Australian Government
Department of Health and Aged Care
Office of the Gene Technology Regulator

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

Image: flowering canola plants. Photo courtesy of Brian Weir.

Version 2.2: July 2024

This document provides an overview of baseline biological information relevant to risk analysis of genetically modified forms of the species that may be released into the Australian environment.

This document is an update of Version 2.1 (February 2017).

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ABBREVIATIONS USED IN THIS DOCUMENT

ABCA	Agricultural Biotechnology Council of Australia
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
CRISPR	Clustered regularly interspaced short palindromic repeats
DNA	Deoxyribonucleic acid
DPI	Department of Primary Industry
e.	Expected
f.	Forecasted
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GRDC	Grains Research & Development Corporation
ha	Hectare
HOLL	High Oleic, Low Linolenic
IT	Imidazolinone tolerant
ITSA	International Seed Testing Association
kt	Kilo tonnes
max.	maximum
Mbp	Megabase pair
MT	Million tonnes
n	Haploid number of chromosomes
NGS	Next generation sequencing
NSW	New South Wales
NT	Northern Territory
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
Prod.	Production
Qld	Queensland
QTL	Quantitative Trait Locus
RFLP	Restriction Fragment Length Polymorphisms
RNA	Ribonucleic acid
SA	South Australia
SNP	Single Nucleotide Polymorphism
spp.	Species
T	Tonnes
Tas	Tasmania
TILLING	Target Induced Local Lesions in Genomes
TT	Triazine Tolerant
TuMV	*Turnip mosaic virus*
Vic	Victoria
WA	Western Australia

GLOSSARY

Term	*Definition*
Allelochemicals	Secondary metabolites which are not required for plant metabolism. They are often involved in plant defence against herbivores
Allelopathy	A biological phenomenon by which an organism produces one or more molecules that influence the growth, survival and reproduction of other organisms
Amphidiploids	Tetraploids containing the diploid chromosome set of both parents
Cleistogamy	The trait of certain plants to propagate by using non-opening, self-pollinating flowers
Diploid	An organism made up of cells containing 2 sets of chromosomes (2N). Most species whose cells have nuclei (eukaryotes) are diploid, meaning many of their cells have 2 sets of chromosomes—one set inherited from each parent
Environmental weeds	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
F_1, F_2…	F_1 are the (hybrid) offspring (generation) resulting from a cross between 2 parent individuals. If F_1 hybrids are crossed, the resulting offspring are the F_2 generation; if F_2 hybrids are crossed, the offspring are the F_3 generation and so on
Haploid	Cells or organisms having a single set of chromosomes (1N), such as the gametes of higher plants
Heterosis	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 (*heterotic* may be used as an adjective)
Hexaploid	An organism made up of cells containing 6 sets of chromosomes (6N)
Homologous	Having the same structure, relation or relative position, or evolution (Greek *homo* – the same). Homologous genes may have a similar, but not the same function
Homologous chromosomes	Chromosomes with the same or allelic genes with genetic loci usually arranged in the same order
Indeterminate	Plant growth that will continue to grow and flower until limited by abiotic factors such as temperature, water stress or nutrient availability
Interspecific	Existing, arising or occurring between species
Isohyet	A line on a geographical map connecting points having the same amount of rainfall in a given period
Multilines	Mixtures of lines differing in a specific disease or pest resistance and bred for phenotypic uniformity of agronomic traits
Napins	Proteins consisting of a small and large protein chain linked by disulphide bonds that are highly resistant to pepsin digestion or temperature/pH denaturation
Naturalised	Non-native species that have been introduced and become established, and that reproduce naturally in the wild
Phylogenetics	The study of the evolutionary history and relationships among individuals or groups of organisms

Polyploid	Cells or organisms containing more than 2 paired (homologous) sets of chromosomes. Polyploids (see below) are labelled according to the number of chromosome sets in the nucleus, with the letter N used to represent the number of chromosomes in a single set. Thus, a diploid would have 2N chromosomes, a tetraploid 4N and so on
Progenitor	An ancestor or parent of an organism
Provisional Tolerable Daily Intake	A permissible human daily exposure to contaminants associated with the consumption of otherwise wholesome and nutritious food (FSANZ 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
Quantitative Trait Loci	Genetic loci that correlate with variation in a given phenotype; often the abbreviation QTLs is used
Silique	2-celled elongated seed capsules (pods)
Sodicity	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
Syntenic genes	Conserved blocks of genes within sets of chromosomes that are being compared
Tetraploid	An organism made up of cells containing 4 sets of chromosomes (4N)
Variety	A group of cultivated plants of significance in agriculture, forestry or horticulture, which have distinct and heritable characteristics. Often used interchangeably with *cultivar*
Volunteers	Unwanted plants in succeeding crops emerging from the soil seedbank

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 analysis 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 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, rai or juncea canola). 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 in industry, such as animal feed. Worldwide, canola production is the second highest of oilseed crops after soybean (FAO, 2022, 2023) 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, Canada, China, and India (Livingston et al., 2009). Australia is a major exporter of canola, exporting an estimated 6.4 million tonnes of canola in 2022/2023 (ABARES, 2023b). Initial trials in Australia of *B. napus* and *B. rapa* began in the early 1960s, with the 2 crops first grown commercially in 1969. It was another decade before canola varieties became available. Today, commercial *B. napus* canola production occurs mainly in Western Australia, New South Wales, Victoria, and South Australia, with an area of 3.5 million hectares estimated to have been planted in 2023/2024 (ABARES, 2023b). The distribution of *B. napus* canola production coincides with the wheat belt, with *B. napus* often grown as a break crop between cereal rotations (GRDC, 2018a).

B. juncea 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 less than 10,000 ha planted annually in western Victoria, central New South Wales and/or South Australia (McCaffery, personal communication, 2022).

SECTION 1 TAXONOMY

1.1 Brassicaceae family

The *Brassicaceae* family consists of more than 350 genera and more than 4000 accepted species worldwide (World Flora Online, 2022). 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 was 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 or introduced *Brassicaceae* are present in Australia (Australian National Botanic Gardens, accessed July 2022). Species used as food crops are introduced and belong to the genus *Brassica*. Other introduced *Brassicaceae* include 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 and Cuthbertson, 2001).

Other introduced species of *Brassicaceae* are used as ornamental plants, such as *Arabis albida* (rock cress), *Cheiranthus cheiri* (wallflower) or *Iberis amara* (candytuft) (Parsons and Cuthbertson, 2001). Native Australian *Brassicaceae* are present in several genera, including *Arabidella*, *Blennodia*, *Cardamine*, *Lepidium* and *Stenopetalum* (Australian National Botanic Gardens, accessed July 2022).

1.2 Brassica genus

The *Brassica* genus consists of approximately 50 species worldwide (World Flora Online, 2022). 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; Jat et al., 2019). *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, 1926; U, 1935). *Brassica* species have 1 or 2 of 3 different types of haploid genomes, (A, B, and C), i.e. AA (*B. rapa*), BB (*B. nigra*), CC (*B. oleracea*), AABB (*B. juncea*), AACC (*B. napus*), and BBCC (*B. carinata*). It was proposed that *B. juncea* (2n=36), *B. napus* (2n=38) and *B. carinata* (2n=34) were natural amphidiploid 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, derived from multiple hybridisation events, with *B. oleracea* one of several maternal ancestors (Allender and King, 2010; Chalhoub et al., 2014). Interspecific hybridisation for *Brassica* species has been described as being unidirectional when happening naturally (Purty et al., 2008).

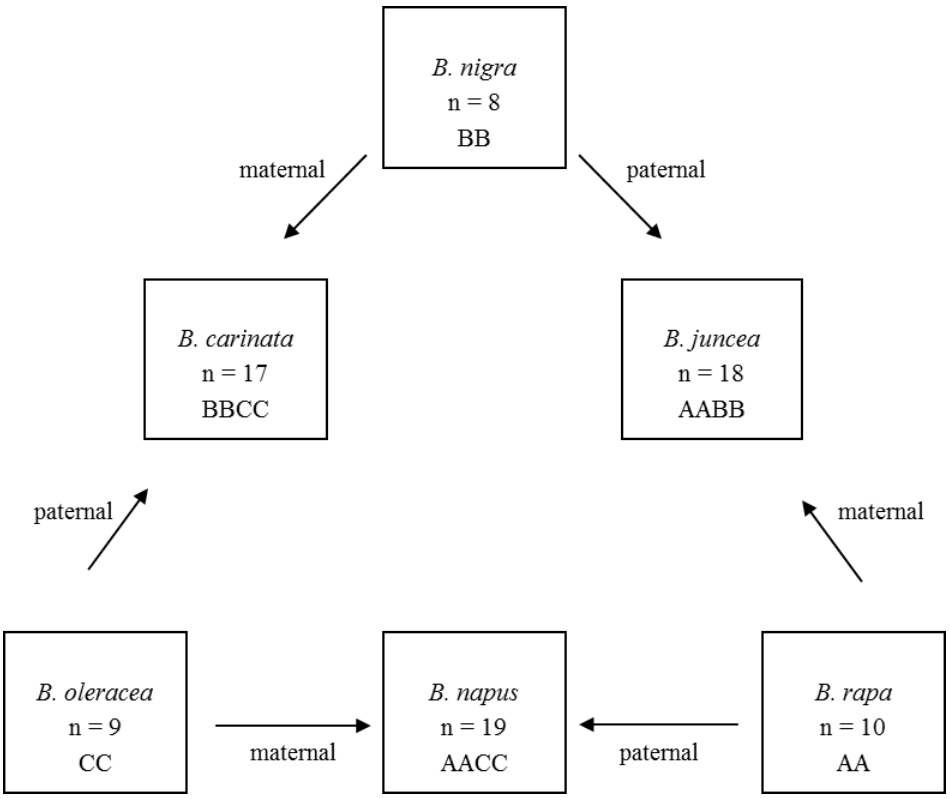

Figure 1: Genomic relationships between the main cultivated Brassica species, also known as U's triangle

According to Morinaga (1934) and U (1935), n refers to the haploid number of chromosomes. Adapted from 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 and 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* (japonica rice) are estimated to be 157 Mbp and 577 Mbp, respectively (Bennett and Leitch, 2011).

Linkage group identification studies have shown that *Brassica* species 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* (Wang and Fristensky, 2001; Lysak et al., 2005). This triplication event has been supported by identification of syntenic genes 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 (Lagercrantz, 1998; Johnston et al., 2005). 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 about 7.9 million years ago.

Genome rearrangements (chromosome fusion, inversions, non-reciprocal translocations) have been widely described in artificial (re-synthetised) amphidiploid *Brassica* (Song et al., 1990; Parkin et al., 1995; Allender

and King, 2010). Panjabi et al. (2008) have shown that natural allopolyploid *Brassica* species 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 in China (Wu et al., 2009; OECD, 2012). *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 (Gomez-Campo and Prakash, 1999; Edwards et al., 2007; Chen et al., 2013b). Afghanistan (and adjoining regions) has been described as a primary centre of origin for oilseed forms (Chen et al., 2013b). China, where the largest diversity of subspecies is observed, is considered as a probable primary centre for vegetable types (Wu et al., 2009; OECD, 2012). India, Pakistan and Asia Minor have been described as secondary centres. Using Simple Sequence Repeat, Amplified Fragment Length Polymorphism (AFLP) and Sequence Related Amplified Polymorphism, it was demonstrated that oilseed varieties cultivated in China, India, Europe, Australia, Japan and Canada could be divided into two genetically distinct groups. 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 (Srivastava et al., 2004; Wu et al., 2009; Chen et al., 2013a).

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. There is no reference to *B. napus* in the ancient literature, unlike *B. rapa* and *B. juncea*. The first record of cultivation of rapeseed in Europe dates back to the Middle Ages but it is not clear if the species grown was *B. napus* or *B. rapa (Appelqvist and 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 and Ohlson, 1972; Daun et al., 2015).

Two main components of *Brassica* plant material are erucic acid, a 22-carbon monounsaturated fatty acid, and glucosinolates, which are allelochemicals. *Brassica* ssp. seeds naturally contain up to 40% erucic acid and more than 60 micromoles per gram of glucosinolates (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; Section 5).

Both forage and vegetable varieties of *B. napus* and of *B. rapa* were introduced to North and South America in the 18th century. The oilseed form of *B. rapa* 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. 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, **Can**adian **o**il, **l**ow **a**cid (Eskin, 2013; Fleury, 2013).

B. juncea canola varieties are more recent, dating back to 2002. *B. juncea* canola cultivars show good growing characteristics, less pod shattering and more drought tolerance 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

The world oil crop production for the 2022/2023 growing season was estimated at 640.9 million tonnes (MT), and is forecast to reach 666.7 MT in 2023/2024, with forecast production of 89.2 MT for rapeseed[1] (FAO, 2023). The 4 major production areas for rapeseed oil are; the European Union, Canada, China, and

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

India, with each producing approximately 12.5 to 20 MT of rapeseed oilseed in 2023/2024 (USDA, 2023, 2024). Rapeseed represents 13.3% of oil production worldwide and is the second largest oil producing crop after soybean (which accounts for 60% of total oil production) (USDA, 2024). Global production of rapeseed for oil reached a record high of around 89 MT in 2022/2023, increasing by 17.2% compared to the previous year (FAO, 2023) and is estimated to remain at around 88 MT 2023/2024 (USDA, 2024).

This increased rapeseed oilseed production coincided with increased consumption. As mentioned, 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 the main rapeseed produced in India, representing 90% of rapeseed production and one third of total oil production (Kumar et al., 2009; Jat et al., 2019). The oil from *B. juncea* is used for cooking, while whole seeds and 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 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). Australian exports of canola oil to the EU biodiesel market account for approximately 75% of total canola exports (CSIRO, 2019). The EU biodiesel market prefers non-GM canola which complies with strict restrictions on greenhouse gas emissions. As Australia's canola is minimum to no-till (preserving soil carbon and reducing nitrous oxide emissions) and is mainly rain-fed rather than reliant on irrigation, Australia has a competitive edge over most other canola suppliers of non-GM canola (CSIRO, 2019; AOF, 2022).

B. juncea has been described as a potential tool for phytostabilisation for metal-contaminated soils (Perez-Esteban et al., 2014). See Section 6.1.2.

Canola meal is the second major oilseed meal produced worldwide (second to soybean meal), with around 48.6 MT produced in 2022/2023 (USDA, 2024). It is widely used as animal feed, e.g. for dairy cattle, pig and poultry (Daun et al., 2015). It is also considered as a potential substitute to fish meal for fish farms (Enami, 2011). Industry standards require canola meal to be low in glucosinolates (up to 30 micromoles per gram of seed) and erucic acid (less than 2%) to be suitable for animal feed (AOF, 2007; CODEX, 2009; Canola Council of Canada, 2019).

Canola meal is also used as a fermenting substrate for the production of industrial enzymes, such as phytases or xylanases used in food, paper or biofuel production (Bonnardeaux, 2007; Daun et al., 2015; Konkol et al., 2019; Tene Tayo et al., 2022).

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, although hay quality can vary and quality testing is recommended before using as a feed (GRDC, 2018b).

2.3 Cultivation in Australia

Canola is the major broadleaf crop in temperate cereal rotations and the third largest broad acre crop after wheat and barley, representing approximately 85% of Australia's oilseed production in 2021/2022 (ABARES, 2023b). 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) (Figure 2) (ABARES, 2023b). Since 1980, the area canola has been produced on has increased from 49,700 ha to 3.9 million hectares (ha) (ABARES, 2023b), and this has coincided with increased canola production, domestic consumption and exports.

In 2021/2022 and 2022/2023, Australia's canola production reached record highs of 6.8 and an expected 8.3 MT, respectively (ABARES, 2022, 2023b). The major domestic demands from canola are for oil and meal with canola oil predominately used as a food-grade oil source and the meal as a high protein feed for livestock and fish. Domestic uses in 2020/2021 and 2021/2022, were estimated to average a little more than 1.2 MT (ABARES, 2024).

Australia exports a large volume of its canola, mainly to the EU, Japan and China. Between 2016/2017 and 2019/2020 Australia exported an average of 2.3 MT at a value of $1.4 billion per year (AEGIC, 2021). In the years of 2021/2022 and 2022/2023, Australia's canola exports more than doubled to 5.6 million and an expected 6.4 MT, respectively (FAO, 2023), at a value of $6.49 and $5.91 billion (ABARES, 2023a), accounting for roughly 80% of Australia's total canola production.

There is limited information available for *B. juncea* production in Australia. In 2022, it was estimated that *B. juncea* was grown over an area of less than 10,000 ha in NSW and western Vic (McCaffery personal communication 2022).

2.3.1 Commercial propagation

Both *B. napus* and *B. juncea* reproduce via seeds. Modern cultivars are mostly F_1 hybrids but the Australian industry started mainly with open-pollinated genotypes (Lemerle et al., 2014). Open-pollinated cultivars were estimated to represent 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). In 2015, it was estimated that up to 40% of total canola seeds were retained by farmers, mainly for conventional, open pollinated cultivars (N. Goddard, personal communication, 2015[2]).

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 (OECD, 2022) and International Seed Testing Association (ITSA) (ITSA, 2022). Australia also has its own seed certification scheme, following the same rules as those for the OECD Seed Scheme. The Australian Seeds Authority (ASA; accessed 21 May 2024) administers the OECD and Australian Seed Certification Schemes.

Seed certification is a 4-step process. Breeders' seed is sown to produce pre-basic seed, which is 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* spp., the land used to produce seeds must not have grown another *Brassica* spp. crop (unless it was the same variety and certification class) for the previous 3 years to produce certified seed, or the previous 5 years to produce basic seed. 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 200 m and 100 m, respectively (Seed Services Australia, 2020). To meet 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 kilogram (ASA, 2011; Seed Services Australia, 2020).

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 (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 (GRDC, 2018a). Canola production was 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). Due to its strong competition with weeds, canola is also an important tool in the management of herbicide resistance in weeds by reducing reliance on herbicides (Matthews et al., 2022).

[2] Nick Goddard is a former Executive Director of the Australian Oilseeds Federation.

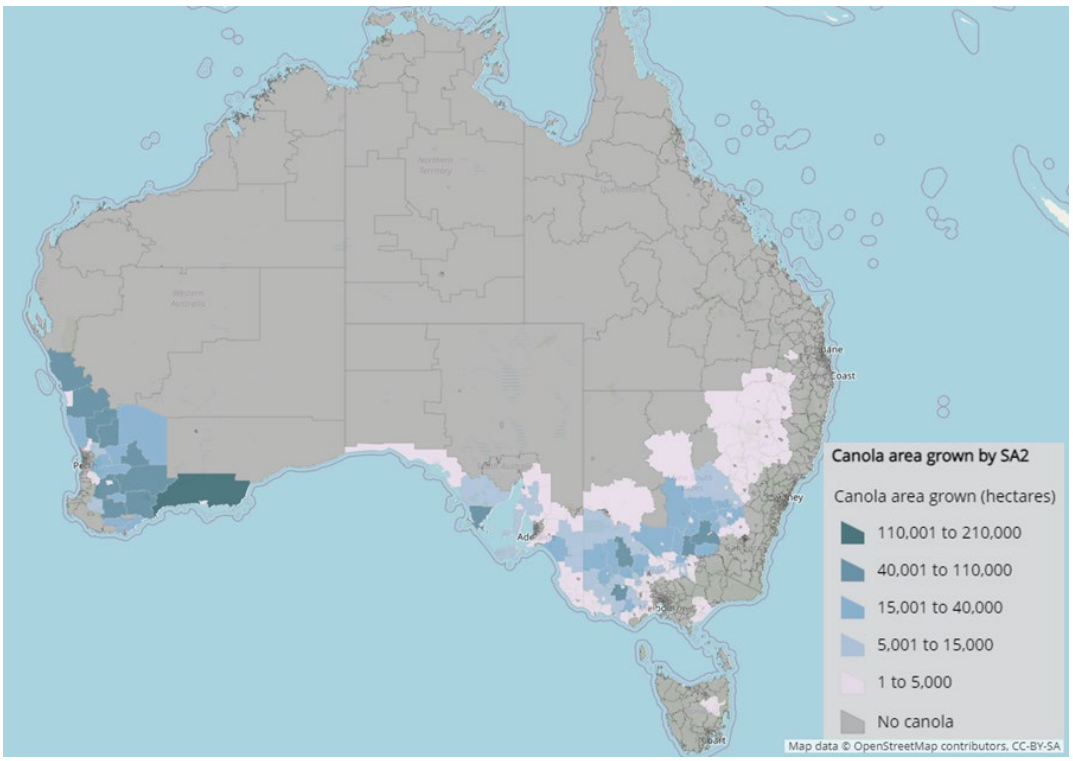

Figure 2. Canola areas grown in Australia based on 2019/2020 experimental regional estimates (Statistical Area 2/SA2)

According to the Australian Bureau of Statistics (accessed on 21 May 2024)

Canola production areas have grown significantly in Australia from 49,700 ha in 1989/1990 to an estimated 3.9 and 3.5 million ha in 2022/2023 and 2023/2024, respectively (ABARES, 2023b). Throughout this time, canola production volumes have also generally increased (Table 1). As with any agricultural crop, the area planted and seed production can fluctuate from year to year. Further, for any year, national figures can hide wide variations within and between states.

ABARES (2023b) reported that the 5-year national average to 2022/2023 saw 4.9 MT of canola produced over 2.74 million ha with approximately:

- 46.5% of the production in WA
- 24.1% in NSW
- 20.6% in Vic
- 8.5% in SA.

While canola production is forecast to drop in 2023/2024, WA is forecast to remain the predominant producer of Australia's canola, followed by NSW, Vic and SA (see Table 1) (ABARES, 2023b) with overall canola production forecast to account for 11.5% of the total area of Australia planted with winter crops.

Table 1: Canola area and production in Australian states between 2019 and 2024

Year	WA		NSW		Vic		SA	
	area	prod.	area	prod.	area	prod.	area	prod.
	'000 ha	kt	'000 ha	kt	'000 ha	kt	'000 ha	kt
2019–20	1 148.2	1 117.1	327.1	206.2	404.6	731.1	152.6	241.5
2020–21	1 183.6	1 689.3	731.0	1 532.2	493.9	1 127.1	202.7	402.3
2021–22	1 512.6	2 953.8	940.7	2 114.1	569.1	1 302.8	221.9	434.9

Year	WA		NSW		Vic		SA	
	area	prod.	area	prod.	area	prod.	area	prod.
	'000 ha	kt	'000 ha	kt	'000 ha	kt	'000 ha	kt
2022–23	2 100.0	4 300.0	900.0	1 800.0	600.0	1 382.5	290.0	770.0
2023-24	1 800.0	2 500.0	840.0	1 095.0	550.0	1 075.0	285.0	475.0
5-year average*	1 414.8	2 281.3	660.8	1 183.1	496.4	1 010.9	207.2	418.3

Adapted from ABARES (2023b). Western Australia (WA), New South Wales (NSW), Victoria (Vic), South Australia (SA), Production (prod.), hectares (ha), estimate (e), forecast (f), *until 2022-2023.

Some Australian states have a 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 51 canola varieties available in 2022, 13 being newly released varieties (Matthews et al., 2022). Further information guides published by seed companies and DPIs also provide data on canola variety characteristics including mean seed yields, pest resistance, and agronomic statistics such as most suitable rainfall regimes.

Information on new *B. napus* and *B. juncea* varieties currently being trialled in Australia can be found at the National Variety Trial Online (accessed on 21 May 2024). Canola varieties are currently classified based on herbicide tolerance:

- conventional (non-genetically modified (non-GM), not tolerant to any major herbicide)
- triazine tolerant (non-GM, triazine tolerant: tolerant to group 5 herbicides, i.e. inhibitors of photosystem II)
- imidazolinone tolerant (non-GM, Clearfield®: tolerant to group 2 herbicides, i.e. inhibitors of acetolactate synthase)
- glyphosate tolerant (GM, Roundup Ready®, TruFlex®: tolerant to group 9 herbicides, i.e. inhibitors of EPSP synthase)
- glufosinate tolerant (GM, Liberty link®: tolerant to group 10 herbicides, i.e. inhibitors of glutamine synthetase).

Varieties that are tolerant to multiple herbicides are known as “stacked” varieties.

Forty six percent of canola grown in 2023/2024 are GM canola varieties (ABCA, 2022; Bayer, 2024).

2.3.3 Efforts to expand B. napus and B. juncea growing regions

2.3.3.1 Cultivation of B. napus canola in northern NSW and southern Qld

Canola production in northern NSW and southern Qld started in the late 1980s but it took time for it to become established. Canola is now considered to play an important role in northern NSW cropping, particular in areas with higher rainfall and when followed by a crop of winter wheat (GRDC, 2017a). Variety selection and optimising sowing time are important factors for a successful canola crop in the northern region (GRDC, 2019).

2.3.3.2 Cultivation of B. napus canola in Western Australia

Canola production volume in WA, accounts for 40-50% of national production (ABARES, 2023b). In WA, canola was traditionally grown in areas of at least 450 mm rainfall, but it can also be grown profitably in the lower rainfall areas (approximately 325 mm) of the northern grain belt (Carmody and Cox, 2001). Expansion into lower rainfall areas has encouraged the selection of early maturing varieties (GRDC, 2015a). 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 and Cox, 2001).

2.3.3.3 B. juncea canola

B. juncea has been studied as a potential alternative 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 that of *B. napus*, or the two products can be blended (GRDC, 2009).

The first Australian *B. juncea* canola variety "Dune" was released in 2007, to be grown in low rainfall zones (Burton et al., 2007). However, due to lower oil content, it was recommended that farmers grow this *B. juncea* canola only where long-term average *B. napus* yields are less than 1.2 to 1.5 t/ha (Haskins et al., 2009; Hunt and Norton, 2011). Using cropping system models, these regions were 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 and Norton, 2011). The historic delineation boundary between *B. napus* and *B. juncea* areas essentially followed that of the 100 mm winter rainfall isohyet (Figure 3).

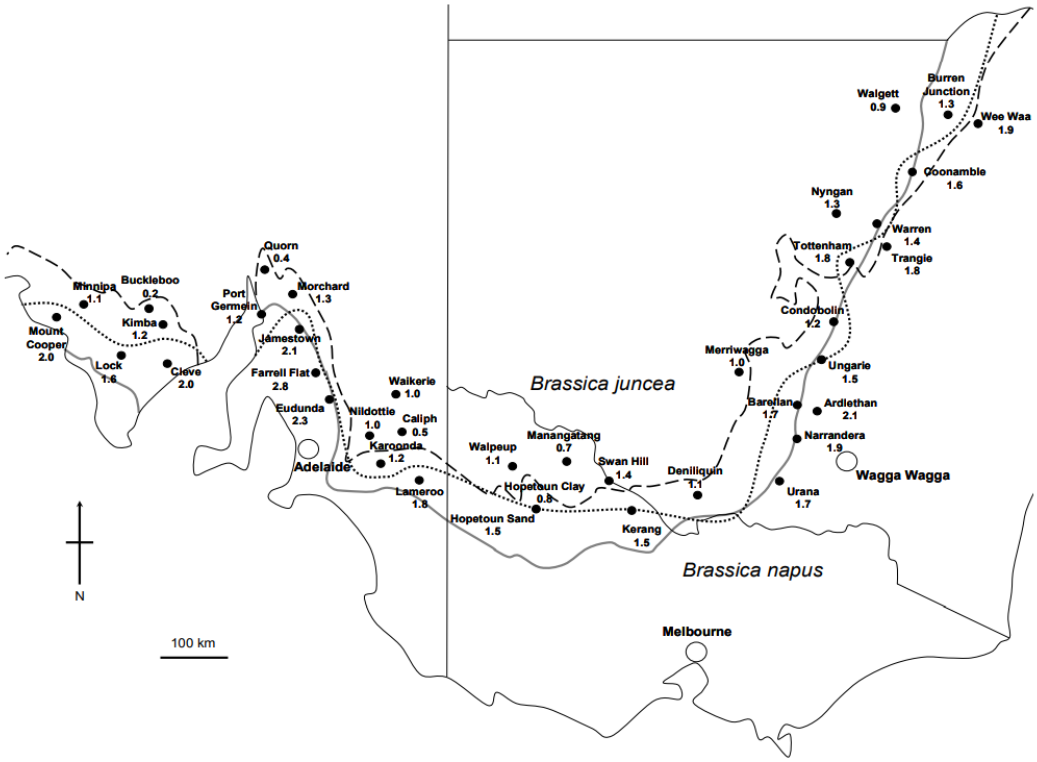

Figure 3: Proposed growing areas for B. juncea cultivation in the south-eastern region in Australia

Source: Hunt and 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 smaller seed size. *B. juncea* seed is smaller than *B. napus* under good growing conditions and can be even smaller and lighter under drought conditions. 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: prior to the commercial release of the first *B. juncea* variety in Australia, it was estimated that 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). A few years following *B. juncea's* commercial release, Potter (2011) suggested that new herbicide-tolerant, high-yield cultivars would be needed to compete with *B. napus* cultivars. Breeding of novel *B. juncea* canola varieties led to the release of the first herbicide and drought tolerant hybrid *B. juncea* canola variety in 2013. This cultivar was described as having a similar oil content, profile and quality to

B. napus canola (Matthews et al., 2015). More recently, in 2022, a GM glufosinate tolerant *B. juncea* variety was authorised for commercial release in Australia (OGTR, 2022).

2.3.4 Cultivation practices

2.3.4.1 Canola in crop rotations

Canola is considered the most profitable break crop available to grain growers in southern Australia. Canola can be grown every 4 years in cereal paddocks (GRDC, 2018).

Canola is usually grown in rotation with wheat as the follow-on crop, providing an important disease and weed break. Studies have shown an average wheat 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 3 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 into the soil. These molecules are derived from glucosinolate degradation (Angus et al., 2015). Isothiocyanates could have an indirect impact on pathogenic fungi, by influencing the composition of the rhizosphere's microbial communities (Watt et al., 2006). Increased populations of plant symbiotic fungi (such as *Trichoderma* sp., an antagonist of *F. 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; Hunt and Norton, 2011). 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* is also considered to be competitive with *B. napus* in regions where canola yields are likely to be below 1.2 t/ha (Haskins et al., 2009; Hunt and Norton, 2011). *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 areas affected by drought (Potter, 2011).

2.3.4.2 Cultivation conditions and practices

Canola is mostly grown as a winter annual in winter-dominant rainfall environments between 30°S and 38°S (Norton et al., 1999). Average yields for broad acre production are 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; GRDC, 2015a, b, 2017b). Spring maturing 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; GRDC, 2015b). This compares to 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 has been predicted to be of interest for farmers in high rainfall zones (GRDC, 2015b; 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 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 (GRDC, 2015a). 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, 2015a, b). These sowing rates are used to achieve a density of approximately 20 to 60 plants/m^2 depending on the region and rainfall. Recommended plant densities for hybrid varieties are usually on the lower end of this range and open pollinated on the higher end (GRDC, 2015a). A density of less than 20 plants/m^2 is not recommended due to adverse effects on leaf area development, reduced biomass at flowering, reduced yield, and increased weed growth (GRDC, 2015a).

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 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). However, dry sowing can be successful in areas with reliable rainfall (Matthews et al., 2022).

The ideal time to sow depends on a range of environmental factors but also on the relative time to maturity of a variety (GRDC, 2015a) and sowing time is a compromise. Mid and late-maturing varieties should be sown early in the recommended sowing window, while early maturing varieties should be sown late. 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 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 or late sowing is also compounded by 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. Fertiliser requirements depend on yield expectation and needs to be assessed against environmental variations. On average, *Brassica* crops remove nutrients from the soil (per T per ha):

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

(Colton & Sykes (1992)).

Nitrogen fertiliser rates vary depending on paddock fertility and expected yield (see GRDC (2015a, b)) for calculations of nitrogen fertiliser rates).

Both *B. napus* and *B. juncea* conventional varieties are very sensitive to Group 2 herbicides (inhibitors of acetolactate synthase, such as chlorsulfuron or triasulfuron) and Group 5 herbicides (inhibitors of photosystem II, such as atrazine and simazine). Cultivation should avoid residues of these herbicides as they damage canola (Agriculture Victoria, Avoiding crop damage from residual herbicides factsheet; accessed on 21 May 2024).

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, 2010). A canola crop is ready when the majority of pods are dry and rattle when shaken. *B. napus* crops are usually 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, 2010; DPI Vic, 2012). 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 and Cox, 2001; GRDC, 2010). However, the use of chemical desiccants can be expensive.

2.4 Crop Improvement

Australian canola was initially improved through recurrent selection in a closed population. This led to inbreeding and genetic drift, with a loss of potentially valuable alleles (Cowling, 2007). One of the major challenges Australian breeders face is how 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 Oilseeds 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, defining 5 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 (Salisbury et al., 2007; GRDC, 2013).

Some more traits for germplasm enhancement, defined by the National Brassica Germplasm Improvement Program as preliminary and future traits are:

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

2.4.1 Breeding in Australia

Canola has moved in less than 40 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 (Salisbury and Wratten, 1999; Buzza, 2007). Private breeding began

in 1980, a major focus being the development of hybrids (Salisbury and Wratten, 1999). The first *B. napus* canola cultivars adapted to Australia growing conditions, Marnoo (Vic) and Wesroona (WA), 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.

2.4.1.1 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 and Wratten, 1999). The identification of Quantitative Trait Loci (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., 2016) the identification of QTL associated with yield and flowering time was also reported (Raman et al., 2016).

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 (Salisbury and Wratten, 1999; Hossain et al., 2012). 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. For example, a commercially available non-GM pod shattering resistance trait, referred to as PodGuard® which is available in GM herbicide tolerant canola varieties, increases the strength of the dehiscence zone of the pod and is reported to reduce shattering in response to high heat, windy/adverse weather events and during windrowing and harvest.

Cleistogamy does not exist naturally among the genetic resources of *B. napus* and *B. juncea*. However, lines of cleistogamous *B. napus* have been obtained via chemical mutagenesis (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 remain 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).

2.4.1.2 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). At that time, 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 being studied, using winter germplasm and polygenic resistance (Salisbury et al. 2007). Modified cropping practices, such as reducing fungicide use, play an important role in reducing the risk of development of new resistances, as detailed in the GRDC blackleg management guide (GRDC, 2022). Another proposed strategy to minimise

disease in crops is to use canola multiline cultivars or mixtures that have different resistance genes (Van de Wouw et al., 2014). Available lines with similar maturity time and herbicide resistance could be grown as a single crop, as this is done for wheat, barley and rice. However, for many of these resistance genes there is at least one virulent pathogen strain existing. Rotation of canola varieties is used to prevent the development of virulent strains, limiting the exposure of the same resistance genes for consecutive years (GRDC, 2024).

2.4.1.3 Non-GM herbicide tolerance

Canola is highly susceptible to weed competition during the early stages of growth, which can lead 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 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 closed the yield gap (Amjad and Pritchard, 2010). The first IT canola, also known as Smart or Clearfield® canola, was released in Australia in 2000. IT varieties do not carry a yield penalty and have been widely adopted (Agriculture Victoria Report, Review of the moratorium of genetically modified canola in Victoria; accessed on 21 May 2024).

Both TT and IT canola varieties are non-GM. The TT trait is derived from natural mutations observed in a wild biotype of *B. rapa*, transferred to *B. napus* through hybridisation (Beversdorf et al., 1980; Beversdorf and Kott, 1987). 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 and Straus, 1987). IT was developed through chemical mutagenesis. The observed tolerance phenotype is due to mutations in the enzyme acetohydroxyacid synthase, 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 have been trialled in SA (EPARF, 2015). Non-GM herbicide tolerant varieties represent a little more than half of Australian *B. napus* canola-quality production (Bayer, 2024). Little detail is available for *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 21 May 2024).

2.4.1.4 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. Another 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). High Oleic, Low Linolenic (HOLL) *B. napus* cultivars have been developed, with up to 70% oleic acid content and less than 3.5% linolenic acid (Gororo, 2007). 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 DPI, 2014). Canola-quality plants, particularly *B. juncea* canola could be used for biodiesel production (Haskins et al., 2009; McCaffery et al., 2009b). See Section 2.2.

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

2.4.1.5 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; Redden et al., 2007; Rahman et al., 2013; Raman et al., 2014a). Intraspecific, interspecific and intergeneric crosses have been used by breeders to improve both oilseed and vegetable *Brassica* species. Hybrids are also widely used in breeding seeds for commercial planting due to heterosis, leading to increased yield performance and early vigour.

B. napus and *B. juncea* are largely self-pollinating (see Section 4.2) 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 3 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 genetically identical except that B possesses normal cytoplasm and is therefore male-fertile)
- 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, several hybrid *B. napus* varieties with improved yields have since become available to growers (McCaffery et al., 2006; Potter et al., 2007). CMS lines have also been developed for *B. juncea*, through wide hybridisation (Malik and Saroha, 1999). Gene technology has been used to develop hybrid production systems.

Haploids and doubled haploids can be 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 homozygous and can be used in interspecific crosses, especially when these crosses involve parents with different levels of ploidy (Rahman et al., 2013; Mason et al., 2015). 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).

2.4.1.6 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 Simple Sequence Repeats are used routinely to identify QTL. These 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 publicly available. Such tools help gene discovery and breeding of *Brassicas* (Wang and Freeling, 2013). Computational methods have been used to analyse the structure of the *B. rapa* genome and compare it with *Arabidopsis* (Tang and Lyons, 2012).

Advances in molecular techniques, such as Next Generation Sequencing, have assisted in the characterisation of candidate resistance genes. 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).

Next Generation Sequencing has led to Single Nucleotide Polymorphisms (SNPs) being widely used for QTL mapping and comparative genomics. In particular, deep transcriptome RNA sequencing has reduced costs as SNP detection can focus on coding regions only (Devisetty et al., 2014). *B. napus*, *B. juncea* and *B. rapa* genomes have been investigated using SNP-based fine mapping methods (Devisetty et al., 2014; 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., 2014). 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 mutagenised populations (following treatment with a chemical mutagen such as ethyl methanesulfonate). Combining TILLING and NGS helps identifying mutants in polyploid species (Gilchrist et al., 2013).

More recently, clustered regularly interspaced short palindromic repeats/Cas (CRISPR/Cas) genome editing has been applied to accelerate the breeding of desirable traits in *B. napus* and *B. juncea*. CRISPR/Cas is an RNA-guided gene editing technique that allows for the precise removal, addition or alteration of DNA. Depending on the application, the CRISPR/Cas system does not necessarily produce a GMO(as defined under the Australian gene technology scheme). CRISPR/Cas could be used to not only to improve the yield and quality of *B. napus* and *B. juncae*, but also as a tool to analyse candidate gene functions and mechanisms. The CRISPR/Cas system offers the advantage of being able to target multiple copies of the same gene at once to overcome redundancy.

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* (Wang et al., 2003b; Dutta et al., 2008; Chhikara et al., 2012). Hypocotyls, cotyledons, stems, leaf discs, microspores or protoplasts can be used to regenerate GM plants (see Dutta et al. (2008) for details).

Agrobacterium-mediated transformation of *B. napus* and *B. juncea* can be done by floral dip, by vacuum-infiltrating immature floral buds (Wang et al., 2003a; Chhikara et al., 2012). Floral dip transformation efficiency is quite low: about 0.8% of seeds analysed by Chhikara et al., 2012 (2012) were found positive by Southern blot. Floral dip is routinely used as no tissue culture is required, thus reducing time and cost of transformation.

A protocol for chloroplast transformation of *B. napus* has been described (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 likelihood of transgene non-expression is reduced compared to nuclear transformation (Clarke and Daniell, 2011).

So far, GM canola varieties commercially released worldwide have been genetically modified for herbicide tolerance, altered oil content and/or a hybrid breeding system. Ongoing 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., 2005) or yield (Kant et al., 2015).

In Australia, field trials of GM canola were first approved in 2002 for *B. napus* and 2007 for *B. juncea*. There have been 10 approvals for commercial releases of GM canola in Australia between 2003 and 2024. The GM canola authorised in Australia include herbicide tolerant GM canola, GM canola with a modified omega-3 oil content and GM canola with a hybrid breeding system.

See the Office of the Gene Technology (OGTR) website for further details.

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 and Hertel, 2011).

A well-developed plant produces between 10 and 15 leaves (Colton and 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 and 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 and 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 (Figure 4) 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 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 and 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 and Bailey, 1976; Edwards and Hertel, 2011). In *B. juncea*, pods are held in a more upright position 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 and Hertel, 2011).

Figure 4: Flowering raceme of B. napus canola

Photo courtesy of Dr 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[3] and mainly self-pollinating, with a self- to cross-pollination ratio of about 70:30 (Downey and Rakow, 1987; Treu and 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 and 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 (Westcott and Nelson, 2001; Damgaard and Kjellsson, 2005). Pollen can be dispersed by physical contact between neighbouring plants. Hoyle and Cresswell (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 (Steffan-Dewenter, 2003; Bommarco et al., 2012; Gavloski, 2012). *B. napus* and *B. juncea* flowers produce nectar with relatively high concentrations of sugars which makes them particularly attractive to feral and managed honeybees (*Apis mellifera*) (Hüsken and 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 (Cunnigham, 2002). Hoverflies

[3] Self-incompatibility is the ability of a fertile hermaphrodite plant to recognise and reject its own pollen, preventing self-fertilisation (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.

have been described as alternative pollinators but their impact on canola pollination also appears to be quite low compared to honey bees (Jauker and 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 is reported to be viable for up to 5 days under natural conditions, with a viability rate of 20% measured 72 hours after emission (Bots and Mariani, 2005). Pollen viability varies with environmental conditions, particularly temperature and humidity. *B. napus* pollen longevity and germinability is reduced by 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 60°C (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 expansion begins about 15 days after fertilisation 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 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, or 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 remained a substantial 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; Rameeh, 2013; Raman et al., 2014b). 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). A pod shattering resistance trait (PodGuard®) is now commercially available in GM herbicide tolerant canola varieties both in Australia and overseas (Bayer, 2019).

B. napus and *B. juncea* seeds lack adaptations to facilitate 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 and 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 and 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. When sheep were placed on a diet containing 10% of whole canola seed for 10 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., 2008; Twigg et al., 2009; 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 (von der Lippe and Kowarik, 2007; Munier et al., 2012). Surveys done in North Dakota, United States' 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 and Clarke, 2000; Garnier et al., 2008). *B. napus* populations from seed spillages have also been detected in WA on a 3.5 km roadside transect from the delivery site (Busi and Powles, 2016). Plants were counted on road margins and/or in the median strip. Sohn et al. (2021) reviewed the scientific literature reporting occurrence of GM canola volunteers across the world which indicates that canola volunteers have also been observed in countries that do not grow GM canola and that do not grow or import GM canola, such as Switzerland, Austria and France.

The above information indicates common occurrence of inadvertent long-distance dispersal of canola seed via human-mediated transport. The persistence of volunteers is discussed in section 4.4.

4.4 Seed germination and seed dormancy

Very little information is available on *B. juncea* seed germination and dormancy. *B. juncea* seed is able to germinate in drier conditions than *B. napus* and is 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 -20°C 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 11 years.

B. napus seed can germinate under a variety of conditions (Pekrun et al. 1998). However, germination rates are reduced at low temperatures (Nykiforuk and Johnson-Flanagan, 1999). 50% of seed had germinated by one and 4 days post imbibition for seeds kept at 22°C and 10°C, respectively, whereas only 10% had germinated by 8 days post imbibition when kept at 6°C (Nykiforuk and 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 and Johnson-Flanagan, 1999).

Because seed is lost during harvest (see Section 4.3.2), seed viability under field conditions is used to predict the presence and amount of volunteers in subsequent crops.

Seeds lost at harvest can enter the soil seedbank when they are buried by tillage (Gruber et al., 2009; Gulden and Shirtliffe, 2009). Most seeds present in the seedbank will die, decompose or be eaten by predators (beetles, rodents and birds) before germination (Gulden and 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 cycles prior to germination (Gulden and Shirtliffe, 2009).

4.4.1 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. In this study 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. 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 no primary dormancy (Lutman et al., 2003). This can lead to pre-harvest sprouting in regions characterised by high humidity during the harvest season. Occurrence and levels of pre-harvest sprouting (or precocious germination) depend on both the environment (e.g. high humidity) and genetics, as well as their interaction (reviewed in Brown et al., 2023). Pekrun et al. (1998) describe *B. napus* seeds as having a high potential to build up secondary dormancy in darkness, sub-optimal oxygen supply and water.

Darkness/burial seems crucial for the development of secondary dormancy: seeds left on the soil surface for 4 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 as 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°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 and cultivars can be classified as low, medium or high dormancy types (Gulden et al., 2000; Gruber et al., 2009). 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 (Gulden et al., 2000; Gruber et al., 2009).

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 9 years. The likelihood of the presence of more than 2 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 10 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 4 years after a GM trial. However, data presented were obtained from a very small area (0.4 ha) 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 4 weeks has been described as a means of reducing the future seedbank (Pekrun et al., 1998; Lutman et al., 2003). 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).

4.4.2 Australian studies

In Australia, *B. napus* does not 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 3 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 2 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 and 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 and Preston, 2008; D'Emden et al., 2008). Furthermore, in SA, fields are rarely cultivated in the months after harvest (Baker and 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 and Preston, 2008).

Fewer volunteers of *B. juncea* than of *B. napus* have been reported in subsequent crops during field trials in the Australian Capital Territory (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 5):

- 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.

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).

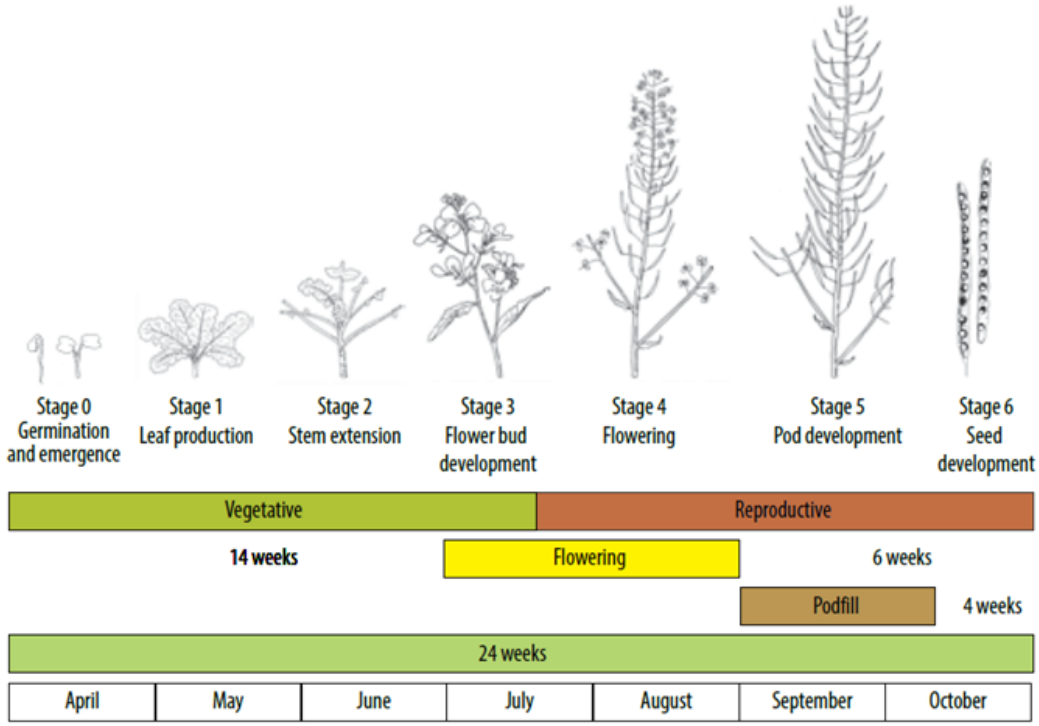

Figure 5: Growth stages of B. napus

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 at least 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 (Section 4.3). It is during this stage that the canola crop reaches physiological maturity and harvesting occurs (Section 2.3.3).

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 extent the gene pool of *B. juncea*) has been subjected to strong selection for low erucic acid and low seed glucosinolate content (Section 2.2). 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 and Potter, 1999).

Erucic acid and glucosinolate content 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 and 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 and Kumar, 2011).

5.1.1 Erucic acid

Erucic acid is a 22-carbon monounsaturated fatty acid (omega-9 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 synthesised in the cytosol by elongation of oleic acid, which is produced in plastids (Bao et al., 1998). Studies demonstrating a correlation between exposure to dietary erucic acid and number and severity of heart lesions in rats have led to human health concerns (Sauer and 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 and 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 (Sauer and Kramer, 1983; Monsalve et al., 2001).

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 of 750 mg/kg bw/day, based on results obtained for nursling pigs. A provisional tolerable daily intake was derived from this, using a safety risk factor of 100 (10 for extrapolating data from pigs to humans and 10 for variations within humans). The tolerable level for human exposure is thus 7.5 milligram per kilogram body weight per 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 milligram per day or 28% of the provisional tolerable daily intake.

5.1.2 Glucosinolates

Glucosinolates are plant secondary metabolites synthesised 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 (Clossais-Besnard and Larher, 1991; Bellostas et al., 2004; Bellostas et al., 2007; 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 80-90˚C as rapidly as possible. Thioglucosidase-catalysed hydrolysis of glucosinolates will proceed with increasing temperature until the enzyme is deactivated (Canola Council of Canada 2015).

Glucosinolates have allelopathic 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 *Rhizoctonia solani* AG8 on wheat production (Handiseni et al., 2011, 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 their 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; Puumalainen et al., 2006; Poikonen et al., 2008). 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 included in the list of 14 allergenic foods that must be declared on food labels of pre-packaged foods in the EU (EFSA, 2013). Of relevance, rapeseed protein isolate was recently approved by FSANZ as a novel food source, with FSANZ advising that people with a mustard allergy may react to this protein isolate (FSANZ, 2020).

Occupational exposure to *B. napus* and *B. junea* pollen, dust and/or flour has also been implicated in allergic reactions in people (Monsalve et al., 1997; Suh et al., 1998; Alvarez et al., 2001; Chardin et al., 2001). Allergic sensitisation to canola can occur *via* the respiratory tract or through skin contact, for example 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* (Toriyama et al., 1995; Okada et al., 1999; Chardin et al., 2001; Chardin et al., 2003; Focke et al., 2003). Proteins belonging to the 2S albumin class of seed storage proteins (napins), characterised 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 Peña et al., 1991; Monsalve et al., 1993). However, there is poor evidence that *B. napus* or

B. juncea pollen actively sensitise as only 0.2% of patients with respiratory allergies displayed a monovalent sensitisation to *B. napus* pollen (Hemmer et al., 1997; Hemmer, 1998). Hemmer (1998) speculated that cross reactivity between *B. napus* or *B. juncea* and other allergens is the main explanation for the observed allergic symptoms. Hypersensitivity to *B. napus* has mainly been observed in patients with atopic dermatitis and a history of pollen allergy (Chardin et al., 2001; Poikonen et al., 2008; Moneret-Vautrin et al., 2012). 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 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 and 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

5.4.1 Compositional analysis of canola seed

A summary of the composition of canola seed is given in Table 2.

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).

A comparison of the main seed quality characteristics of *B. napus* and *B. juncea* is provided in Table 3.

Table 2: Canola quality parameters, oil content and composition

Quality parameter	Mean
Oil content (% in whole seed, 6% moisture)	41.5
Protein content (% in oil-free meal, 10% moisture)	39.2
Total glucosinolates (µmol/g of meal, 6% moisture)	20.0
Energy (kcal per 100g of oil)	884
Saturated fats (% in oil)	7.6
Monounsaturated fats (% in oil)	61.5
Polyunsaturated fats (% in oil)	29.3
Erucic acid (% in oil)	0.1
Vitamin E (mg/100 g oil)	17.5
Vitamin K (mg/100 g oil)	71.3

Source: Adapted from GRDC (2009), FoodData Central by USDA (accessed 5 July 2024).

Table 3: B. napus and B. juncea seed characteristics

Characteristic	*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

Source: Adapted from Edwards & Hertel (2011)

5.4.2 Oil composition

Reported oil compositions of *B. napus* and *B. juncea* may vary in the literature due to differences in detection methods, plant quality, growing conditions, maturity and *B. napus* and *B. juncea* varieties studied.

A summary of the typical reported composition of canola oil is given in Table 4. Oil content is expressed as a percentage of whole seed at 6 or 8.5% moisture (Mailer, 1999; GRDC, 2009). 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.

Table 4: Average fatty acid profile of canola oil

Fatty acid	Common 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
24:1	Nervonic	0.1

Source: Adapted from GRDC (2009).

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 3) (Edwards and Hertel, 2011).

Due to these characteristics and a low concentration of low-density lipoproteins, the United States Food and Drug Administration now allows manufacturers to claim potential health benefits for canola oil due to reduced risk of coronary disease (Douaud, 2006).

5.4.3 Tocopherols

Tocopherols are naturally occurring antioxidants in vegetable oils and have a role in reducing cardiovascular diseases (ODS, 2016).There are 4 natural tocopherol isomers (all found in canola) that, together with 4 corresponding tocotrienols, make up the 8 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.

5.4.4 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 similar to that of soybeans which are the standard comparator due to their importance as feed source; the seed meal is slightly lower in lysine but higher in all sulphur-containing amino acids than soymeal. 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 and 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 and 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 other crops and will not produce high yields unless all three elements are present. Canola needs approximately (per T per ha) 40 kg of nitrogen, 7 kg phosphorus and 10 kg sulphur (Colton and Sykes, 1992). Gypsum is often applied to sodic soils to improve soil structure and alleviate sulphur deficiencies (Potter et al., 1999).

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 and 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 water stress (GRDC 2009). The main symptoms of heat and water 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 and 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 and Ladiges, 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.

Common high-impacting Australian subsoil constraints include salinity, sodicity, alkalinity and toxic ion levels (Zhang et al., 2000). Salinity is an aggravating factor for water and temperature stress. Soil salinity stresses plants via dehydration and toxicity (Zhang et al., 2000). 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., 2000).

B. napus and *B. juncea* are relatively frost tolerant. Although uncommon, damage can occur at the cotyledon stage 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 and 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).

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 and Cox, 2001; McCaffery et al., 2009c). For example, in the northern agricultural region of WA, silver grass (*Vulpia myuros* and *V. bromoides*), wild radish (*Raphanus raphanistrum*) and turnip (*Brassica rapa* var. *rapa*) can devastate early sown crops. Competition from these weeds leads to significant yield losses. Registered herbicides for use in *B. napus* and *B. juncea* crops are either grass specific or for limited broadleaf weed control. 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 and Cox, 2001).

Varieties 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 including the redlegged earth mite (*Halotydeus destructor*), blue oat mite (*Penthaleus major*, *P. falcatus*, and *P. tectus* sp. n), lucerne fleas (*Sminthurus viridis*), cutworms (*Agrotis infusa*), aphids (*Brevicorne brassicae*, *Lipaphis pseudobrassicae* and *Myzus persicae*; as viral vectors), diamondback moths or cabbage moths (*Plutella xylostella*), heliothis caterpillars (*Helicoverpa punctigera* and *H. armigera*) and Rutherglen bug (*Nysius vinitor*) (OGTR, 2022).

Significant insect damage to *Brassica* crops is most likely to occur during establishment, and from flowering to maturity (Miles and McDonald, 1999).

7.2.2 Pathogens

B. napus and *B. juncea* can be infected by several pathogens in Australia, leading to diseases ranging from root rots to leaf and crown to stem infections. The most important pathogens currently affecting canola within Australia are listed below (GRDC canola disease update, accessed 22 May 2024 and Agriculture Victoria, accessed 22 May 2024):

- Blackleg (caused by *Leptosphaeria maculans*) – the most widespread disease affecting canola in Australia
- Sclerotinia stem rot (caused by *Sclerotinia sclerotiorum*) – a growing problem affecting canola production in medium to high rainfall zones
- White leaf spot (caused by *Mycosphaerella capsellae*) – sporadic disease in southern canola production areas
- Powdery mildew (caused by *Erysiphe cruciferarum*) – important disease affecting NSW canola producers in the Northern region and to a lesser extent in southern NSW
- Club root (caused by *Plasmodiophora brassicae*) – detected sporadically in NSW and Vic
- White rust or Staghead (caused by *Albugo candida*) - uncommon in Australian *B. napus* varieties but does infect *B. juncea.*

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 and Brennan, 2012).

7.2.2.1 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, causing 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 and 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). Other sources of resistance are studied, using winter germplasm and polygenic resistance (Salisbury et al., 2007). See Section 2.4.1.

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., 2014; Van de Wouw et al., 2016).

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 characterised 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 (Somers et al., 2002; GRDC, 2007; Li et al., 2007a; Kaur et al., 2008). 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 (Howlett et al., 1999; Oilseeds WA, 2006; GRDC, 2009; Murray and Brennan, 2012).

7.2.2.2 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*), *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 because *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 important in breeding (Nyalugwe et al., 2015).

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

7.2.2.3 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 (Somers et al., 2002; Sharma et al., 2009). In some instances, it has also been possible to use resistance that occurs in other *Brassica* species. For example, in India, natural resistance that occurs 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 and Powles, 2016).

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 (Sections 2.4 and 4.3.2).

As with all crops cultivated and harvested at the field scale, *B. napus* and *B. juncea* seed is lost during 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, for example along roadsides and storage facilities, as a result of spillage during transport (Kawata et al., 2009; Schafer et al., 2011; Busi and Powles, 2016). See Section 4.3.2.

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, Kaminski (2001) reported *B. napus* as the fifth ranked weed in Manitoba. However, *B. napus* is not considered a significant weed, nor invasive of natural undisturbed habitats, in Canada (Canadian Food Inspection Agency, 1994; Warwick and Small, 1999; Beckie et al., 2001). *B. juncea* has been reported as an escapee in Canada since the late 19th century but is not considered to be a problem weed, which may be due to the small scale of cultivation (CFIA, 2012). *B. napus* is not listed as a weed in the Invasive Plant Atlas of the United States whereas *B. juncea* appears on the invasive species list or law in New Hampshire, Minnesota and Michigan (accessed on 24 June 2024).

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 or Weeds of National Significance (Weeds Australia; accessed on 24 June 2024). 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). Weeds were described as naturalised and were defined as environmental or agricultural weeds depending on how they impact either ecosystem. The weeds were further categorised 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 5.

Table 5: Categories for assessing the status of naturalised non-native species in natural ecosystems

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
Source: Adapted from Groves et al. (2003).

B. napus and *B. juncea* are classified as category 5 weeds in agricultural ecosystems, with variations between states (Table 6). However, WA and Vic state governments do not consider *B. napus* and/or *B. juncea* as weeds. 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[4], personal communication, 2016). *B. napus* and *B. juncea* are classified as category 2 and 3 weeds in natural ecosystems, respectively.

Table 6: B. napus and B. juncea weed classification in agricultural and natural ecosystems in Australia

Agricultural ecosystems		Natural ecosystems
By state	National	National
B. napus		
Qld 1		
NSW 3		
Vic 3		
Tas 1	5	2
SA n/a		
WA 5		
NT n/a		
B. juncea		
Qld 2		
NSW 3	5	3
Vic 5		
Tas n/a		

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

	Agricultural ecosystems		Natural ecosystems
	By state	National	National
SA	2		
WA	5		
NT	1		

Source: Adapted from Groves et al. (2003). Risk ratings from the same source as defined in Table 5 above.

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., 2009b).

Both *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). If dispersed seed germinates, it is unlikely to persist. Seedlings established in adjacent fields would likely be destroyed by normal agricultural practices unless *B. napus* or *B. juncea* is grown in the field (herbicide application, cultivation). However, poor management practices can result in severe volunteer problems in succeeding crops.

Seedlings established in non-agricultural areas are not likely to spread and persist, as *B. napus* and *B. juncea* plants are poor competitors and do not establish well in unmanaged areas (Salisbury, 2002c; Oram et al., 2005). Unless the habitat is regularly disturbed, or seed replenished due to 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

Both *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 and Powles, 2016). However, *B. napus* and *B. juncea* are considered poor competitors (Section 4.3.2).

Only optimal agronomic conditions will promote the establishment of *B. napus* and 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, with observations made every 10 km along designated roads (Agrisearch, 2001). 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[5]: 0%
- Southern NSW: 31.2%
- Vic: 12.6%
- Tas: 3.6%
- SA: 8.6%
- WA: 20.3%.

The authors found no evidence of canola forming self-sustaining populations. Average distance between plants was 2.6 m.

[5] 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.

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 15 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 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 from a range of mode of action groups are registered for use on *B. napus* and *Brassica* ssp., including:

- Group 2 (flumetsulam, sulfosulfuron or metosulam)
- Group 6 (bromoxynil)
- Group 14 (carfentrazone)
- Group 12 (diflufenican)
- Group 9 (glyphosate)
- Group 4 (MCPA - 2-methyl-4-chlorophenoxyacetic acid, 2,4-D or clopyralid) (APVMA website, accessed on 22 May 2024).

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, 2008).(Weber et al., 2009) 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 evaluation of dealings with 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, 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 and 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 (Rieger, 2002; Walklate et al., 2004; Hayter and Cresswell, 2006; Bommarco et al., 2012). Hoyle and Cresswell (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 shows an increase in bee-borne cross-pollination (Hoyle and Cresswell, 2007).

Most studies describe *Brassica* pollen dispersal as leptokurtic[6], with the majority of cross-pollination occurring over very short distances (less than 10 m) from the source (Eastham and Sweet, 2002). Because of this distribution, any foreign pollen in a given field will quickly be diluted into the massive local pollen production (Damgaard and 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 and 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* (Singhal et al., 2005; Salisbury, 2006) showed that no wind 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-sustaining populations over long periods of time (Section 8).

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

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 geneflow 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 generally constant around 0.05% after 20 to 50 m. Decline observed using discontinuous design is slower and steadier, and hybridisation rate is constant at 0.1% beyond 100 m. The relative size of 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 et al., 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).

When 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 5 m and 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 and Sweet, 2002; Hüsken and 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 (Hüsken and 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[7]. Isolation distances are effective for self-fertile plants but not for male-sterile crops, where discarded border zones should be preferred (Damgaard and Kjellsson, 2005; Hüsken and 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 recognised 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, 2002a).

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

9.3 Interspecific crossings

Potential gene flow between *B. napus* and *B. juncea* and Australian *Brassicaceae* weed species is summarised in Table 7.

The direction of a cross is an important parameter to consider when evaluating the likelihood of hybridisation 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, 2002a). Progeny viability and fertility through several generations are also factors influencing crosses (Mallory-Smith and 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 6). 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* (Navabi et al., 2011; Rahman, 2013; Mason et al., 2015). See Section 2.4.1.

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

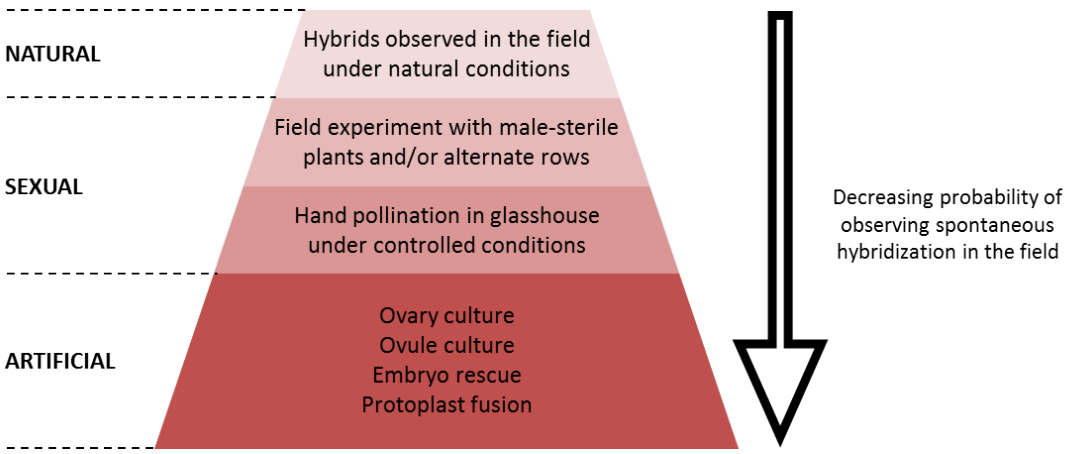

Figure 6: Intraspecific, interspecific and intergeneric hybrids can be obtained naturally, sexually or artificially in the tribe Brassiceae

Adapted from Warwick et al. (2009b).

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 hybridise with each other (FitzJohn et al., 2007; Warwick et al., 2009a). However, natural hybrids in fields and riversides were reported only for *B. napus* x *B. rapa* hybrids (Warwick et al., 2009a). There is no other evidence suggesting that hybrids formed between *B. napus* and other wild relatives could establish in nature (Wei and Darmency, 2008).

Table 7: Potential gene flow between B. napus and B. juncea and Australian Brassicaceae weed species

Tribe	Genus	Main species of concern in Australia[1]	Means of propagation	Considered as weed in Australia?			Hybridisation in the field[7]			
				Groves et al. (2003)[2]		Department of the Environment[3]	Overseas[4]		In Australia[5,6]	
				Agricultural	Natural		*B. napus*	*B. juncea*	*B. napus*	*B. juncea*
Brassiceae	*Brassica*	*Brassica rapa*	Seed	5	4	No	Likely		Likely*	
		Brassica tournefortii		5	5		Unlikely		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*	Seed	5	3	No	Unlikely		Unlikely	
		Sinapis arvensis		5	5		Possible[#]			
Cardamineae	*Cardamine*	*Cardamine flexuosa*	Seed	5	3	No	n/a		n/a	
		Cardamine hirsuta		5	5					
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] Source (Salisbury, 2002b) the Department of the Environment website (accessed on 22 May 2024)

[2] See Table 5 for detailed description of the different categories

[3] Source the Department of the Environment website (accessed on 22 May 2024)

[4] Source (FitzJohn et al., 2007; Warwick et al., 2009a and references therein)

[5] Source (Salisbury, 1991, 2002b)

[6] *B. napus* x *B. rapa* hybrids have not been reported to date in Australia. However, hybridisation and subsequent introgression are possible where the two species grow in sympatry and when flowering periods overlap (Salisbury, 2002b)

[7] 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 (Salisbury, 1991; Eber et al., 1994; Lefol et al., 1996; FitzJohn et al., 2007; Warwick et al., 2009b; Warwick and Martin, 2013).

Rates of natural hybridisation between *B. napus* and *B. rapa* vary across 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 hybridisation rates up to 42.5% in feral populations growing at the margin of *B. napus* fields. Hybrid rates dropped to 2.5% within 3 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 hybridisation rates (9-93%) were observed by Jorgensen et al. (1996). However, these hybridisation 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 and Sweet, 2002; Hüsken and 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 (Hansen et al., 2001). The extent and direction of hybridisation 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, hybridisation and subsequent introgression are possible where the two species grow in sympatry and when flowering periods overlap.

B. napus x *B. jun*cea hybrids 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 hybridisation 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 highly unlikely, limiting the gene flow to some extent (Wei and 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 and 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 (Salisbury, 2002b; FitzJohn et al., 2007). The potential of gene flow from *B. napus* or *B. juncea* to *B. nigra* is thus considered highly 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 highly unlikely, due to pre-fertilisation barriers. Some hybrids have been obtained using of artificial crossing methods (Figure 6). Furthermore, these hybrids have been shown to be sterile (Salisbury (2002b) and references therein). *B. tournefortii* x *B. juncea* hybrids were obtained using embryo rescue. No *B. juncea* x *B. tournefortii* hybrids

were produced as embryos aborted at early development stages (Kumar and Abbo, 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 7.

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).

Pre-and post-fertilisation barriers exist between *B. napus* or *B. juncea* and their weedy relatives in Australia (Salisbury, 2006). Gene flow 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).

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. (2009b) for an extensive review of available interspecific and intergeneric hybridisation data.

This section focuses mainly on *R. raphanistrum*, *S. arvensis* and *H. incana*. These species are recognised as major weeds of commercial *Brassica* crops and have been described as potentially compatible with *B. napus* (Eastham and Sweet, 2002). Relative weediness of these 3 species in agricultural ecosystems is summarised in Table 8.

Table 8: Relative weediness of R. raphanistrum, S. arvensis and H. incana in Australia

	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

Source: Adapted from Groves et al. (2003).

*Raph*anus *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 the glasshouse, using a male sterile *B. napus* (Darmency et al., 1998; Gueritaine et al., 2003; Ammitzboll and Jorgensen, 2006). 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., 2009b). Transfer of genes of *B. napus* or *B. juncea* to *R. raphanistrum* is 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*, hybridisation rates of 0.12-0.18% were observed (Chèvre 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., 2009b). *B. juncea* x *S. arvensis* hybrids were generated using co-cultivation under field conditions, at a rate of 0.0018% (Warwick and

Martin, 2013). Hybrids showed reduced fertility and no backcross progeny was obtained using *S. arvensis*. The likelihood of transgene introgression from *B. juncea* to *S. arvensis* is highly unlikely to unlikely (Warwick and Martin, 2013). Gene flow is unlikely to occur between either *B. napus* or *B. juncea*, and *S. arvensis* (Eastham and 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. Backcrossing 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 and Fleury, 2000). Potential gene flow from *B. juncea* to *H. incana* under Australian conditions has also been described as extremely unlikely (Salisbury, 1991, 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 and deVicente, 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 hybridise 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 and deVicente, 2010). Crossing between *B. juncea* and *B. nigra* is possible because they share a common genome (the B genome, see Figure 1). However, hybridisation 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 hybridise 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 hybridise with *B. tournefortii*, *H. incana*, *R. raphanistrum* or *S. arvensis* (Warwick et al., 2009b). 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

WEED RISK ASSESSMENT OF CANOLA

Species: *Brassica napus* L and *Brassica juncea* L

Relevant land uses:

1. Production from dryland agriculture and plantations (ALUM classification 3.3: cropping)

2. Production from irrigated agriculture and plantations (ALUM classification 4.3: irrigated cropping)

3. Intensive uses

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 traits, 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 dispersal. These 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?	**Rating: Low** *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 areas***. 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** *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 areas***. 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

Invasiveness Questions	***B. napus***	***B. juncea***
		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* (Canadian Food Inspection Agency 2007; Oram et al. 2005a).
2. What is the species' tolerance to average weed management practices in the land use?	**Rating: Low** **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** *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 s*eed 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: Low** *B. napus* is an annual crop and generally takes at most 7 months to complete its life cycle under standard agricultural conditions of ***dryland & irrigated cropping areas***. The lifecycle	**Rating: Low**

Invasiveness Questions	***B. napus***	***B. juncea***
	is similar in other land uses. However, stresses such as competition or drought may hasten reproduction and shorten the lifecycle.	*B. juncea* is an annual crop and generally takes less than 7 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.

[a] In Western Australia, mustard lines can reach maturity in 4.5 to 5 months (Gunasekera et al. 2001; Oram et al. 2005a).

Invasiveness Questions	*B. napus*	*B. juncea*
3b. What is the annual seed production in the land use per square metre?	**Rating: High** 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 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 2.6 m. Seed production may be ≥ 1000 seeds/m^2.	**Rating: High** 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. 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 be ≥ 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:		

[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*
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, dissemination of *B. napus* seed by wild birds consuming seed directly from a crop would occur occasionally. 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* volunteers 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* volunteer seeds 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.
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	**Rating: Occasional** Dispersal by water is possible but no data is available for *B. juncea* or other *Brassica* species. Seeds may be transported

Invasiveness Questions	***B. napus***	***B. juncea***
	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 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.	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 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, windrowing of *B. napus* volunteers does not occur and 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 for *B. juncea* crops. 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***.

Invasiveness Questions	*B. napus*	*B. juncea*
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 (even by volunteers) 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 number of *B. napus* volunteers would be minimised by standard weed management. *B. napus* seed is accidentally spread *via* transport along roadsides, railway lines and processing sites. 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.	**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 (even by volunteers) 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 number of *B. juncea* volunteers would be minimised by standard weed management. *B. juncea* seed may be accidentally spread *via* transport along roadsides, railway lines and processing sites. 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.

Invasiveness Questions	*B. napus*	*B. juncea*
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	**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

Impact Questions	***B. napus***	***B. juncea***
	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.	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.	**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.

[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*
	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. 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.	*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. 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** 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** 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,	**Rating: None** *B. napus* may grow in all the relevant land use areas as a volunteer at a low population density. No self-sustaining	**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.*

Impact Questions	*B. napus*	*B. juncea*
animals, vehicles, machinery and/or water?	volunteer *B. napus* population has been reported under Australian conditions.	*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** *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 that cross reactivity between *B. napus* and other allergens is the main explanation for allergies observed.	**Rating: Low** 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. 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	**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

Impact Questions	*B. napus*	*B. juncea*
	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.	*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** The number and density of *B. napus* volunteers is expected to be low and would not be expected to affect fire regimes.	**Rating: Minor or no effect** The number and density of *B. juncea* volunteers is expected to be low 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** The number and density of *B. napus* volunteers is expected to be low and would not be expected to affect nutrient levels.	**Rating: Minor or no effect.** The number and density of *B. juncea* volunteers is expected to be low 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** The number and density of *B. napus* volunteers is expected to be low and would not be expected to affect soil salinity.	**Rating: Minor or no effect** The number and density of *B. juncea* volunteers is expected to be low 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** The number and density of *B. napus* volunteers is expected to be low for and would not be expected to affect soil stability.	**Rating: Minor or no effect.** The number and density of *B. juncea* volunteers is expected to be low 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** The number and density of *B. napus* volunteers is expected to be low and would not be expected to affect the soil water table.	**Rating: Minor or no effect** The number and density of *B. juncea* volunteers is expected to be low and would not be expected to affect the soil water table.
11g. Does the species alter the structure of nature conservation areas by adding a new strata level?	**Rating: Minor or no effect** The number and density of *B. napus* volunteers is expected to be low and would not be expected to add a new strata level.	**Rating: Minor or no effect** The number and density of *B. juncea* volunteers is expected to be low and would not be expected to add a new strata level.