The Biology of *Brassica juncea* (Canola/Mustard)

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Biology Document BIO2007-01: A companion document to the Directive 94-08 (Dir94-08), Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits

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Part A - General Information

A1. Background

The Canadian Food Inspection Agency (CFIA) is regulating the field testing of crop plants with novel traits (PNTs) in Canada. PNTs are defined as a plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct, stable population of a cultivated species of seed in Canada and that have been intentionally selected, created or introduced into a population of that species through a specific genetic change. Familiarity is defined as the knowledge of the characteristics of a plant species and experience with the use of that plant species in Canada. Substantial equivalence is defined as the equivalence of a novel trait within a particular plant species, in terms of its specific use and safety to the environment and human health, to those in that same species, that are in use and generally considered as safe in Canada, based on valid scientific rationale.

The PNTs may be developed using traditional plant breeding techniques or other methodologies such as recombinant DNA technologies. Regulated field testing is necessary when PNTs are: (1) considered unfamiliar when compared with products already in the market; (2) not considered substantially equivalent to similar, familiar plant types already in use, and regarded as safe.

Before PNTs may be authorized for unconfined release, they must be assessed for environmental safety. CFIA's Directive 94-08 (Dir94-08), entitled "*Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits*", describes the criteria and information requirements that must be considered in the environmental assessment of PNTs to ensure environmental safety in the absence of confined conditions.

A2. Scope

The present document is a companion document to the Dir94-08. It is intended to provide background information on the biology of *Brassica juncea*, its centres of origin, its related species and the potential for gene introgression from *Brassica juncea* into relatives, and details of the life forms with which it may interact.

Such species-specific information will serve as a guide for addressing some information requirements of Part D of Dir94-08. Specifically, it will be used to determine if there are significantly different/altered interactions with other life forms resulting from the PNT's novel gene products, which could potentially cause the PNT to become a weed of agriculture, become invasive of natural habitats, or be otherwise harmful to the environment.

The conclusions drawn in this document about the biology of *Brassica juncea* only relate to plants of this species with no novel traits.

Part B - The Biology of *Brassica juncea*

B1. General Description, Cultivation and Use as a Crop Plant

Brassica juncea (L.) Czern. belongs to the Cruciferae (Brassicaceae) plant family, commonly known as the mustard family. The name crucifer is derived from the shape of the flowers that have four diagonally opposed petals in the form of a cross. *B. juncea* has pale green foliage, with a few hairs on the first leaves and leaf blades that terminate well up the petiole. Mature *B. juncea* plants grow to a height of one to two meters. The lower leaves are deeply lobed, while the upper leaves are narrow and entire. *B. juncea* is distinct from its close relatives *B. napus* and *B. rapa* in that the upper leaves of *B. juncea* are not clasping. The inflorescence is an elongated raceme and the flowers are pale yellow and open progressively upwards from the base of the raceme. The seed pods are slightly appressed and 2.5 to 5 cm in length exclusive of the beak. The beak is 0.5 to 1 cm long. Seeds are round and can be yellow or brown.

There are both vegetable and oilseed varieties of *B. juncea* that possibly have different origins (Hemingway, 1976). Both types are considered to be natural amphidiploids (AABB genome, 2n=36) of *B. rapa* (AA genome, 2n=20) by *B. nigra* (BB genome, 2n=16) crosses. Axelsson *et al.* (2000) have shown by molecular analysis that *B. juncea* contains conserved genomes of the progenitor species.

Brassica juncea can be divided into four sub-species, with different morphology, quality characteristics and uses (Spect and Diederichsen, 2001).

1. ssp. *integrifolia*, used as a leaf vegetable in Asia.
2. ssp. *juncea*, cultivated mainly for its seed, occasionally as fodder.

3. ssp. *napiformis*, used as a root-tuber vegetable.
4. ssp. *taisai*, stalks and leafs are used as vegetables in China

Oilseed *B. juncea* is grown as a spice crop in North America, but is also used as a source of cooking oil in Asia. Within Canada the primary production areas are located in the prairie provinces of Manitoba, Saskatchewan, and Alberta. Western Canada has become a major producer of mustard seed since World War II when supplies from Western Europe, the historic base of production, were disrupted. For spice purposes, appearance of the seed is particularly important, and therefore, the crop is grown in more southerly, drier regions where the risk of green seed in the harvested crop is reduced. Compared to the more widely grown canola species *B. napus* and *B. rapa*, *B. juncea* is more tolerant to heat and drought stress (Woods *et al.*, 1991). The species does not shatter as readily as *B. napus* and so it can be straight cut or swathed and combined (Hemingway, 1995). Low glucosinolate and low erucic acid varieties have recently been developed for use as edible (canola quality) oil (Potts *et al.*, 1999).

B2. Brief Outlook at Breeding, Seed Production and Agronomic Practices for mustard and canola-quality *Brassica juncea*

Brassica juncea breeders aim to make simultaneous improvement of agronomic performance, disease resistance and quality traits. Agronomic traits include yield, lodging, maturity, herbicide tolerance, drought tolerance, shattering resistance and seed size. Disease resistance breeding efforts may include blackleg, white rust, alternaria blackspot and Fusarium wilt resistance. Improvements in quality traits will depend on whether the aim is to develop canola or mustard varieties. For canola, high oil content, low glucosinolate content, high protein content, and a fatty acid profile that is canola-quality with low erucic and low saturated fatty acid content are desired. For mustard varieties, low oil content, high glucosinolate content and a fatty acid profile with a moderate level of erucic acid are desired.

Most varieties of canola/mustard are pure lines, developed through repeated cycles of inbreeding or through the use of doubled haploid technology. As with most inbred crops, developing a new variety begins with the creation of F1 hybrid seed by crossing two or more parents. The aim is to combine the desirable traits from the parents into a new variety. In pedigree breeding programs, F2 populations are created by selfing the F1. Selection of highly heritable traits may begin in the F2 generation. Selection will continue on either a single plant or family basis for several generations. Selection for complex traits such as yield will wait until the breeding line is reasonably uniform. Some breeding programs will use contra-season nurseries or greenhouses to decrease the time required to reach genetic uniformity. When doubled haploid technology is used, immature pollen grains from F1 plants are grown through tissue culture techniques to produce haploid plants. A mitotic inhibitor such as colchicine is used to develop homozygous breeding lines. One or two generations of seed increase and selection are used before doubled haploid lines are advanced to yield trials. Molecular markers are sometimes employed to help select for traits that are difficult to measure, and to help select parents or pollen donor plants.

Lines chosen in nurseries are entered into small plot performance trials at one or more locations for one or more years. Based on data from these trials, selected lines are advanced to registration trials, which are conducted over several locations for at least two years. Using established guidelines, committees of experts evaluate the data from registration trials and recommend varieties to be registered to the Canadian Food Inspection Agency.

Pure seed increase may begin before a variety is registered. The first increases are often done under tents to prevent outcrossing. Once registration is granted, Breeder seed is distributed to seed growers for increase and production of Foundation seed. Foundation seed is used to produce Certified seed, from which the commercial crop will be grown. Breeder, Foundation and Certified seed are grown under regulations established by the Canadian Seed Growers Association.

Currently, there are no commercial *B. juncea* hybrids in Canada. In the case of hybrids, the commercial crop is grown from F1 seed produced by crossing two inbred lines, one of which is male sterile.

Agronomic practices are similar for canola and mustard types of *B. juncea*. Both are grown primarily in the southern prairies of western Canada to take advantage of the superior heat and drought tolerance of this species in comparison to B. napus canola. Mustard and canola are usually grown in rotation with cereal and pulse crops. Summerfallowing to store moisture and control weeds is a common practice in the southern prairie and is often part of a rotation. Mustard, and canola that is not tolerant to wide spectrum herbicides, is generally grown in fields that are relatively free of weeds. Weeds such as wild mustard and cow cockle are difficult to remove from the harvested crop and result in downgrading. Diseases of mustard and canola do not generally affect cereal crops and vice versa, so it is beneficial for disease control to include both types of crops in a rotation. It is generally recommended not to grow a canola or mustard crop more than once every three or four years to prevent the build up of disease and weed problems.

Small seeded crops such as mustard and canola require shallow seeding into a firm, moist seedbed to achieve good emergence. Seed rates of four to seven kilograms per hectare are usually used. Early seeding is usually beneficial to make use of soil moisture and avoid high heat at flowering time. *Brassica juncea* is quite tolerant to frost in the seedling stage (Dhillon and Larsson, 1985). Seed treatments are usually used to control flea beetles and seedling diseases.

A limited number of herbicides are registered for use on *B. juncea* (Sask. Ag. and Food, 2007). Soil incorporated herbicides, such as trifluralin, are effective against a wide spectrum of weeds, but require cultivation which dries the seedbed and can lead to soil erosion. Post-emergence herbicide options are limited to several graminicides (clethodim, fenoxaprop-p-ethyl, fluazifop-p-butyl, sethoxydim) and one broadleaf weed chemical (ethametsulfuron-methyl).

Mustard and canola-quality *B. juncea* may be either swathed or straight combined. Straight combining is possible if the crop is reasonably uniform and free of green seed. *Brassica juncea* is more resistant to shattering than *B. napus* and therefore is often straight combined.

B3. The Reproductive Biology of *Brassica juncea*

All *B. juncea* varieties have an annual growth habit. Fertilization of ovules usually results from self-pollination, although interplant outcrossing rates of 20-30% have been reported (Rakow and Woods, 1987). Bees are the primary pollen vector because the pollen is heavy and sticky and is not carried great distances by wind. Cross-pollination of nearby plants can also result from physical contact of the flowering racemes.

Using a herbicide resistance marker, GhoshDastidar *et al.* (2000) found outcrossing up to 35m when a plot of resistant *B. juncea* was surrounded by non-resistant plants. Since *B. juncea* has similar flower structure, pollen volume and outcrossing rate as *B. napus*, it is likely that, as with *B. napus*, occasional outcrossing to distances beyond 35m occurs (Salisbury, 2006).

Beckie *et al.* (2003) summarized gene flow data for *B. napus* and conducted a study involving gene flow between commercial fields of cultivars with different herbicide resistance traits. Many variables affect outcrossing between fields, including, size of fields, fertility of recipient plants, environmental conditions and presence of insect pollinators. Previous experiments had shown that outcrossing is sharply reduced with distance between fields, but relatively rare outcrossing events can occur at distances greater than 200 m. In their study, Beckie *et al.* (2003) found outcrossing to be approximately 1.4% at the border between fields, dropping to 0.04% at 400 m. In one year, gene flow was detected at the 800 m limit of the experiment.

B4. The Centres of Origin of the Species

The origins of *B. juncea* are unclear. *B. juncea* is an amphidiploid with a chromosome number of 18. It is believed to have derived from natural interspecific hybridization between B. nigra (n=8) and *B. rapa* (n=10). *B. juncea* likely originated where distributions of *B. nigra* and *B. rapa* overlap, such as in the Middle East and neighbouring regions (Prakash, 1980). The foliage types (Chinese) and oleiferous types (Indian) may have had separate origins, a concept supported by the glucosinolate studies of Vaughn *et al.* (1963).

Spect and Diederichsen (2001) support the view that *B. juncea* probably evolved somewhere between Eastern Europe and China, where the progenitor species are sympatric. Most of the variation occurs in western and central China. It occurs as a weed in the south of the European part of Russia, the Caucasus, central Asia and southern Siberia and as a casual or feral plant in south and southeast Asia, Africa and America.

B5. Cultivated *Brassica juncea* as a Volunteer Weed

As with all crops that are cultivated and harvested on a field scale, some *B. juncea* seed may escape harvest and remain in the soil until the following season when it will germinate either before or following seeding of the successive crop. As a result, *B. juncea* volunteers could grow and become weedy in subsequent crops. Harvest losses in *Brassica napus* grown in Saskatchewan average approximately 3000 seeds m-2 (Gulden *et al.*, 2003a). Losses in *B. juncea* may be somewhat less due to greater pod shatter resistance, but are assumed to be substantial.

Bibbey (1948) compared dormancy in weedy species such as *Brassica arvensis* (syn. *Sinapis arvensis*) and *Thlaspi arvense* (stinkweed) with cultivated species, including *B. juncea*. He found that the cultivated species germinated readily, even when buried, whereas the weedy species would stay dormant until brought to the surface. He attributed the difference to sensitivity to oxygen and carbon dioxide concentration in the weedy species that was not present in the cultivated species.

Secondary dormancy has been found to be a factor in persistence of volunteer *B. napus* in western Canada (Gulden *et al.*, 2003b). Genotype and seed size were both found to be factors affecting secondary seed dormancy in *B. napus* (Gulden *et al.*, 2004).

Brassica juncea has been reported as an escape for many years (Scoggan, 1957) with the earliest such report in Manitoba in 1896. Although *B. juncea* occurs throughout the prairies and parklands (Looman and Best, 1979), and is listed as a weed in Canada (Darbyshire, 2003), it has not become a problematic weed. It is not listed in the Weed Seeds Order (2005) of Canada. In a review of prairie weed surveys (Leeson *et al.*, 2005), found that in surveys since 2000, *B. juncea* (Indian mustard) was found in less than 0.1% of surveyed fields and ranked 131st out of 148 weeds in overall abundance. In contrast, canola/rapeseed (*B. napus*/*B. rapa*) ranked 14th and wild mustard (*Sinapis arvensis*) ranked 24th.

Despite a long history of cultivation in western Canada, *B. juncea* has not become an abundant weed, and therefore there is good reason to conclude that it does not have the weedy characteristics of wild mustard and may be less prone than *B. napus* and *B. rapa* to become a problem as a volunteer weed. The difference in weed ranking among the cultivated species can be largely attributed to differences in cultivated acreage and, more recently, herbicide resistance in *B. napus*. However, *B. juncea* has some attributes that may reduce its weediness in comparison to *B. napus*, such as shatter resistance, small seed size and thin seed coat in yellow-seeded cultivars.

Part C - The Close Relatives of *Brassica juncea*

C1. Inter-Species / Genus Hybridization

While many interspecific and intergeneric crosses have been made between *B. juncea* and its relatives in the mustard family, most have required human intervention in the form of ovary culture, ovule culture, embryo rescue, or protoplast fusion. Listed here, mainly from the extensive review by Warwick *et al.* (2000b), are the interspecific and intergeneric hybrids obtained from sexual crosses between *B. juncea* and its relatives.

Table 1. Reports of sexual hybridization between *Brassica juncea* and related species. Where attempted, reciprocal crosses are classed as successful (S) or unsuccessful (U). From Warwick *et al.* (2000b) unless noted by an asterisk.

Cross	Reciprocal	Reference
B. juncea x *B. carinata*	S	Alam *et al.* (1992)

B. juncea x *B. carinata*		Barcikowska *et al.* (1994)
B. juncea x *B. carinata*	S	GoshDastidar &Varma (1999)
B. juncea x *B. carinata*		Gupta (1997)
B. juncea x *B. carinata*		Katiyar &Chamola (1995)
B. juncea x *B. carinata*		Krishnia *et al.* (2000)*
B. juncea x *B. carinata*		Kumar *et al.* (2002)*
B. juncea x *B. carinata*		Rao & Shivanna (1997)
B. juncea x *B. carinata*		Sharma & Singh (1992)
B. juncea x *B. carinata*		Singh *et al.* (1997)
B. juncea x *B. maurorum*		Bijral *et al.* (1995)
B. juncea x *B. napus*	S	Alam *et al.* (1992)
B. juncea x *B. napus*	S	Bing *et al.* (1991; 1996)
B. juncea x *B. napus*	S	Choudhary & Joshi (1999)
B. juncea x *B. napus*	S	GoshDastidar & Varma (1999)
B. juncea x *B. napus*	S	Gupta (1997)
B. juncea x *B. napus*		Shen *et al.* (2006)*
B. juncea x *B. napus*		Warwick (2007)*
B. juncea x *B. napus*		Rao & Shivanna (1997)
B. juncea x *B. napus*		Sharma & Singh (1992)
B. juncea x *B. napus*		Vijayakumar *et al.* (1994)
B. juncea x *B. nigra*	S	Bing *et al.* (1991)
B. juncea x *B. nigra*		Prasad *et al.* (1997)
B. juncea x *B. nigra*		Rao & Shivanna (1997)
B. juncea x *B. oleracea*		Gupta (1997)
B. juncea x *B. oxyrrhina*		Bijral & Sharma (1999)
B. juncea x *B. rapa*		Choudhary *et al.* (2002)*
B. juncea x *B. rapa*		Gupta (1997)
B. juncea x *B. rapa*	S	Gupta *et al.* (2006)*
B. juncea x *B. rapa*		Katiyar & Chamola (1995; 1998)
B. juncea x *B. rapa*	S	Rhee *et al.* (1997)
B. juncea x *B. rapa*		Sharma & Singh (1992)
B. juncea x *B. rapa*	S	Choudhary & Joshi (1999)
B. juncea x *B. rapa*	S	GoshDastidar & Varma (1999)
B. rapa x *B. juncea*		Prasad *et al.* (1997)

B. juncea x *Diplotaxis muralis*		Bijral & Sharma (1995)
D. muralis x *B. juncea*		Gupta (1997)
B. juncea x *Eruca sativa*		Bijral & Sharma (1999)
B. juncea x *Eruca sativa*	U	GoshDastidar & Varma (1999)
B. juncea x *Orychophragmus violaceus*		Li *et al.* (1998, 2003*)
B. juncea x *Raphanus sativus*	U	Gupta (1997)
B. juncea x *Raphanus sativus*	S	Rhee *et al.* (1997)
B. juncea x *Sinapis alba*		Bijral *et al.* (1991)
B. juncea x *Sinapis alba*		Sharma & Singh (1992)
B. juncea x *Sinapis arvensis*	U	Bing *et al.* (1991)
B. tournefortii x *B. juncea*		Gupta (1997)
Diplotaxis siifolia x *B. juncea*		Gupta (1997)
D. tenuifolia x *B. juncea*	U	Salisbury (1989)
(*D. erucoides* x *B. rapa* F1) x *B. juncea*		Malik *et al.* (1999)*
(*B. juncea* x *B. napus* F1) x *B. juncea*		Frello *et al.* (1995)
(*B. carinata* x *B. juncea* F1) x B. carinata		Getinet *et al.* (1994; 1997)
(*B. napus* x *B. juncea* F1) x *B. juncea*		Kirti *et al.* (1995)

Sexual hybrids derived through crosses among the crop relatives of *B. juncea* and the most prevalent weedy species found in Canada have been reported as follows in Warwick *et al.* (2000b):

Table 2. Reports of sexual hybridization among Brassica napus, B. rapa and related species. Where reciprocal crosses were attempted, they are classed as either successful (S) or unsuccessful (U). From Warwick *et al.* (2000b).

Cross	Reciprocal	Reference
B. napus x *B. nigra*	S	Bing *et al.* (1991)
B. napus x *D. muralis*		Bijral & Sharma (1996)
D. muralis x *B. napus*		Fan *et al.* (1985)
D. muralis x *B. napus*		Gupta (1997)
D. muralis x *B. napus*		Ringdahl *et al.* (1987)
D. muralis x *B. napus*	U	Salisbury (1989)
B. napus x *E. gallicum*	U	Lefol *et al.* (1997)
B. napus x *R. raphanistrum*		Baranger *et al.* (1995)
B. napus x *R. raphanistrum*		Chadoeuf *et al.* ((1998)
B. napus x *R. raphanistrum*	S	Darmency *et al.* (1998)
B. napus x *R. raphanistrum*		Eber *et al.* (1994)
B. napus x *R. raphanistrum*	U	Lefol *et al.* (1997)

B. napus x *R. raphanistrum*	S	Rieger *et al.* (1999)
B. napus x *R. sativus*	U	Gupta (1997)
B. napus x *S. alba*		Bijral *et al.* (1993)
B. napus x *S. arvensis*	U	Moyes *et al.* (1999)
S. arvensis x *B. napus*		Leckie *et al.* (1993)
(*B. napus* x *R. raphanistrum* F1) x *R. raphanistrum*		Chèvre *et al.* (1997; 1998)
(*B. napus* x *S. alba* F1) x *B. napus*		Bijral *et al.* (1994)
(*B. napus* x *S. arvensis* F1) x *B. napus*		Inomata (1997)
B. rapa x *B. nigra*	U	Bing *et al.* (1991)
B. rapa x *B. nigra*		Mattsson (1988)
B. rapa x *B. nigra*		Prasad *et al.* (1997)
B. rapa x *E. gallicum*	U	Lefol *et al.* (1997)
B. rapa x *R. sativus*	S	Ellerström (1978)
B. rapa x *R. sativus*	U	Gupta (1997)
B. rapa x *R. sativus*	S	Rhee *et al.* (1997)
D. muralis x *B. rapa*	U	Salisbury (1989)

Seed from interspecific crosses should be checked for hybridity, as matromorphic seed is often produced rather than true hybrid seed (Salisbury, 2006).

For a trait to become incorporated into a species genome, recurrent backcrossing of plants of that species by the hybrid intermediates and survival and fertility of the resulting offspring would be required.

C2. Potential for Introgression of Genes from *Brassica juncea* into Relatives

For successful gene transfer to occur between species, a number of requirements must be met. Pre-fertilization factors include physical proximity, pollen movement, pollen longevity, synchrony of flowering, breeding system, floral characteristics and competitiveness of foreign pollen. Post-fertilization factors include sexual compatibility, hybrid fertility, viability and fertility of progeny through several generations of backcrossing and successful introgression of the gene into the chromosomes of the recipient species (Salisbury, 2006).

Bing *et al.* (1991) reported that of the crosses with *B. juncea* that they attempted, there was a potential for hybrids with *B. napus*, and *B. rapa* to produce viable seed that could survive to the next generation. All three species are grown as canola crops in western Canada, with *B. napus* currently the dominant species. In field co-cultivation experiments with *B. juncea* and *B. napus*, Bing *et al.* (1996) found five interspecific hybrids out of 469 plants when *B. napus* was the female and three out of 990 when *B. juncea* was the female. Successful transfer of genes from *B. rapa* to *B. juncea* has been reported (Love *et al.*, 1990) as well as transfer of genes from *B. juncea* to *B. carinata* (Getinet *et al.*, 1994). Warwick (2007) reported on field experiments where gene flow from herbicide resistant *B. napus* to neighbouring fields of *B. juncea* was measured and found to be 0.245% at the field border and 0.005% at 200 m

Sinapis arvensis is the worst of the weedy relatives of *B. juncea* in Canada. Hybrids (2.5% frequency) were obtained between *Brassica juncea* and *Sinapis arvensis* in controlled greenhouse studies (Bing *et al.* 1991), when emasculated plants of *B. juncea* served as the female parent, but not for the reciprocal cross. Seed produced on backcross F1 x *B. juncea* failed to germinate and the one seed from F1 x *S.*

arvensis developed into a weak, male sterile plant which produced no seed on open pollination. No gene flow was detected from *B. juncea* to 45 plants of *S. arvensis* grown together in a small field plot experiment in Saskatchewan (Bing *et al.* 1991, 1996); however hybrid detection was based primarily on morphological characters and very small sample sizes. In a field co-cultivation experiment between *B. juncea* and *S. arvensis* (pollen recipient), where use of a herbicide resistance marker allowed for screening of larger numbers of seedlings, hybrid plants were detected but at a very low frequency (Warwick, 2005). Only two hybrids were obtained from 109,951 screened seedlings, i.e frequency of 1.8 x 10-5. One F1 hybrid was able to set seed when selfed, and many of the subsequent selfed F2, F3 and F4 hybrid generation plants derived from this plant showed vigorous growth and high pollen fertility levels. Herbicide resistance persisted in the F2, F3 and F4 hybrid generations. However, no backcross progeny were produced when the F1 hybrid nor when self-derived hybrids were backcrossed to *S. arvensis*, confirming the results obtained by Bing *et al.* (1991). The likelihood of introgression of traits from *B. juncea* to *S. arvensis* appears to be low to negligible.

Lefol *et al.* (1997) investigated the production of hybrid seeds between *B. juncea* and *Erucastrum gallicum* or *Raphanus raphanistrum* using reciprocal crosses. They did not use embryo rescue so their measurements were of seed production that might occur under natural conditions. The *R. raphanistrum* x *B. juncea* cross failed to produce any seed and the viable seed produced from all the other crosses were not considered to be hybrids. Therefore, the probability of intergeneric crosses between these two weedy species and *B. juncea* appears to be low.

Brassica nigra is an uncommon weed in western Canada. Bing (1991) reported that *B. juncea* and *B. nigra* had relatively high cross compatibility in hand-crossing, especially when *B. juncea* was used as the female. Extensive back-crossing to *B. nigra* failed to produce seed. No interspecific hybrids were found with field crossing. It was concluded that there are strong natural barriers to gene flow from *B. juncea* to *B. nigra*.

C3. Occurrence of Related Species of *Brassica juncea* in Canada

Of the species in the above listed crosses, *B. carinata*, *B. maurorum*, *B. oxyrrhina*, *B. tournefortii*, *Diplotaxis siifolia* and *Orychophragmus violaceus* are not reported as present in Canada (Darbyshire, 2003, Frankton and Mulligan, 1987, Warwick, 1999). *Brassica oleracea*, apart from the wild types in its original habitats in Europe, is rarely found outside of cultivation, but there are a few small, naturalized populations in coastal areas of BC. Of the other species:

Brassica juncea is recorded in Northwest Territories, (NT), Newfoundland (NF), Prince Edward Island (PE), Nova Scotia (NS), New Brunswick (NB), Quebec (QC), Ontario (ON), Manitoba (MB), Saskatchewan (SK), Alberta (AB), and British Columbia (BC). Frankton and Mulligan (1987) report that *B. juncea* occurs in every province and reaches its greatest abundance in the western provinces.

Brassica napus is recorded in NT, Labrador (LB), NF, PE, NS, NB, QC, ON, MB, SK, AB, and BC. *B. napus* is not listed in Frankton and Mulligan (1987) but has become increasingly abundant as a volunteer weed. It ranked 18th in abundance during the 1970s and had risen to 14th since 2000 (Leeson *et al.*, 2005).

Brassica nigra is recorded in NF, NS, PE, NB, QC, ON, SK, AB, and BC. Frankton and Mulligan (1987) suggest that it is not very common in western Canada.

Brassica rapa is recorded in NT, Yukon Territory (YK), LB, NF, NS, PE, NB, PQ, ON, MB, SK, AB, and BC. Frankton and Mulligan (1987) suggest that it is sometimes abundant and that in some parts of the East, the wild form, bird rape, supplants *S. arvensis* over large areas.

Diplotaxis erucoides is recorded in QC only (Warwick, 1999).

Diplotaxis muralis is recorded in NS, PE, NB, QC, ON, MB, SK, AB, and BC. Darbyshire (2003) lists it as an occasional weed of shores, railway lines, roadsides and disturbed areas.

Diplotaxis tenuifolia is recorded in NS, NB, QC, ON and BC. It is a perennial found occasionally on railway lines, roadsides and other disturbed areas (Darbyshire, 2003).

Eruca sativa is recorded in AB, SK, ON, QC and possibly MB as a rare escape and contaminant in cultivated fields, roadsides and waste places (Warwick, 1999).

Erucastrum gallicum is recorded in NF, NS, PE, NB, QC, ON, MB, SK, AB, and BC. Frankton and Mulligan (1987) state that it reaches its greatest abundance in MB and SK where it inhabits fields, waste areas, railways, gardens and orchards. It is very common on road sides and is an abundant field weed in many localities in western Canada (Warwick and Wall, 1998).

Raphanus raphanistrum is recorded in LB, NF, NS, PE, NB, QC, ON, MB, SK, AB, and BC.

Frankton and Mulligan (1987) state that this species is very abundant in all provinces on the Atlantic seaboard. In Quebec and Ontario, it is of less importance and is reported to occur in the moister areas of Manitoba and Saskatchewan. According to Warwick and Francis (2005), it is no longer present in Saskatchewan and Manitoba and has a limited distribution in Alberta.

Raphanus sativus is recorded in NF, NS, PE, NB, PQ, ON, MB, and BC. Frankton and Mulligan (1987) indicate that this species is occasionally persistent in gardens as a result of cultivation.

Sinapis alba is recorded in NT, YK, LB, PE, NB, QC, ON, MB, SK, AB, and BC. It is cultivated as yellow or white mustard and is an uncommon weed in cultivated fields, prairies, roadsides and disturbed areas (Darbyshire, 2003).

Sinapis arvensis is recorded in NT, YK, LB, NF, NS, PE, NB, PQ, ON, MB, SK, AB, and BC. Frankton and Mulligan (1987) list it as one of the common annual weeds. It occurs in all provinces, but is a minor weed east of Manitoba. In the prairie provinces, it is most abundant in Manitoba, with lower relative abundance in Saskatchewan and Alberta (Warwick *et al.*, 2000a). Its habitats include grain fields, cultivated fields, waste areas, fence rows, and road sides.

C4. Summary of the Ecology of Relatives of *Brassica juncea*

Of the *B. juncea* relatives already discussed, *S. arvensis* and *R. raphanistrum* are listed as primary noxious weeds in the Weed Seeds Order (2005) and *E. gallicum* is listed as a secondary noxious weed. These three species are potentially the weediest in the agricultural croplands. All are relatively easily controlled in crops other than those of the Brassica group by the use of selective herbicides.

The abundance of these three species in agricultural croplands is partly determined by cropping practices. The prominence of weed species can be dramatically affected by cropping systems and cultivation practices. The recent adaptation of minimum or no-till crop production system and the abandonment of cultivated summer fallow practices as a means of soil cultivation have caused a shift in the prominence of different weed species.

The above listed species are all plants of "disturbed land" habitats. Their success will be dependent on their ability to compete for space with other primary colonizers, in particular with other successful weedy plant types. This in turn will depend on how well suited they are to the particular climate, soil conditions, environment, etc. of individual sites.

Part D - Potential Interactions of *Brassica juncea* with Other Life Forms During its Life Cycle

Table 3 is intended to guide applicants in their considerations of potential impacts that the release of the PNT in question may have on non-target organisms, but should **not be considered as exhaustive**. Where the impact of the PNT on another life form (target or non-target organism) is significant, secondary effects may also need to be considered.

Table 3. Examples of potential interactions of *Brassica juncea* with other life forms during its life cycle in a natural environment. Pathogens and insect pests are from Diseases of Field Crops in Canada (Canadian Phytopathological Society, 2003) and the Canola Growers Manual (Thomas, 2003).

Other Life Forms	Interaction with *Brassica juncea* (Pathogen; Symbiont or Beneficial Organism; Consumer; Gene transfer)
Albugo candida (white rust, staghead)	Pathogen
Alternaria spp. (alternaria blackspot)	Pathogen
Fusarium spp. (damping off, brown girdling root rot, Fusarium wilt, foot rot)	Pathogen
Leptosphaeria maculans, L. *biglobosa* (blackleg)	Pathogen
Peronospora parasitica (downey mildew)	Pathogen
Plasmodiophora brassicae (clubroot)	Pathogen
Pseudocercosporella capsellae (grey stem)	Pathogen
Pythium spp. (damping off)	Pathogen
Rhizoctonia solani (damping off, brown girdling root rot, foot rot)	Pathogen
Agrotis orthogonia (pale western cutworm)	Consumer
Autographa californica (alfalfa looper)	Consumer
Ceutorhynchus obstrictus (cabbage seedpod weevil)	Consumer
Delia spp. (root maggot)	Consumer
Dicestra trifolii (clover cutworm)	Consumer
Entomoscelis americana (red turnip beetle)	Consumer
Euxoa ochrogaster (red-backed cutworm)	Consumer
Laxostege sticticalis (beet webworm)	Consumer
Lygus spp. (Lygus bugs)	Consumer
Mamestra configurata (bertha armyworm)	Consumer
Plutella xylostella (diamondback moth)	Consumer
Phyllotreta spp. (flea beetles)	Consumer
Pollinators	Symbiont, Consumer

Mycorrhizal fungi	Symbiont
Birds	Consumers
Animal browsers	Consumers
Soil microbes	Symbiont
Earthworms	Beneficial organism
Other *Brassica juncea*	Gene transfer
Brassica napus	Gene transfer
Brassica rapa	Gene transfer
Brassica nigra	Gene transfer
Raphanus raphanistrum	Gene transfer
Erucastrum gallicum	Gene transfer
Sinapis arvensis	Gene transfer

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