stress (GRDC 2009). The main symptoms linked to heat and drought stress are the same and will occur either independently or in combination.

B. napus is most susceptible to heat and drought stresses during grain fill (October/November). The stresses lead to lower yields and oil content (Potter et al. 1999). High temperatures can induce both male and female sterility (Polowick & Sawhney 1988; Young et al. 2004).

B. juncea is known to be more heat and drought tolerant than commercial *B. napus* varieties (Woods et al. 1991). Some varieties of *B. juncea* have been recorded as germinating in soils too dry for the germination of seeds of *B. napus* (Sharma et al. 2009). Under water stress conditions, *B. juncea* produces more seeds than *B. napus*, mainly because of its greater production of dry matter (Wright et al. 1995; Wright et al. 1996; Wright et al. 1997). In Australia, *B. juncea* has been flagged as an alternative to canola in regions that have particularly low rainfall (Javid et al. 2012). See Section 2.3.3 for more details.

Common and high impacting Australian subsoil constraints include salinity, sodicity[26], alkalinity and toxic ion levels (Zhang et al. 2014). Salinity is an aggravating factor for water and temperature stress. Soil salinity stresses plants *via* dehydration and toxicity (Zhang et al. 2014). Salts on the outside of roots make it more difficult for the plant to extract water, leading to dehydration. Toxicity occurs when salt accumulation in plant tissues reaches a certain threshold. Growth and seed yield of *B. napus* is greatly reduced by drought and salinity stress (Zhang et al. 2014).

B. napus and *B. juncea* are relatively frost tolerant. However, damage can occur at the cotyledon stage (this is uncommon) and affected seedlings will blacken and may die. Plants become more frost tolerant as they develop. Low 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 occurs relatively infrequently (Colton & Sykes 1992).

Abiotic stress tolerance in *Brassica* is being addressed by two approaches – screening of existing germplasms and associated conventional breeding, and/or generation of GM plants expressing genes of interest (Purty et al. 2008). For example, attempts have been made to integrate drought tolerance traits from species such as *B. carinata* into *B. juncea* (Singh et al. 2011).

[26] Sodicity refers to the amount of sodium held in a soil. A sodic soil is defined as a soil containing sufficient sodium to negatively impact crop production and soil structure.

SECTION 7 BIOTIC INTERACTIONS

7.1 Weeds

Certain weeds, particularly those from the Brassicaceae family and plants such as annual ryegrass (*Lolium rigidum*) and volunteer wheat, are the most problematic in *B. napus* and *B. juncea* crops. Both *B. napus* and *B. juncea* can face many weed problems (Carmody & Cox 2001; McCaffery et al. 2009b). For example, in the northern agriculture region of WA, silver grass, wild radish and turnip can devastate early sown crops. Registered herbicides for use in *B. napus* and *B. juncea* crops are either grass specific or for limited broadleaf weed control. Consequently, competition from these weeds leads to significant yield losses. Furthermore, seeds of certain Brassicaceae species can contaminate canola seed, compromising seed quality by increasing levels of erucic acid and glucosinolates. Weeds are best controlled by the sowing of herbicide tolerant varieties (Carmody & Cox 2001).

Varieties frequently differ in their ability to grow in the presence of weeds. Some varieties can suppress the growth of weeds and maintain high levels of yield. In general, it appears that varieties that are high yielding in monoculture are also high yielding in the presence of weeds such as annual ryegrass and wheat (Lemerle et al. 2014).

7.2 Pests and pathogens

7.2.1 Pests

A number of insects and mites can damage *B. napus* and *B. juncea* 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 *Brassica* crops is most likely to occur during establishment, and from flowering to maturity (Miles & McDonald 1999).

7.2.2 Pathogens

B. napus and *B. juncea* can be infected by a number of pathogens in Australia, leading to diseases ranging from root rots to leaf and crown to stem infections (Table 6). As with all diseases, the severity of infection depends on pathogen strain, plant susceptibility and favourable climatic conditions (Karunakar et al. 2002). Pathogens have a high potential to damage *B. napus* and *B. juncea* crops but are reasonably well-controlled. Losses in 2012 were an estimated AUD $113 per ha (Murray & Brennan 2012).

Table 6. Main diseases affecting *B. napus* and *B. juncea* in Australia. Adapted from GRDC (2009; 2012).

Type of disease	Main pathogen	Average annual loss (% of total loss)
Root and crown fungal disease	Blackleg (*Leptosphaeria maculans*)	AUD$ 83.3 million (64%)
Necrotrophic fungal leaf disease	*Sclerotinia* stem rot (*Sclerotinia sclerotiorum*)	AUD$ 18.0 million (14%)
	White leaf spot (*Mycosphaerella capsellea*)	
Virosis	Beet Western Yellows Virus (BWYV)	AUD$ 15.4 million (12%)
Biotrophic leaf fungal disease	Downy mildew (*Peronospora parasitica*)	AUD$ 13.2 million (10%)
	White rust (*Albugo candida*)	

Fungi

Blackleg

Blackleg disease, caused by *Leptosphaeria maculans*, is one of the most devastating diseases of canola worldwide (Howlett et al. 2001; Tollenaere et al. 2012; Van de Wouw et al. 2016). Blackleg can be carried over from year to year on infected stubble, from where spores are released. Spores germinate on cotyledons and young leaves, and lead to lesions. Once the lesions have formed, the fungus will grow within the plant's vascular system. This causes the crown of the plant to rot, resulting in a canker. Severe cankers will sever the roots from the stem whereas a less severe infection will result in a restriction of water and nutrient flow within the plant (GRDC 2009).

Blackleg disease incidence in *B. napus* and *B. juncea* is very high, with the disease occurring 99% of years and affecting 92% or more of *B. napus* and *B. juncea* growing areas (Murray & Brennan 2012). Although not common, yield losses of 50% and greater have been recorded in some seasons (GRDC 2009). In the early 1970s, blackleg wiped out the emerging canola industry in Australia (Kaur et al. 2008). Initial resistance to blackleg came from polygenic resistance genes. In the 1990s, a resistance gene from *B. rapa* spp. *sylvestris* was introduced. This resistance was overcome by 2003 (Kaur et al. 2008). New sources of resistance are currently studied, using winter germplasm and polygenic resistance (Salisbury et al. 2007). See Section 2.4.1 for more details.

Monitoring for the breakdown of resistance to blackleg is necessary for the canola industry. The selection of specific varieties prevents substantial yield losses (Van de Wouw et al. 2016; Van de Wouw et al. 2014).

B. juncea is more resistant to blackleg than *B. napus*, and breeding has been used to transfer identified resistances (Oram et al. 2005). However, there has been a decline in the resistance of *B. juncea* to blackleg, perhaps reflecting selection pressures for strains of blackleg with greater virulence. Other *Brassica* species, such as *B. carinata*,

may be better sources of resistance to this pathogen for *B. napus* than *B. juncea* (Marcroft et al. 2002).

White rust

White rust, caused by the fungal pathogen *Albugo candida*, can be a devastating disease in crops of both *B. juncea* and *B. rapa*. Infection by *A. candida* is characterized by formation of white to cream pustules on cotyledons, leaves, stems and inflorescences. Combined infection of leaves and inflorescences causes yield losses of up to 20% in Australia, particularly in WA (Kaur et al. 2008). White rust is considered less of a problem in *B. napus*, as resistance in common (GRDC 2007a; Kaur et al. 2008; Li et al. 2007a; Somers et al. 2002). Proteins involved in host resistance to white rust have been identified in *B. juncea*, potentially leading to the engineering of durable resistance (Kaur et al. 2011). This is considered of importance by breeders and growers as *B. juncea* is seen as an alternative to *B. napus* in drier, hotter cropping systems.

Other fungi

Other fungal diseases include *Sclerotinia* stem rot (*Sclerotinia sclerotiorum*), downy mildew (*Peronospora parasitica*), club root (*Plasmodiophora brassicae*), and alternaria leaf spot (*Alternaria brassicae*), any of which can cause serious yield loss to canola in wet seasons (GRDC 2009; Howlett et al. 1999; Murray & Brennan 2012; Oilseeds WA 2006).

Viruses

Viral diseases have been found in production areas across Australia (Hertel et al. 2004). Three main viruses have been reported, *Beet western yellows virus* (BWYV, synonym *Turnip yellows virus*, TuYV), *Turnip mosaic virus* (TuMV) and *Cauliflower mosaic virus* (CaMV). Infection with BWYV is widespread in *B. napus* crops in south-western Australia, where losses up to 46% have been recorded (Coutts et al. 2006; Oilseeds WA 2006). However, these losses have been described as "worst case scenario" (Hertel et al. 2004). A QTL for resistance to BWYV was identified in *B. napus* double haploid lines, and thought to be used for marker-assisted selection (Dreyer et al. 2001).

TuMV has not been detected in *B. napus* but is seen as potentially able of becoming a threat: Brassicaceae weeds are naturally infected and could become a reservoir for more virulent strains (Hertel et al. 2004; Schwinghamer et al. 2014). Some *B. juncea* accessions are highly susceptible to TuMV, potentially leading to severe seed losses. A resistance gene was recently identified in *B. juncea* crosses (Nyalugwe et al. 2015). Development of TuMV-resistant *B. juncea* cultivars is seen as becoming an important part of breeding programs in the coming years (Nyalugwe et al. 2015).

CaMV has not been described as a current threat for canola in Australia. Potential loss linked to CaMV has been estimated to be of $ 0.14 per ha (whereas BWYV potential losses have been an estimated $66.7 per ha) (Murray & Brennan 2012).

Disease management and resistance

Introducing resistance to many of these pathogens has focused on identifying natural sources among the available germplasm of *B. napus* and *B. juncea*, and using conventional breeding to move these resistance genes into commercial varieties (Sharma et al. 2009; Somers et al. 2002). In some instances, it has also been possible to use resistances that occur in other *Brassica* species. For example, in India, natural

resistances that occur in *B. carinata* to both white rust and alternaria have been bred, via ovule culture, into *B. juncea* (Gupta et al. 2010).

Nonetheless, best management practices, such as weed and aphid control, are seen as particularly important to help limit the spread of diseases (Hertel et al. 2004).

SECTION 8 WEEDINESS

B. napus and *B. juncea* share some characteristics with known weeds, such as self- and wind-pollination, the ability to produce large numbers of seeds and the potential for short- and long-distance seed dispersal. However, *B. napus* and *B. juncea* lack other characteristics that are common to many weeds, such as the ability to reproduce vegetatively. *B. napus* and *B. juncea* are also considered to be poor competitors (Busi & Powles 2016 and references therein).

The domestication of many common crop plants has involved the loss of natural shattering (Sang 2009). However, in the case of cultivated *B. napus*, shattering of siliques remains a problem. *B. juncea* is more shatter-resistant, which may reduce its likelihood of spread (see Sections 2.4 and 4.3.2 for more details).

As with all crops cultivated and harvested at the field scale, *B. napus* and *B. juncea* seed may escape harvest. Seed remains in the soil until the following season when it germinates either before or after seeding of the succeeding crop. In some instances these volunteers may provide considerable competition to the seeded crop and warrant chemical and/or mechanical control. Volunteers can also be expected outside the planting site, e.g. along roadsides and storage facilities, as a result of spillage during transport (Busi & Powles 2016; Kawata et al. 2009; Schafer et al. 2011). See Section 4.3.2 for more details.

8.1 Weediness status on a global scale

An important element in predicting weediness is a plant's history of weediness in any part of the world (Panetta 1993; Pheloung 2001). Both *B. napus* and *B. juncea* have been cultivated throughout the world for decades or centuries.

In Canada, *B. napus* is considered a minor weed (Canadian Food Inspection Agency 1994). Kaminski et al. (2001) reported *B. napus* as being the fifth ranked weed in Manitoba. However, *B. napus* is not considered a significant weed, nor invasive of natural undisturbed habitats, in Canada (Beckie et al. 2001; Canadian Food Inspection Agency 1994; Warwick et al. 1999). *B. juncea* has been reported as an escapee in Canada since the late 19th century but is not considered to be an abundant weed (CFIA 2007).

In the US, both *B. napus* and *B. juncea* have been classified as being or having the potential to become weedy or invasive (USDA Plants Database; accessed on 16 March 2016). *B. juncea* is classified as a noxious weed in Alaska, Florida and Michigan (Invasive Plant Atlas of the United States; accessed on 16 March 2016).

See Randall (2012) for an extensive review of *B. napus* and *B. juncea*'s weediness status at a global scale.

8.2 Weediness status in Australia

B. napus and *B. juncea* are not classified in Australia as noxious weeds (Weeds Australia; accessed on 16 March 2016) or as weeds of national significance (Department of Environment National Weeds Lists; accessed on 16 March 2016).

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[27] and were defined as environmental or agricultural weeds[28] depending on how they impact either ecosystem. The weeds were further categorized based on their status within each ecosystem on a scale from 0 (naturalised, but the population no longer exists or has been removed) to 5 (naturalised and known to be a major problem at four or more locations within a State or Territory) (see Table 7).

B. napus and *B. juncea* are classified as category 5 weeds in agricultural ecosystems, with variations between States (Table 8). However, WA and VIC State governments do not consider *B. napus* and/or *B. juncea* as weeds, nor does the Department of the Environment (accessed on 29 March 2016). The weediness rankings for Groves et al. (2003) were made by experts from each State or Territory and represent the best personal judgements available. However, according to Dignam (2001), canola is more often reported as a weed when prompted than when not. Neither *B. napus* nor *B. juncea* volunteers are considered as problematic weeds for Australian agricultural and natural ecosystems (N. Ainsworth[29], personal communication, 2016). *B. napus* and *B. juncea* are classified as category 2 and 3 weeds in natural ecosystems, respectively.

Table 7. Categories for assessing the status of naturalised non-native species in natural ecosystems. Adapted from Groves et al. (2003).

Category	Description
0	Reported as naturalised but only known naturalised population now removed or thought to be removed
0?	Uncertainty as to whether any plants exist
1	Naturalised; may be a minor problem but not considered important enough to warrant control at any location
1?	Uncertainty as to whether a small number of plants remain
2	Naturalised; known to be a minor problem warranting control at 3 or fewer locations within a State or Territory
3	Naturalised; known to be a minor problem warranting control at 4 or more locations within a State or Territory
4	Naturalised; known to be a major problem at 3 or fewer locations within a State or Territory
5	Naturalised; known to be a major problem at 4 or more locations within a State or Territory

? Information not available at present

[27] 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).

[28] 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.

[29] Nigel Ainsworth is Principal Officer for the Agriculture and Rural Division, Department of Economic Development, Jobs, Transport and Resources, Victoria State Government.

Table 8. *B. napus* and *B. juncea* weed classification in agricultural and natural ecosystems in Australia. Adapted from Groves et al. (2003).

	Agricultural ecosystems			Natural ecosystems
	State		Australia-wide	Australia-wide
B. napus	QLD	1 – Naturalised; may be a minor problem but not considered important enough to warrant control at any location	5 – Naturalised; known to be a major problem at 4 or more locations within a State or Territory	2 – Naturalised; known to be a minor problem warranting control at 3 or fewer locations within a State or Territory
	NSW	3 – Naturalised; known to be a minor problem warranting control at 4 or more locations within a State or Territory		
	VIC	3 – Naturalised; known to be a minor problem warranting control at 4 or more locations within a State or Territory		
	TAS	1 – Naturalised; may be a minor problem but not considered important enough to warrant control at any location		
	SA	n/a		
	WA	5 – Naturalised; known to be a major problem at 4 or more locations within a State or Territory		
	NT	n/a		
B. juncea	QLD	2 – Naturalised; known to be a minor problem warranting control at 3 or fewer locations within a State or Territory	5 – Naturalised; known to be a major problem at 4 or more locations within a State or Territory	3 – Naturalised; known to be a minor problem warranting control at 4 or more locations within a State or Territory
	NSW	3 – Naturalised; known to be a minor problem warranting control at 4 or more locations within a State or Territory		
	VIC	5 – Naturalised; known to be a major problem at 4 or more locations within a State or Territory		
	TAS	n/a		
	SA	2 – Naturalised; known to be a minor problem warranting control at 3 or fewer locations within a State or Territory		
	WA	5 – Naturalised; known to be a major problem at 4 or more locations within a State or Territory		
	NT	1 – Naturalised; may be a minor problem but not considered important enough to warrant control at any location		

8.2.1 Cultivated areas

Surveys have shown that *B. napus* occurs as a volunteer weed in up to 10% of cereal crops in southern Australia (Lemerle et al. 1996). The limited extent of *B. juncea* cultivation in Australia, and its shatter resistance may reduce its ability to behave as a weed. However, *B. juncea* has excellent seedling vigour and is drought and heat-resistant, two characteristics found in weeds (McCaffery et al. 2009a).

B. napus and *B. juncea* seed can be dispersed to neighbouring non-agricultural areas by mechanisms such as strong winds blowing windrows across or off a field, or seed may be dispersed with straw and chaff during mechanical harvest (see Section 4.3.2 for more details). If dispersed seed germinates, it is unlikely to persist. Seedlings established in adjacent fields would likely be destroyed by normal agricultural practices (herbicide application, cultivation). However, poor management practices can result in severe volunteer problems in succeeding crops.

Seedlings established in non-agricultural areas would not likely spread and persist, as *B. napus* and *B. juncea* plants are poor competitors and do not establish well in unmanaged areas (Oram et al. 2005){Salisbury, 2002 1612 /id}. Unless the habitat is

regularly disturbed, or seed replenished due to harvest/transport spillage, *B. napus* and *B. juncea* will be displaced by other plants (Salisbury 2002c). Predation by slugs and snails and infection by blackleg have been reported as hampering the survival of *Brassica* volunteers (Scott & Wilkinson 1998; N. Ainsworth personal communication, 2016).

8.2.2 Non-cropped disturbed habitats

B. napus and *B. juncea* seeds can be disseminated to neighbouring, non-agricultural habitats, such as roadsides or railway line verges, field margins and wastelands (Busi & Powles 2016). However, *B. napus* and *B. juncea* are considered poor competitors. See Section 4.3.2 for more details.

According to Salisbury (2002b), only optimal agronomic conditions will promote the establishment of *B. napus*. These conditions are not generally available in non-cultivated areas (Salisbury 2002b). Unless the habitat is regularly disturbed, or seed replenished from outside, canola will be displaced by other plants (Salisbury 2002c).

A survey run in spring 2001 in NSW, VIC, TAS, SA and WA recorded the incidence of volunteer *B. napus* and *B. juncea* plants growing within 5 m of the roadside (Agrisearch 2001). Observations were made every 10 km along the designated roads. The presence of *B. napus* in the surveyed areas for the different growing regions was as follows (expressed in percentage of surveyed areas):

- Northern NSW[30]: 0%
- Southern NSW: 31.2%
- VIC: 12.6%
- TAS: 3.6%
- SA: 8.6%
- WA: 20.3%

The occurrence of predominantly isolated plants suggested they had originated from individual seeds that had fallen to the ground during transportation rather than from plants grown the previous season. Average distance between plants was 2.6 m.

No data is available regarding the persistence or dispersal of the populations described in the 2001 survey (Agrisearch 2001). However, spatial dispersion was not observed for persistent volunteer *B. napus* populations in Germany over a fifteen year period despite growing in high quality soil conditions (Belter 2016). Dignam (2001) surveyed 103 local councils across Australia. When asked about the main weed types present in councils, National Parks and along roads and rail lines, *B. napus* was only cited by 8% of respondents. However, when prompted, *B. napus* was reported as a weed by 30% of councils (Dignam 2001). Only 5% of councils reported that *B. napus* was present in large numbers.

8.2.3 Undisturbed natural habitats

B. napus and *B. juncea* are not considered to be significant weeds, nor invasive of natural undisturbed habitats in Australia (Dignam 2001). 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

[30] The authors surveyed two different areas in NSW, referred to as northern and southern NSW. Northern NSW covers Narrabri, Gunnedah, Tamworth, Glen Innes, Inverell and Moree. Southern NSW covers Culcairn, Wagga Wagga, Cowra, West Wyalong, Narrandera and Tocumwal.

habitats, resulting in poor fitness (Salisbury 2002b). In the absence of disturbance, *B. napus* and *B. juncea* are unable to compete with other plants and/or weeds and do not persist (Salisbury 2002b).

8.3 Control measures

B. napus and *B. juncea* may be grown in rotation with wheat as the follow-on crop. Volunteer plants can be controlled in the post-emergent wheat crop by spraying herbicides or by using mechanical means.

A number of herbicides are registered for use on *B. napus* and *Brassica* ssp., including:

- B (flumetsulam, sulfosulfuron or metosulam)
- C (bromoxynil)
- E (carfentrazone)
- F (diflufenican)
- G (glyphosate)
- O (MCPA - 2-methyl-4-chlorophenoxyacetic acid, 2,4-D or clopyralid) (APVMA website).

Flumetsulam, sulfosulfuron, MCPA 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).

8.4 Weed risk assessment of *B. napus* and *B. juncea*

The weed risk potential of *B. napus* and *B. juncea* has been assessed (Appendix 1) using methodology based on the Australian/New Zealand Standards HB 294:2006 National Post-Border Weed Risk Management Protocol. The National Post-Border Weed Risk Management Protocol rates the weed risk potential of plants according to properties that strongly correlate with weediness (Virtue et al. 2008). These properties relate to invasiveness, impacts and potential distribution.

In summary, as volunteers (rather than crops) *B. napus* and *B. juncea* are considered to:

- have low ability to establish amongst existing plants
- have low tolerance to average weed management practices
- have short time to seeding
- have a high annual seed production in dryland and irrigated cropping areas
- have a low ability to establish in any land use, except in some cultivated and disturbed areas
- only reproduce by sexual means
- be unlikely to spread long distance by natural means
- be commonly spread long distance by people
- have limited ability to reduce establishment or yield of desired vegetation
- have low ability to reduce the quality or characteristics of products, diversity or services available from the land use

- have low potential to restrict the physical movement of people, animals, vehicles, machinery and/or water
- have low potential to negatively affect the health of animals and/or people
- have minor or no effect on degradation of the landscape or ecosystems.

This is consistent with previous assessments of *B. napus* and *B. juncea* in Australia described in Section 8.2 and provides a baseline for the assessment of GM canola-quality crops.

SECTION 9 POTENTIAL FOR VERTICAL GENE TRANSFER

Vertical gene transfer is the transfer of genetic material from parent to offspring by reproduction. Reproduction may occur by sexual or asexual means. Gene transfer can be intraspecific, interspecific or intergeneric. This section deals with gene transfer by sexual reproduction only (as *B. napus* and *B. juncea* do not reproduce by any asexual mechanism) and focuses on gene flow *via* pollen. For gene flow *via* seed, which is likely to occur in agronomic environments, see Section 4.3.2.

Under natural conditions, most plants are capable of crossing with members of the same species. Crossing with other species, which can form part of the evolutionary origin of new species, is usually rarer but can often be facilitated by human intervention. Although *B. napus* and *B. juncea* are self-compatible and mainly self-pollinating, they are both capable of crossing with a limited number of other species (Downey & Rakow 1987; FitzJohn et al. 2007).

9.1 Pollen flow and cross-pollination rates

B. napus and *B. juncea* are predominantly self-pollinating, with an average of 70% of seeds resulting from self-fertilisation. Up to 30% of *B. napus* and *B. juncea* seeds result from cross-pollination. Outcrossing can be mediated by insects, wind or physical contact. The relative importance of wind and bee-mediated pollination is as yet unresolved (Bommarco et al. 2012; Hayter & Cresswell 2006; Rieger et al. 2002; Walklate et al. 2004). Hoyle et al. (2007) proposed a mixed pollination model, based on seasonal and spatial variations in bee abundance. Winter cultivars flowering in early spring are more prone to wind-borne cross-pollination whereas spring ones, flowering in summer show an increase in bee-borne cross-pollination (Hoyle et al. 2007).

Most studies described *Brassica* pollen dispersal as leptokurtic[31], with the majority of cross-pollination occurring over very short distances (less than 10 m) from the source (Eastham & Sweet 2002). Because of this distribution, any foreign pollen in a given field will quickly be diluted into the massive local pollen production (Damgaard & Kjellsson 2005). However, low to very low pollen movements can occur at long distances, meaning that complete genetic isolation is difficult to maintain. Pollen dispersal profiles are highly dependent on topographical and environmental conditions (Eastham & Sweet 2002). This has led to variable pollen-mediated gene flow being reported, from 0.00034% at 47 m to 0.08% at 2.5 km (Scheffler et al. 1993; Timmons et al. 1995). The pattern of *B. juncea* pollen movement is considered to be very similar to *B. napus* (Salisbury 2006). Singhal et al. (2005) showed that no wind

[31] A leptokurtic distribution is a statistical distribution with a more acute peak and fatter tails than found in a normal distribution.

pollination occurred over a 40 m distance for *B. juncea* under Indian conditions. No information is available regarding *B. juncea*'s pollen movement in Australia.

The sections below focus on intraspecific, interspecific and intergeneric crossings.

9.2 Intraspecific crossing

Intraspecific crossing refers here to hybridisation between two plants of the same species, e.g. two *B. napus* or two *B. juncea* plants. These crosses can occur within a field, between fields, with wild populations or volunteer plants (Klein et al. 2006). *B. napus* and *B. juncea* are not considered weeds and do not establish self-sustainable populations over long periods of time (see Section 8 for more details).

Intraspecific gene flow is considered more likely than interspecific gene flow (FitzJohn et al. 2007). There are no sexual barriers to cross-pollination between *B. napus* or *B. juncea* crops, as these species are mainly self-compatible (Cui et al. 1999; Salisbury 2002b; Stone et al. 2003).

9.2.1 Crosses with oilseed subspecies

Hüsken and Dietz-Pfeilstetter (2007) compared methods measuring pollen-mediated intraspecific gene-flow in *B. napus*. The authors describe two experimental designs:

- a continuous design where the recipient field is surrounding the donor field
- a discontinuous design where the recipient field is located as a patch at different distances from the donor field.

Using a continuous design, average values of cross-fertilisation decline sharply and are frequently constant around 0.05% after 20 to 50 m. Decline observed using discontinuous design is slower and steadier, and hybridization rate is constant at 0.1% beyond 100 m. Size of relative donor and recipient fields impacts the level of outcrossing: a combination of a small pollen source and a large recipient population may lead to an underestimation of the level of outcrossing.

Under Australian conditions, a large study found that outcrossing rates between neighbouring commercial fields averaged less than 0.1% over whole fields (Rieger 2002). Tracking cross-pollination at the landscape level in NSW, VIC and SA, and using donor and recipient fields of similar sizes (25-100 ha), Rieger et al. (2002) showed that random cross-pollination was recorded at low frequencies to distances of up to 3 km from the pollen source. On a field basis, the highest outcrossing frequency observed was of 0.07%, with no outcrossing observed in 36.5% of the fields studied (Rieger et al. 2002). The authors suggested that roaming insects may target single plants flowering early or late, resulting in sporadic pollen movement (Rieger et al. 2002).

Outcrossing in *B. juncea* was studied using a continuous design, with a small-sized donor field (GhoshDastidar et al. 2000). The outcrossing rate was 0.244% at 5m. No outcrossing was observed beyond 35 m. The use of a continuous design may underestimate the outcrossing rate. However, rates observed for *B. juncea* are very similar to those observed for *B. napus*.

Male sterile plants and individual pollen traps have been used to measure gene flow. However, they lead to an overestimation of outcrossing rates, as they do not reflect the usual levels of pollen competition in open-pollinating varieties (Eastham & Sweet 2002; Husken & Dietz-Pfeilstetter 2007). Male sterile plants can be used to determine

maximum levels of gene flow but do not provide information on actual outcrossing rates (Husken & Dietz-Pfeilstetter 2007).

To keep cross-pollination between fields below 0.3%, Damgaard and Kjellsson (2005) proposed using 200 m isolation zones or 10 m discarded border crops[32]. Isolation distances are effective for self-fertile plants but not for male-sterile crops, where discarded border zones should be preferred (Damgaard & Kjellsson 2005; Husken & Dietz-Pfeilstetter 2007). Damgaard and Kjellsson (2005) also discussed the practicality of increasing field width when possible, in order to dilute the foreign pollen to a lower proportion.

9.2.2 Crosses with vegetables and forage rape subspecies

B. napus canola and *B. juncea* canola can also cross with subspecies including forage rape or vegetables such as swedes, rutabaga or kale (for *B. napus*) or condiment-quality and leafy vegetables such as gai choy or mustard greens (for *B. juncea*). Such crosses are possible if subspecies are in close proximity and if there is synchrony of flowering. *Brassica* vegetables are not recognized as weeds in agricultural environments. They are generally harvested prior to flowering, unless the plants are grown for seed production. Whenever plants are grown for seed production, isolation distances are in place to maintain seed purity (see Section 2.3.1 for more details regarding seed certification). For these reasons, hybrids between canola-quality and vegetable *B. napus* or *B. juncea* are unlikely to occur (Salisbury 2002b).

9.3 Interspecific crossings

Potential gene flow between *B. napus* and *B. juncea* and Australian Brassicaceae weed species is summarised in Table 9.

The direction of a cross is an important parameter to consider when evaluating the risks linked to hybridization with weedy relatives. Gene dispersal and introgression of genes present in *B. napus* or *B. juncea* into weedy populations will only be possible with *B. napus* or *B. juncea* as the pollen donor.

Interspecific crosses are limited by both pre- and post-fertilisation barriers. Pre-fertilisation barriers include pollen longevity, synchronicity of flowering, breeding system, floral characteristics and competitiveness of pollen. Post-fertilisation barriers include sexual compatibility, hybrid viability and fertility (Salisbury 2002b). Progeny viability and fertility through several generations are also factors influencing crosses (Mallory-Smith & Sanchez Olguin 2011).

Modern breeding techniques have overcome natural pre- and post-fertilisation barriers to interspecific crosses (OECD 2012). They do not occur naturally, i.e. in the field. Sexual and artificial, *in vitro* breeding techniques such as ovary, ovule or embryo culture, as well as protoplast fusion, have produced hybrids that would otherwise have failed (Figure 7). Such techniques have been used to integrate important agronomic or quality traits into cultivated *B. napus* and *B. juncea*. For example, *B. napus* and *B. juncea* crop improvement has involved breeding with several *Brassica* species, such as *B. carinata*, *B. oleracea* or *B. nigra* (Mason et al. 2015; Navabi et al. 2011; Rahman 2013). See Section 2.4.1 for more details.

While success using *in vitro* techniques is not an indication that such crosses could occur under natural conditions, failure to cross even with such assistance may give

[32] This study focused on GM-pollination of non-GM crops.

some indication about which species will not cross (FitzJohn et al. 2007; OECD 2012). See Warwick et al. (2009) for an extensive review of available interspecific and intergeneric hybridization data.

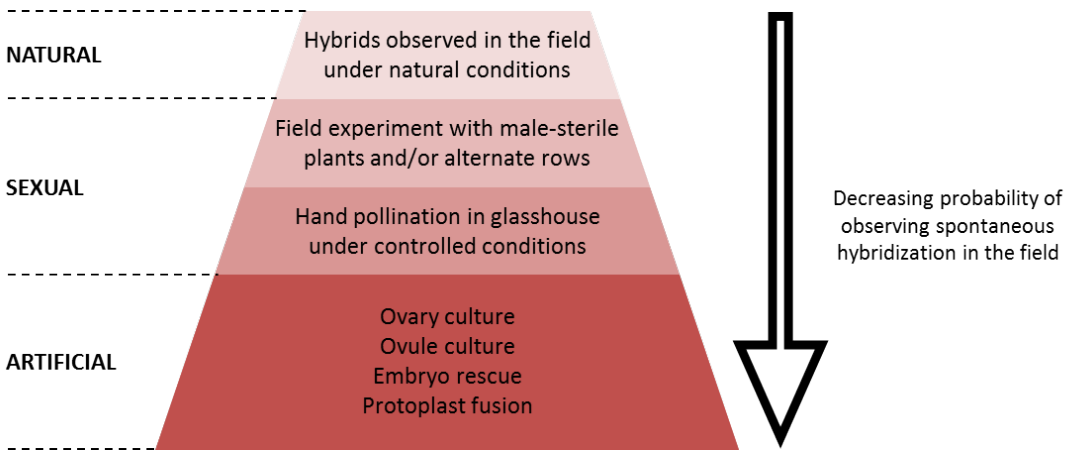

Figure 7. Intraspecific, interspecific and intergeneric hybrids can be obtained naturally, sexually or artificially in the tribe Brassiceae. Adapted from Warwick et al. (2009).

B. napus, *B. juncea* and *B. rapa* share a common set of chromosomes (the A genome, see Figure 1), increasing the likelihood of interspecific hybridisation and gene flow (Salisbury 2002a). Gene introgression is expected to occur *via* the A genome shared by these species (Salisbury 2006). All three species have been reported to hybridize with each other (FitzJohn et al. 2007; Warwick et al. 2009). However, natural hybrids in fields and riversides were reported only for *B. napus* x *B. rapa* hybrids (Warwick et al. 2009). There is no other evidence suggesting that hybrids formed between *B. napus* and other wild relatives could establish in nature (Wei & Darmency 2008).

Table 9. Potential gene flow between *B. napus* and *B. juncea* and Australian Brassicaceae weed species. This table focuses specifically on species considered to be potentially weedy in Australia (Groves et al. 2003; Salisbury 2002b).

Tribe	Genus	Main species of concern in Australia[1]	Means of propagation	Considered as weed in Australia?			Hybridization in the field			
				Groves et al. (2003)[2]		Department of the Environment[3]	Overseas[4]		In Australia[5]	
				Agricultural	Natural		*B. napus*	*B. juncea*	*B. napus*	*B. juncea*
Brassiceae	*Brassica*	*Brassica rapa* *Brassica tournefortii*	Seed	5 5	4 5	No	Likely Unlikely		Likely* Unlikely	
	Diplotaxis	*Diplotaxis tenuifolia*	Seed	5	3	Yes	Unlikely		Unlikely	
	Hirschfeldia	*Hirschfeldia incana*	Seed	5	4	Yes	Unlikely		Unlikely	
	Raphanus	*Raphanus raphanistrum*	Seed	5	5	Yes	Possible*		Unlikely	
	Rapistrum	*Rapistrum rugosum*	Seed	5	5	No	n/a		Unlikely	n/a
	Sinapis	*Sinapis alba* *Sinapis arvensis*	Seed	5 5	3 5	No	Unlikely Possible#		Unlikely	
Cardamineae	*Cardamine*	*Cardamine flexuosa* *Cardamine hirsuta*	Seed	5 5	3 5	No	n/a		n/a	
Isatideae	*Myagrum*	*Myagrum perfoliatum*	Seed	5	2	Yes	n/a		Unlikely	
Lepidieae	*Lepidium*	*Lepidium draba*	Seed Vegetative	5	5	Yes	n/a		n/a	
Sisymbrieae	*Sisymbium*	*Sisymbium thellungii*	Seed	5	5	Yes	Unlikely		Unlikely	
Vellinae	*Carrichtera*	*Carrichtera annua*	Seed	5	5	n/a	n/a		n/a	

[1] According to Salisbury, 2002 and the Department of the Environment website (accessed on 29 March 2016)
[2] See Table 7 for detailed description of the different categories
[3] According to the Department of the Environment website (accessed on 29 March 2016)
[4] According to (FitzJohn et al. 2007; Warwick et al. 2009 and references therein)
[5] According to (Salisbury 1991; Salisbury 2002b)
* *B. napus* x *B. rapa* hybrids have not been reported to date in Australia. However, hybridization and subsequent introgression are possible where the two species grow in sympatry and when flowering periods overlap (Salisbury 2002b)
# Hybridisation has been described in the field under experimental settings such as use of male-sterile *B. napus* or *B. juncea*, alternate rows and/or caged crop plant and weedy relatives (see Eber et al. 1994; FitzJohn et al. 2007; Lefol et al. 1996; Salisbury 1991; Warwick et al. 2009; Warwick & Martin 2013).

Rate of natural hybridization between *B. napus* and *B. rapa* varies depending on studies. Gene flow measurements by Scott and Wilkinson (1998) from *B. napus* to *B. rapa* populations growing outside field boundaries showed hybridisation frequencies of 0.4-1.5% and seedling establishment of less than 2%. Hybrids were identified in populations growing 2-5 m from 12-15 ha *B. napus* fields. However, Warwick et al. (2008) described hybridization rates up to 42.5% in feral populations growing at the margin of *B. napus* fields. Hybrid rates dropped to 2.5% within three years. Plants were collected along two edges of the original *B. napus* field. No data is available regarding the spatial distribution of the hybrids observed, making comparison with other studies difficult. High hybridization rates (9-93%) were observed by Jorgensen et al. (1996). However, these hybridization rates were obtained using co-cultivation methods in field conditions, with, e.g. single *B. rapa* plants grown in *B. napus* fields. Such experimental settings have been shown to overestimate outcrossing levels (Eastham & Sweet 2002; Husken & Dietz-Pfeilstetter 2007).

B. napus x *B. rapa* hybrids are fertile, with lower pollen fertility and seed set than the parents (Hansen et al. 2001 and references therein). The extent and direction of hybridization may depend on the relative abundance of the two species (Hauser et al. 1997). Under normal field conditions, the larger number of *B. napus* stigmas in a given area compared to *B. rapa* increases the chance of *B. napus* becoming the female parent (Hauser et al. 1997). However, the authors noted that hybrids formed on *B. rapa* survive and reproduce. As these hybrids can backcross with *B. rapa*, Hauser et al. (1997) suggested that gene introgression was a likely process. *B. rapa* is no longer grown commercially in Australia and is not considered as a widespread agricultural weed (Salisbury 2002b). *B. napus* x *B. rapa* hybrids have not been reported to date in Australia. However, hybridization and subsequent introgression are possible where the two species grow in sympatry and when flowering periods overlap.

B. napus x *B. juncea* have been produced using caged plants (Liu et al. 2010) or alternate rows (Bing et al. 1996; Tsuda et al. 2012). These crosses have been described as spontaneous as they did not require human intervention such as hand pollination. However the use of caged plants or alternate rows does not mimic natural field conditions. A maximum hybridization rate of 1% was observed for *B. napus* x *B. juncea* co-cultivation experiments under field conditions, using alternate rows, with plants grown with 25-61 cm spacing between rows (Bing et al. 1996). No hybrids were detected beyond 20 m from the pollen source when co-cultivating *B. napus* and *B. juncea* (Tsuda et al. 2012).

B. napus x *B. juncea* hybrids can be backcrossed with both parents. Liu et al. (2010) showed that backcrosses with *B. juncea* produced fewer, smaller seeds than backcrosses with *B. napus*. Self-pollinated hybrids also produced small seeds, with a germination equivalent to those observed for backcrosses (Liu et al. 2010). In most cases, small-seeded hybrids make interspecific hybrid establishment in the field very unlikely, limiting the gene flow to some extent (Wei & Darmency 2008). Small seed size has a strong effect on early seedling growth through reduced capacity to germinate and reduced reserves for seedling development (Gueritaine et al. 2003).

Some *B. napus* x *B. juncea* hybrids have been described as growing taller and producing more flowers than both parents, suggesting that these hybrids could establish and compete better with other plants (Di et al. 2009). However, this change in plant height and flower production was not linked to an increased above ground

biomass or seed number. On the contrary, hybrids produced 3-24 times less seeds than the parents (Di et al. 2009).

Co-cultivation experiments did not yield hybrids between *B. napus* or *B. juncea* and *B. nigra* (Bing et al. 1996). Hybrids have been produced using hand pollination under controlled conditions but outcrossing rates were very low and no further generation was observed (FitzJohn et al. 2007; Salisbury 2002b). The potential of gene flow from *B. napus* or *B. juncea* to *B. nigra* is thus considered extremely unlikely under natural conditions.

The potential of gene introgression from *B. napus* to *B. fructiulosa*, *B. oxyrrhina* and *B. tournefortii* under Australian conditions has been assessed by Salisbury (2002b). *B. fructilosa* is a relatively uncommon weed of disturbed soils, *B. oxyrrhina* a potential weed of canola and *B. tournefortii* a significant weed of canola crops in all States. Salisbury (2002b) qualifies the potential of gene introgression as extremely unlikely, due to pre-fertilisation barriers. Some hybrids have been obtained using of artificial crossing methods (Figure 7). Furthermore, these hybrids have been shown to be sterile (Salisbury 2002b and references therein). *B. tournefortii* x *B. juncea* were obtained using embryo rescue. No *B. juncea* x *B. tournefortii* hybrid was produced as embryos aborted at early development stages (Kumar et al. 2001). Thus, the potential of gene flow from *B. juncea* to *B. tournefortii* is considered extremely unlikely.

9.4 Intergeneric crossings

Potential gene flow between *B. napus* or *B. juncea*, and Australian Brassicaceae weed species is summarised in Table 9.

The flowering periods of many weedy Brassicaceae species overlap with those of *B. napus* and *B. juncea*. Depending on the season and region, the synchrony of flowering between species can also influence the rate of outcrossing in the field. Generally, in Australia commercially grown *Brassica* species flower 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).

Significant pre-and post-fertilization barriers exist between *B. napus* or *B. juncea* and their weedy relatives in Australia (Salisbury, 2006). Gene movement between *B. napus* or *B. juncea*, and other members of the Brassicaceae family is rare, and in most cases probably never occurs. It is considered that, if such hybrids were to be produced under natural conditions, their chance of survival would be extremely low (Salisbury, 2006).

As for interspecific crosses, the use of modern breeding techniques has allowed the production of intergeneric hybrids that would otherwise have failed. Hybrids have been generated *in vitro* by crossing *B. napus* with *Diplotaxis tenuifolia*, *Hirschfeldia incana*, *Raphanus raphanistrum* and *Sinapis arvensis* (FitzJohn et al. 2007). See Warwick et al. (2009) for an extensive review of available interspecific and intergeneric hybridization data.

This section focuses mainly on *Raphanus raphanistrum*, *Sinapis arvensis* and *Hirschfeldia incana*. These species are recognised as major weeds of commercial *Brassica* crops and have been described as potentially compatible with *B. napus*

(Eastham & Sweet 2002). Relative weediness of these three species in agricultural ecosystems is summarized in Table 10.

Table 10. Relative weediness of *R. raphanistrum*, *S. arvensis* and *H. incana* in Australia. Adapted from Groves et al. (2003).

	Australian rating	QLD	NSW	VIC	TAS	SA	WA	NT
R. raphanistrum	5	5	5	5	5	4	5	n/a
S. arvensis	5	2	5	3	5	1	5	n/a
H. incana	5	1	3	5	2	2	1	n/a

Raphanus raphanistrum is a major weed of canola in all canola growing States, especially in WA (Salisbury 2002b). Hybrids have been generated by co-cultivation under field conditions or in glasshouse, using a male sterile *B. napus* (Ammitzboll & Jorgensen 2006; Darmency et al. 1998; Gueritaine et al. 2003). Hybridisation rate observed by Darmency et al. (1998) was of 0.05%. No details were given regarding hybridisation rates for the other studies. Gueritaine et al. (2003) showed that such hybrids are less likely than both parents to emerge and survive competition with other plants, both in agronomic conditions and in disturbed habitats. There is no record of hybrids generated under natural conditions with *B. juncea* as the pollen donor (FitzJohn et al. 2007, and references therein; Warwick et al. 2009). Transfer of genes of *B. napus* or *B. juncea* to *R. raphanistrum* would be highly unlikely (Gueritaine et al. 2003).

Sinapis arvensis is an occasional weed of canola in all canola growing areas (Salisbury 2002b). Using co-cultivation with male-sterile *B. napus*, hybridization rates of 0.12-0.18% were observed (Chevre et al. 1996; Lefol et al. 1996). There is no record of hybrids generated under natural conditions with *B. napus* as the pollen donor (FitzJohn et al. 2007, and references therein; Warwick et al. 2009). *B. juncea* x *S. arvensis* hybrids were generated using co-cultivation under field conditions, at a rate of 0.0018% (Warwick & Martin 2013). Hybrids showed reduced fertility and no backcross progeny was obtained using *S. arvensis*. The authors suggested that the likelihood of transgene introgression from *B. juncea* to *S. arvensis* is low to negligible (Warwick & Martin 2013). Gene flow is not likely to occur between either *B. napus* or *B. juncea*, and *S. arvensis* (Eastham & Sweet 2002).

Hirschfeldia incana is a weed of disturbed soils in eastern Australia and an occasional weed of canola in all canola growing regions (Salisbury 2002b). Using co-cultivation under field conditions, Lefol et al. (1996) obtained 0.36-1.0 *B. napus* x *H. incana* hybrid per plant. Back-crossing the hybrids to *H. incana* produced only non-viable plants. Darmency and Fleury (2000) estimated frequency of hybrid descendants to be as low as 0.002%. Gene introgression was deemed as extremely unlikely (Darmency & Fleury 2000). Potential gene flow from *B. juncea* to *H. incana* under Australian conditions has also been described as extremely unlikely (Salisbury 1991; Salisbury 2006).

9.5 Bridging as a means of gene transfer

When a direct cross between two species is not possible, an intermediate crossing with a third species may bridge the crossing barrier (Andersson & Vicente 2010; van de Wiel et al. 2010). Bridging is used for breeding but could also be a way for *B. napus* or *B. juncea* to transfer genes to related weeds. As described above, *B. napus* and *B. juncea* can hybridize with a few members of the *Brassicacea* family. Such

hybrids could be seen as intermediates. For example, *B. juncea* could act as an intermediate species for *B. napus*. If genes from *B. napus* were to be introgressed into the genome of *B. juncea*, *B. juncea* could act as bridge to transfer these genes into *B. nigra*, and from the latter into *S. arvensis* (Andersson & Vicente 2010). Crossing between *B. juncea* and *B. nigra* is possible because they share a common genome (the B genome, see Figure 1). However, hybridization between these species has not been observed under natural conditions. Hybrids have only been produced under artificial conditions and backcrossing to *B. nigra* does not produce viable plants (Salisbury 2006). Thus, this introgression pathway is considered highly unlikely.

B. rapa has also been proposed as an intermediate species. Indeed, *B. napus* and *B. rapa* have been shown to hybridize in the field under natural conditions (see Section 9.3 for more details). However, such hybrids are less competitive and persistent than their parents, due to lower fertility and reduced dormancy (Bing et al. 1991; Jorgensen et al. 1999). *B. rapa* does not hybridize with *B. tournefortii*, *H. incana*, *R. raphanistrum* or *S. arvensis* (Warwick et al. 2009). Based on the available data, the potential for gene transfer to weed relatives using *B. rapa* as a bridge is considered unlikely.

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Appendix 1 Weed Risk Assessment

Species: *Brassica napus* L.
Brassica juncea L.

Definitions of terms used in this Weed Risk Assessment

Establishment	The perpetuation, in the foreseeable future, of a plant within an area after its entry
Impact	For this document, the negative effects of a plant on human health and safety, and the environment. Impacts are considered on a per unit area basis (the overall consequence of a weed is a function of impacts and potential distribution). For the purpose of this Weed Risk Assessment, impacts of volunteer *B. napus* and *B. juncea* are considered different from the utility obtained when they are grown deliberately
Invasiveness	A relative index measure of the likelihood of spread of a naturalised plant species, being a function of the species' establishment, reproductive and dispersal abilities
Land use	The principal land management objective. In broad terms, an objective may be primary production (e.g. agriculture), conservation or human services (e.g. residential, water supply)
Potential distribution	The geographic area that a plant could occupy if allowed to spread unhindered
Ruderal species	Species that are first to colonise disturbed areas
Weed control	Application of any of a number of methods (e.g. mechanical, chemical or biological) that are designed to reduce the density and reproductive output of plant infestations, so that impacts are reduced or mitigated through suppression, containment or eradication

Land uses:

The Australian Land Use and Management (accessed on 13 April 2016) Classification system provides a nationally consistent method to collect and present land use information for a wide range of users across Australia. The classification has six primary classes of land use that are distinguished in order of generally increasing levels of intervention or potential impact on the natural landscape:

1. Conservation and natural environments: land is used primarily for conservation purposes, based on the maintenance of essentially natural ecosystems already present
2. Production from relatively natural environments: land is used mainly for primary production based on limited change to the native vegetation
3. Production from dryland agriculture and plantations: land is used mainly for primary production, based on dryland farming systems
4. Production from irrigated agriculture and plantations: land is used mainly for primary production, based on irrigated farming
5. Intensive uses: land is subject to substantial modification, generally in association with closer residential settlement, commercial or industrial uses. Intensive uses includes areas of intensive horticulture or animal production, areas of manufacture or industry, residential areas, service areas (e.g. shops, sportsgrounds), utilities (e.g. facilities that generate electricity) areas of transportation and communication (e.g. along roads, railways, ports, radar stations), mine sites and areas used for waste treatment and disposal.
6. Water: although primarily land cover types, water features are regarded as essential to the classification.

The relevant land uses for this Weed Risk Assessment are:

3. **Production from dryland agriculture and plantations** (more specifically 3.3: cropping)
4. **Production from irrigated agriculture and plantations** (more specifically 4.3: irrigated cropping)
5. **Intensive uses**

Neither *B. napus* nor *B. juncea* are known to establish in nature conservation land use areas (Groves et al. 2003; Salisbury 2000) so this land use was not included in this assessment.

Background: The Weed Risk Assessment (WRA) methodology is adapted from the Australian/New Zealand Standards HB 294:2006 National Post-Border Weed Risk Management Protocol. The questions and ratings (see table) used in this assessment are based on the South Australian Weed Risk Management Guide (Virtue 2004). The terminology is modified to encompass all plants, including crop plants.
Weeds are usually characterised by one or more of a number of traits, these including rapid growth to flowering, high seed output, and tolerance of a range environmental conditions. Further, they cause one or more harms to human health, safety and/or the environment. Although *B. napus* and *B. juncea* have some traits associated with weeds and are agricultural and ruderal weeds in Australia, they are not considered as invasive weeds (Groves et al. 2003). Other than agricultural areas where they are cultivated, *B. napus* and *B. juncea* are common along the roadsides and railway lines that have acted as routes for their transportation. These two species are also commonly found in areas used for manufacture (crushing for oil or condiment production), intensive animal production areas that use *B. napus* or *B. juncea* meal as feed stock, around storage areas (grain elevators, inland termini) and occasionally in or near residential areas (particularly along transport routes). Less commonly, they might be found in areas used for intensive horticulture where disturbed land and good growing conditions may occur.
B. juncea is closely related to *B. napus* and the two species can hybridise under natural conditions (Bing et al. 1991; Jorgensen et al. 1996).
Unless specific work is cited, the information provided below is taken from the document *The Biology of* Brassica napus *L. (canola)* and *B. juncea* (L.) Czern. & Coss (Indian mustard) v2.1.

Risk rating for this WRA is conducted according to (Johnson 2009).

This WRA is for non-GM *B. napus* and non-GM *B. juncea* volunteers and includes non-GM herbicide resistant varieties of these crops. References made to *B. napus* and *B. juncea* as cultivated crops are only to inform their assessments as volunteers.

Invasiveness Questions	*B. napus*	*B. juncea*
1. What is the species' ability to establish amongst existing plants in the land use?	Rating: Low, i.e. seedlings mainly need bare ground to establish including removal of stubble/leaf litter. These conditions occur after major disturbances such as cultivation, overgrazing, hot fires, grading, long-term floods or long droughts. *B. napus* is a domesticated crop which grows optimally under managed agricultural conditions, such as high soil fertility, adequate moisture and low plant competition commonly found in ***dryland & irrigated cropping areas***. *B. napus* is known to establish as a volunteer in these areas, taking advantage of disturbed land due to cultivation and sowing. *B. napus* can establish in ***intensive use area***. It can establish in field margins, along roadsides and railway lines, where there has been moderate disturbance to existing vegetation (e.g. mowing or grading) or in areas of more open vegetation. *B. napus* has a low ability to establish in these areas because, under these suboptimal conditions, it has - poor fitness with reduced recruitment - low survivorship - poor competitive ability - low seed production Intensive horticulture areas may provide an optimal growing environment for *B. napus*; it may establish between the rows of desired species. However, areas of intensive horticulture are not used for *B. napus* production so it is unlikely to build up a seedbank.	Rating: Low, i.e. seedlings mainly need bare ground to establish including removal of stubble/leaf litter. These conditions occur after major disturbances such as cultivation, overgrazing, hot fires, grading, long-term floods or long droughts.*B. juncea* is a domesticated crop which grows best under managed agricultural conditions. It is cultivated in ***dryland & irrigated cropping areas***, but on a much smaller scale than *B. napus* (the area planted to *B. juncea* is approximately 2% of that planted to *B. napus*). *B. juncea* is known to establish as a volunteer in these areas taking advantage of disturbed land due to cultivation and sowing. *B. juncea* can establish in ***intensive use area***. It can establish in field margins, along roadsides and railway lines, where there has been moderate disturbance to existing vegetation (e.g. mowing or grading) or in areas of more open vegetation. *B. juncea* has characteristics that may enhance its ability to establish, such as - enhanced seedling vigour compared to *B. napus* - ability to form a ground cover relatively quickly - blackleg resistance - higher resistance to drought and high temperature than *B. napus* However, it also has other attributes reducing its ability to establish, such as - shatter resistance - small seed size - thin seed coat in yellow-seeded varieties Intensive horticulture areas may provide an optimal growing environment for *B. juncea*; it may establish between the rows of desired species. However, areas of intensive horticulture are not used for *B. juncea* production so it is unlikely to build up a seedbank. *B. juncea* is not considered competitive and volunteers are found less frequently in subsequent crops compared to *B. napus* (CFIA 2007; Oram et

Invasiveness Questions	*B. napus*	*B. juncea*
		al. 2005).
2. What is the species' tolerance to average weed management practices in the land use?	Rating: Low, i.e. less than 5% of plants survive. As a crop, *B. napus* is generally cultivated in rotation with cereals or legumes. Consequently, in ***dryland & irrigated cropping areas***, average weed management practices control *B. napus* volunteers in cereal/legume rotations. 75% of non-GM *B. napus* canola production in Australia is herbicide-tolerant but there are no reports of tolerance to average weed management. However, some *B. napus* seeds may germinate after herbicides have been broken down and volunteers may become established. *B. napus* seed can spill during transport, which may result in populations of *B. napus* along roadsides and railway lines or other ***intensive use areas*** where seed is loaded/unloaded, stored or processed. Standard weed management in these areas include herbicide application and/or mechanical control (e.g. mowing, slashing) and these would minimise seed set.	Rating: Low, i.e. less than 5% of plants survive. *B. juncea* is generally cultivated in rotation with cereals or legumes. Consequently, in ***dryland & irrigated cropping areas***, average weed management practices control *B. juncea* volunteers in cereal/legume rotation. There are no reports of tolerance to average weed management. However, some *B. juncea* seeds may germinate after herbicides have been broken down and volunteers may become established. *B. juncea* seed can spill during transport, which may result in populations of *B. juncea* along roadsides and railway lines or other ***intensive use areas*** where seed is loaded/unloaded, stored or processed. Standard weed management practices in these areas include herbicide application and/or mechanical control (e.g. mowing, slashing) and these would minimise seed set.
3. Reproductive ability of the species in the land use:		
3a. What is the time to seeding in the land uses?	Rating: <1 year *B. napus* is an annual crop and generally takes at most seven months to complete its life cycle under standard agricultural conditions of ***dryland & irrigated cropping areas***. The lifecycle is similar in other land uses. However, stresses such as competition or drought may hasten reproduction and shorten the lifecycle.	Rating: <1 year *B. juncea* is an annual crop and generally takes less than seven months[a] to complete its life cycle under standard agricultural conditions of ***dryland & irrigated cropping areas.*** The lifecycle is similar in other land uses. However, stresses such as competition or drought may hasten reproduction and shorten the lifecycle.
3b. What is the annual seed production in the land use per square metre?	Rating: High, i.e. more than 1000 seeds per m^2. As a crop grown under optimal conditions, *B. napus* average yield in Australia is 132g/m^2, or 38280[b] seeds/m^2, assuming an average weight of 3.44 mg per seed. At a recommended rate of about 70 plants/m^2, this represents a yield of about 550 seeds per plant. Harvest seed loss has been measured as 1.5-8.5% of total yield, equivalent of 575-3030 seeds/m^2. Volunteers will generally not occur at the density of cultivated plants in ***dryland & irrigated cropping areas***, due to standard weed management	Rating: High, i.e. more than 1000 seeds per m^2. As a crop plant grown under optimal conditions, *B. juncea* average yield in Australia is 100g/m^2, or 40,000[c] seeds/m^2, assuming an average weight of 2.5 mg per seed. At a recommended rate of about 70 plants/m^2, this represents a yield of about 570 seeds per plant. *B. juncea* is less prone to pod shatter compared to *B. napus* and does not need windrowing, reducing the risk of seed loss. However, it is still likely that approximately 1000 seeds/m^2 remain in the field after harvest.

[a] In Western Australia, mustard lines tested can reach maturity in 4.5 to 5 months (Gunasekera et al. 2001; Oram et al. 2005).
[b] This figure is based on an average 1.32 t/ha yield over the period 2013-2016 (ABARES 2015).
[c] This figure is based on a 1 t/ha yield.

Invasiveness Questions	*B. napus*	*B. juncea*
	practices in subsequent crops. The seed production of volunteers is likely <1000 seeds/m^2. Seed production of volunteers in ***intensive use areas*** is expected to be reduced due to poor competitiveness and suboptimal conditions. According to Agrisearch (2001), the average distance between two volunteer plants along roadsides is of 2.6 m. Seed production may be or exceed 1000 seeds/m^2.	Volunteers will generally not occur at the density of cultivated plants in ***dryland & irrigated cropping areas***, due to standard weed management practices in subsequent crops. The seed production of volunteers may be <1000 seeds/m^2. *B. juncea*'s adaptation to low soil moisture and hot temperatures may enhance survival and seed set in ***intensive use areas***. While seed production in these areas where *B. juncea* is present is expected to be reduced due to poor competitiveness and suboptimal conditions, it is likely to exceed 1000 seeds/m^2.
3c. Does the species reproduce vegetatively?	No	No
4. Long distance dispersal (more than 100 m) by natural means in land uses:		
4a. Are viable plant parts dispersed by flying animals (birds and bats)?	Rating: Occasional Birds can shred or remove pods during development and at maturity. However, it is uncertain if the seeds or pods are dispersed more than 100 m from the source plant. If consumed, some seed may remain viable after passing through the digestive tract of birds and be dispersed further. Viable seeds were only found in faeces from wood ducks, representing less than 0.01% of ingested seeds. Omnivorous/herbivorous species such as ducks are less efficient at digesting seeds compared to most obligate seed-eaters. Parrots are even less likely to pass viable seed because they generally dehusk seeds and consume only the kernel. Therefore, it is likely that dissemination of *B. napus* seed by wild birds consuming seed directly from a crop would be very low. Dispersal by bats is not reported.	Rating: Occasional Specific information for dispersal of *B. juncea* by flying animals is not available. The assumption for this question is that *B. juncea* is dispersed by birds as described for *B. napus*. However, *B. juncea* has a thinner seed coat and thus viability of seed after digestion may be further reduced. Dispersal by bats is not reported.
4b. Are viable plant parts dispersed by wild animals other than birds and bats?	Rating: Unlikely to occasional Wild animals may feed on *B. napus* plants and disperse viable seed in their faeces or transport it in wool/fur or muddy hooves. Whether seed can pass through the gut of wild animals and remain viable is currently unknown. However, up to 1% of *B. napus* seed remains viable after ingestion by sheep and this may be true for other animals.	Rating: Unlikely to occasional Specific information for dispersal of *B. juncea* by wild animals is not available. The assumption for this question is that *B. juncea* is dispersed by wild animals via the same mechanisms as *B. napus*. However, *B. juncea* has a thinner seed coat and thus viability of seed after digestion may be further reduced.

Invasiveness Questions	*B. napus*	*B. juncea*
4c. Are viable plant parts dispersed via water?	Rating: Occasional Dispersal by water is possible but no data is available for *B. napus* or other *Brassica* species. Seeds may be transported as bed load sediment in rivers and creeks. However it is highly unlikely that seed would be carried to areas favourable for establishment. *B. napus* seed is unlikely to remain viable after prolonged exposure to water. Heavy rains or flooding could transport canola seed which remained on the soil surface after harvest. If flooding was not prolonged and displaced seed did not become waterlogged, canola seed would likely germinate. However, in flooded or waterlogged soil, the lack of oxygen for cell respiration would impair germination. Even if germination occurred, the survival of any seedling would be jeopardized due to a reduction in nutrient uptake.	Rating: Occasional Dispersal by water is possible but no data is available for *B. juncea* or other *Brassica* species. Seeds may be transported as bed load sediment in rivers and creeks. However it is highly unlikely that seed would be carried to areas favourable for establishment. *B. juncea* seed is unlikely to remain viable after prolonged exposure to water. Heavy rains or flooding could transport residual canola seed which remained on the soil surface after harvest. If flooding was not prolonged and displaced seed did not become waterlogged, canola seed would likely germinate. However, in flooded or waterlogged soil, the lack of oxygen for cell respiration would impair germination. Even if germination occurred, the survival of any seedling would be jeopardized due to a reduction in nutrient uptake.
4d. Are viable plant parts dispersed via wind?	Rating: Unlikely to occasional Dispersal by wind is possible but no data is available for *B. napus* or other Brassica species. Windrows of *B. napus* plant material including seed may be blown into adjacent fields by high winds. The dispersal distance will depend on the wind strength, the amount of trash on the ground and the moisture content of the seeds. Dispersal beyond 100 m is possible. However, given that the pod is prone to shatter, seed would likely be dispersed at relatively short distances.	Rating: Unlikely to occasional Dispersal by wind is possible but no data is available for *B. juncea* or other Brassica species. *B. juncea* is harvested and processed directly in the field. This is likely to reduce dispersal of seed by wind into distant fields. However, plant material including seed may be blown into adjacent fields by high winds. The dispersal distance will depend on the wind strength, the amount of trash on the ground that could trap the seeds and the moisture content of the seeds. Dispersal beyond 100 m is possible. Dispersal distance would depend on wind strength, amount of trash on the ground and moisture content of the material.
5. Long distance dispersal (more than 100 m) by human means in land uses:		
5a. How likely is deliberate spread by people?	Rating: Common *B. napus* is a crop species purposely introduced for production in ***dryland & irrigated cropping areas***.	Rating: Common *B. juncea* is a crop species purposely introduced for production in ***dryland & irrigated cropping areas***.
5b. How likely is accidental spread by people, machinery and vehicles?	Rating: Common in/from dryland & irrigated cropping areas and unlikely in/from intensive use area In ***dryland & irrigated cropping areas***, *B. napus* seed is commonly accidentally dispersed by people, machinery and vehicles. This is due to the high number of seeds produced per m^2 and the small seed size. Contamination of harvest machinery and vehicles is likely common. Accidental spread of *B. napus* in following crops occurs less often as the	Rating: Common in/from dryland & irrigated cropping areas and unlikely in/from intensive use area In ***dryland & irrigated cropping areas***, *B. juncea* seed is commonly dispersed by people, machinery and vehicles. This is due to the high number of seeds produced per m^2 and the small seed size. It is assumed, that like *B. napus*, contamination of harvest machinery and vehicles is likely common. Accidental spread of *B. juncea* in following crop seed occurs less often as the

Invasiveness Questions	*B. napus*	*B. juncea*
	number of *B. napus* volunteers would be minimised by standard weed management. *B. napus* seed is accidentally spread *via* transport along roadsides and railway lines. Accidental spread by people, machinery and vehicles would be unlikely in or from ***intensive use areas*** as these areas would typically have low *B. napus* population density. Furthermore, management practices such as mowing or herbicide application would reduce or eliminate *B. napus* seed production.	number of *B. juncea* volunteers would be minimised by standard weed management. *B. juncea* seed is accidentally spread *via* transport along roadsides and railway lines. Accidental spread by people, machinery and vehicles would be unlikely in or from ***intensive use areas*** as these areas would typically have low *B. juncea* population density. Furthermore, management practices such as mowing or herbicide application would reduce or eliminate *B. juncea* seed production.
5c. How likely is spread via contaminated produce?	Rating: Common in/from dryland & irrigated cropping areas and occasionally in/from intensive use areas In ***dryland & irrigated cropping areas*** contamination is common: *B. napus* seed may be sown with the seed of the following crop. The amount of *B. napus* seed present as a contaminant would depend on the efficiency of weed management as well as harvest and seed cleaning practices. Long distance dispersal via contaminated hay and forage may also occur occasionally in or from ***intensive use areas***. This could occur from areas purposely producing hay/forage or if roadside vegetation were cut for this purpose.	Rating: Common in/from dryland & irrigated cropping areas and occasionally in/from intensive use areas In ***dryland & irrigated cropping areas*** contamination is common: *B. juncea* seed may be sown with the seed of the following crop. The amount of *B. juncea* seed present as a contaminant would depend on the efficiency of weed management as well as harvest and seed cleaning practices. Long distance dispersal via contaminated hay and forage may also occur occasionally in or from ***intensive use areas***. This could occur from areas purposely producing hay/forage or if roadside vegetation were cut for this purpose.
5d. How likely is spread via domestic/farm animals?	Rating: Common In ***intensive use areas*** such as feedlots or if livestock were to graze ***dryland & irrigated cropping area*** paddocks close to seed set, it is likely that some viable seed might be spread on muddy hooves or in wool/fur. *B. napus* seed and meal can make up a small portion of livestock feed. Up to 1% of *B. napus* seed remains viable after ingestion by sheep. *B. napus* seed meal contains a small amount of viable seed; thus, for sheep fed *B. napus* meal, the amount of viable seed excreted would be extremely low. Whether seed can pass through the gut of other domestic/farm animals and remain viable is currently unknown. Long distance dispersal of viable seed via domestic/farm animals from all the relevant land use areas commonly occurs. However, where *B. napus* grows as a volunteer, it would be managed like other agricultural weeds. In these suboptimal growing conditions, fewer seeds are expected to be produced per plant than when *B. napus* is cultivated as a crop.	Rating: Common Specific information on *B. juncea* is not available. For this question, it is assumed that spread via domestic/farm animals will be similar to that for *B. napus* seed. However, *B. juncea* has a thinner seed coat than *B. napus*, thus it may not remain viable after consumption. The area planted to *B. juncea* is considerably less than that planted to *B. napus*, thus dispersal of viable *B. juncea* seed via domestic/farm animals would occur less frequently compared to *B. napus*. Long distance dispersal of viable seed via domestic/farm animals from all the relevant land use areas commonly occurs. However, where *B. juncea* grows as a volunteer, it would be managed like other agricultural weeds. In these suboptimal growing conditions, fewer seeds are expected to be produced per plant than when *B. juncea* is cultivated as a crop.

Impact Questions	*B. napus*	*B. juncea*
6. Does the species reduce the establishment of desired plants?	Rating: Reduces establishment by <10% Typically *B. napus* establishes where land has been disturbed and in these areas it may impact on the establishment of desired species. The desired species in ***dryland & irrigated cropping areas*** and in intensive horticultural areas are crop plants. These areas are subject to standard weed management practices which would minimise the impact of *B. napus* volunteers on the establishment of desired plants. *B. napus* is a poor competitor. In ***intensive use areas*** such as along roadsides the desired species may be perennial grasses, clover species or remnant vegetation with high ecological value (Rural City of Wangaratta 2011). These species may serve as food sources and shelters for native & non-native fauna. However, roadside vegetation is managed for two main reasons: - the removal of noxious or invasive weeds - the removal of obstructions to line of sight around corners and signs Thus roadside management may focus on safety and removal of specific plants, rather than protection of desired plants.	Rating: Reduces establishment by <10% Typically *B. juncea* establishes where land has been disturbed and in these areas it may impact on the establishment of desired species. The desired species in ***dryland & irrigated cropping areas*** and in intensive horticultural areas are crop plants. These areas are subject to standard weed management practices which would minimise the impact of *B. juncea* volunteers on the establishment of desired plants. *B. juncea* is a poor competitor. In ***intensive use areas*** such as along roadsides the desired species may be perennial grasses, clover species or remnant vegetation with high ecological value (Rural City of Wangaratta 2011). These species may serve as food sources and shelters for native & non-native fauna. However, roadside vegetation is managed for two main reasons: - the removal of noxious or invasive weeds - the removal of obstructions to line of sight around corners and signs Thus roadside management may focus on safety and removal of specific plants, rather than protection of desired plants.
7. Does the species reduce the yield or amount of desired vegetation that does establish?	Rating: Reduces yield/amount by <10% As discussed in question 6, *B. napus* has a low impact on the establishment of desired species in the relevant land use areas. *B. napus* is no more competitive than *B. juncea*, suggesting that in dryland & irrigated cropping *area*, under standard weed management practices, *B. napus*'s negative impact on following crop yield would be very low. Studies show that the root system of *B. napus* has beneficial effects on soil structure and soil moisture infiltration, resulting in higher yield and protein levels in the following cereal crop. In ***intensive use areas*** such as horticulture, standard weed management would minimise crop loss. For other areas such as roadsides or railway tracks, no information is available regarding desired species. However, as indicated in question 6, roadside management focuses on safety and removal of specific plants, rather than protection of desired plants.	Rating: Reduces yield/amount by <10% As discussed in question 6, *B. juncea* would have a low impact on the establishment of desired species in the relevant land use areas. Zerner & Gill (2011) showed that there was no significant impact on wheat yield (compared to weed free treatment) when *B. juncea* was grown at a density of 30 plants/m^2 in wheat fields without standard weed management[d]. In ***dryland & irrigated cropping area***, under standard management practices, *B. juncea*'s negative impact on following crop yield would be very low. *B. juncea*'s root system is considered to have similar beneficial effects on soil structure and soil infiltration as *B. napus*. Similarly, for ***intensive use areas*** such as horticulture, standard weed management would minimise crop loss. For other areas such as roadsides or railway tracks, no information is available regarding desired species.

[d] Observed yield loss ranged from 3 to 21% depending on wheat cultivars. However, these results were shown as not significantly different from those obtained in weed-free fields (Zerner & Gill 2011).

Impact Questions	*B. napus*	*B. juncea*
	Given that *B. napus* is not known to be competitive it is highly likely that it has a negligible impact on the amount of desired vegetation along roadsides. Roadside surveys in the major canola growing districts in Australia have shown that the incidence and density of volunteer *B. napus* is low.	However, as indicated in question 6, roadside management focuses on safety and removal of specific plants, rather than protection of desired plants. Given that *B. juncea* is not known to be competitive it is highly likely that it has a negligible impact on the amount of desired vegetation along roadsides. Roadside surveys in the major canola growing districts in Australia have shown that the incidence and density of volunteer *B. juncea* is low.
8. Does the species reduce the quality or characteristics of products, diversity or services available from the land use or reduce habitats for desirable species?	Rating: Low, i.e. the plant slightly reduces product quality, lowering its price but still passing as a first grade produce. For natural vegetation, the plant has only marginal effects on biodiversity, but is visually obvious and degrades the natural appearance of the landscape. For residential areas, the plant causes negligible structural damage, but reduces the aesthetics of an area through untidy visual appearance and/or unpleasant odour. As discussed in questions 6 and 7 above, *B. napus* has a low impact on both the establishment and yield/amount of desired species. Generally there is no expectation that *B. napus* would reduce the quality or characteristics of products, diversity or services available from any of the land use areas discussed. Volunteer *B. napus* along roadsides has potential to grow to a height of 1.5 m. As noted in question 6, roadside vegetation is managed to remove noxious or invasive weeds and to maintain clear lines of site, so *B. napus* would be controlled if it impacted on these. The presence of *B. napus* may reduce aesthetics in residential areas.	Rating: Low, i.e. the plant slightly reduces product quality, lowering its price but still passing as a first grade produce. For natural vegetation, the plant has only marginal effects on biodiversity, but is visually obvious and degrades the natural appearance of the landscape. For residential areas, the plant causes negligible structural damage, but reduces the aesthetics of an area through untidy visual appearance and/or unpleasant odour. As discussed in questions 6 and 7 above, *B. juncea* has a low impact on both the establishment and yield/amount of desired species. Generally there is no expectation that *B. juncea* would reduce the quality or characteristics of products, diversity or services available from any of the land use areas discussed. Volunteer *B. juncea* along roadsides has potential to grow to a height of 2.5 m. As noted in question 6, roadside vegetation is managed to remove noxious or invasive weeds and to maintain clear lines of site so *B. juncea* would be controlled if it impacted on these. The presence of *B. juncea* may reduce aesthetics in residential areas.
9. What is the species' potential to restrict the physical movement of people, animals, vehicles, machinery and/or water?	Rating: None *B. napus* may grow in all the relevant land use areas as a volunteer at a low population density. No self-sustaining volunteer *B. napus* population has been reported under Australian conditions.	Rating: None *B. juncea* may grow in all the relevant land use areas as a volunteer at a low population density. No self-sustaining *B. juncea* population has been reported under Australia conditions.
10. What is the species' potential to negatively affect the health of animals and/or people?	Rating: Low, i.e. the plant can cause slight physical injuries or mild illness in people, for example hay fever or minor rashes, in livestock, and/or native animals, with no lasting effects. *B. napus* has been specifically bred for reduced levels of glucosinolates and erucic acid. Nonetheless, there are limits on the use of *B. napus* seed meal in livestock feed. Allergies to *Brassica* pollen have been reported but it has been suggested	Rating: Low, i.e. the plant can cause slight physical injuries or mild illness in people, for example hay fever or minor rashes, in livestock, and/or native animals, with no lasting effects. Modern varieties of *B. juncea* canola have been specifically bred for reduced levels of glucosinolates and erucic acid, as these toxins can have a negative impact on human and animal health. Nonetheless, there are limits on the use of *B. juncea* seed meal in livestock feed.

Impact Questions	*B. napus*	*B. juncea*
	that cross reactivity between *B. napus* and other allergens is the main explanation for allergies observed.	Allergies to *Brassica* pollen have been reported but it has been suggested that cross reactivity between *B. juncea* and other allergens is the main explanation for allergies observed.
11. Major positive or negative effect of the species on environmental health in the land use:		
11a. Does the species provide food and/or shelter for pathogens, pests and/or diseases in the land use?	Rating: Major positive and major negative effect In ***dryland & irrigated cropping areas*** *B. napus* is usually grown in rotation with wheat as the following crop. *B. napus* provides an important disease break during which the inoculums of cereal pathogens (such as the take-all fungus) decline. *B. napus* acts as a grass weed competitor, limiting pathogen reservoirs. An indirect effect on wheat pathogenic fungi has also been suggested: *B. napus* is thought to influence the composition of the rhizosphere's microbial communities, reducing fungal inoculum. This constitutes a major positive effect. Conversely, *B. napus* is subject to, and may harbour, numerous pests, pathogens and diseases which could affect other susceptible species. Although in ***dryland & irrigated cropping*** and ***intensive use areas*** the density of volunteer *B. napus* is expected to be low, in some years this population may provide a major source of pests, pathogens and diseases and this would constitute a major negative effect.	Rating: Major positive and major negative effect In ***dryland & irrigated cropping areas*** *B. juncea* is usually grown in rotation with wheat as the following crop. *B. juncea* provides an important disease break during which the inoculums of cereal pathogens (such as the take-all fungus) decline. *B. juncea* acts as a grass weed competitor, limiting pathogen reservoirs. An indirect effect on wheat pathogenic fungi has also been suggested: *B. juncea* is thought to influence the composition of the rhizosphere's microbial communities, reducing fungal inoculum. This constitutes a major positive effect. Conversely, *B. juncea* is also subject to, and may harbour, numerous pests, pathogens and diseases which could affect other susceptible species. Although in ***dryland & irrigated cropping*** and ***intensive use areas*** the density of volunteer *B. juncea* is expected to be low, in some years this population may provide a major source of pests, pathogens and diseases and this would constitute a major negative effect.
11b. Does the species change the fire regime in the land use?	Rating: Minor or no effect in all relevant land uses The number and density of *B. napus* volunteers is expected to be low for all relevant land uses, and would not be expected to affect fire regimes.	Rating: Minor or no effect in all relevant land uses The number and density of *B. juncea* volunteers is expected to be low for all relevant land uses, and would not be expected to affect fire regimes.
11c. Does the species change the nutrient levels in the land use?	Rating: Minor or no effect in all relevant land uses The number and density of *B. napus* volunteers is expected to be low for all relevant land uses, and would not be expected to affect nutrient levels.	Rating: Minor or no effect in all relevant land uses The number and density of *B. juncea* volunteers is expected to be low for all relevant land uses, and would not be expected to affect nutrient levels.
11d. Does the species affect the degree of soil salinity in the land use?	Rating: Minor or no effect in all relevant land uses The number and density of *B. napus* volunteers is expected to be low for all relevant land uses, and would not be expected to affect soil salinity.	Rating: Minor or no effect in all relevant land uses The number and density of *B. juncea* volunteers is expected to be low for all relevant land uses, and would not be expected to affect soil salinity.
11e. Does the species affect the soil stability in the land use?	Rating: Minor or no effect in all relevant land uses The number and density of *B. napus* volunteers is expected to be low for all relevant land uses, and would not be expected to affect soil stability.	Rating: Minor or no effect in all relevant land uses The number and density of *B. juncea* volunteers is expected to be low for all relevant land uses, and would not be expected to affect soil stability.
11f. Does the species affect the soil water table in the land use?	Rating: Minor or no effect in all relevant land uses The number and density of *B. napus* volunteers is expected to be low for all relevant land uses, and would not be expected to affect the soil water table.	Rating: Minor or no effect in all relevant land uses The number and density of *B. juncea* volunteers is expected to be low for all relevant land uses, and would not be expected to affect the soil water table.

Impact Questions	*B. napus*	*B. juncea*
11g. Does the species alter the structure of nature conservation areas by adding a new strata level?	Rating: Minor or no effect in all relevant land uses The number and density of *B. napus* volunteers is expected to be low for all relevant land uses, and would not be expected to add a new strata level.	Rating: Minor or no effect in all relevant land uses The number and density of *B. juncea* volunteers is expected to be low for all relevant land uses, and would not be expected to add a new strata level.