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# Outcrossing Potential for *Brassica* Species and Implications for Vegetable Crucifer Seed Crops of Growing Oilseed *Brassicas* in the Willamette Valley

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# Outcrossing Potential for *Brassica* Species and Implications for Vegetable Crucifer Seed Crops of Growing Oilseed *Brassic*as in the Willamette Valley

James R. Myers

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## Summary

The oilseed mustards known as canola or rapeseed (*Brassica napus* and *B. rapa*) are the same species as some vegetable crucifers and are so closely related to others that interspecific and intergeneric crossing can occur.

**Intraspecific crosses** (within the same species) readily occur among the following:

- *B. napus* canola with rutabaga and Siberian kale
- *B. rapa* canola with Chinese cabbage, Chinese mustard, pai-tsai, broccoli raab, and turnip

**Interspecific crosses** (between different species) can occur among the following:

- **Occur readily:** *B. napus* canola with Chinese cabbage, Chinese mustard, pai-tsai, broccoli raab, and turnip
- **Occur more rarely:** *B. napus* or *B. rapa* canola with the *B. oleracea* cole crops (cabbage, kohlrabi, Brussels sprouts, broccoli, cauliflower, collards, and kale)

**Intergeneric crosses** (between species of different genera) are possible with varying degrees of probability:

- *B. napus* or *B. rapa* canola with wild and cultivated radish (*Raphanus raphanistrum* and *R. sativus*)

Many factors affect the probability of an interspecific cross, but the most important is proximity of the two species. Many interspecific crosses need to occur for a few to succeed. Thus, hybrid seeds rarely are detected more than 50 meters (165 feet) from the pollen-supplying parent. Because both wind and insects transfer pollen, very rare outcrosses can be detected up to 4 kilometers (2.4 miles) away under special circumstances. However, a distance of 2 kilometers (1.2 miles) should be sufficient for stock seed production.

Although it is relatively easy to maintain adequate distance between fields with pinning maps, other sources of canola seed present a greater threat to vegetable seed growers. The two greatest threats are canola seed blown from vehicles onto road shoulders and volunteers in fields previously planted to canola. Detecting and eliminating volunteers from a 2-kilometer radius around a seed field would be onerous and perhaps impossible.

The introduction of genetically modified, herbicide-tolerant canola also constitutes a threat to vegetable seed production. Herbicide resistance is unlikely to become established in weedy species or seed crops. However, transgenes can be detected at very low frequency and would make a seed crop unsuitable for some markets.

The best solution for introduction of canola into the Willamette Valley would be to maintain zones free of canola plantings and from traffic carrying canola seeds to crushing plants.

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## Introduction

The proposal to introduce substantial canola (*Brassica napus*) acreage into the Willamette Valley for biodiesel production has caused concern among *Brassica* and *Raphanus* vegetable seed growers. The vegetable species of the family Brassicaceae readily outcross, with pollen transferred by insects. Seed growers are concerned about the potential for canola pollen to contaminate their seed crops.

In this paper, I describe species relationships, discuss the literature on interspecific hybridization, and describe potential impact on vegetable seed production.

## Identification of Brassicaceae species

The family Brassicaceae has more than 3,000 species in 370 genera, a number of which have been brought into cultivation. Most rapeseed and canola are of the species *B. napus*, although some cultivars are *B. rapa*.

The vegetable Brassicaceae include the following:

- *B. napus* (rutabaga, Siberian kale)
- *B. rapa* (Chinese cabbage, pai-tsai, mizuna, Chinese mustard, broccoli raab, and turnip)
- *B. oleracea* (cabbage, broccoli, cauliflower, Brussels sprouts, kohlrabi, collards, kale)
- *Raphanus sativus* (radish)

Condiment crops include *B. nigra* (black mustard), *B. carinata* (Ethiopian mustard), *B. juncea* (brown or Indian mustard), *Armoracia rusticana* (horseradish), and a number of minor potherbs and salad vegetables.

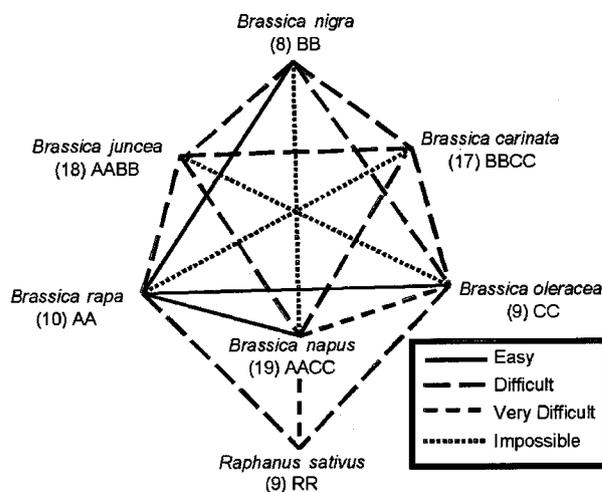
In the Willamette Valley, weedy crucifers of economic significance include black mustard (*B. nigra*), birdsrape mustard (*B. rapa*), wild mustard (*Sinapsis arvensis*; synonyms *B. arvensis*, *B. kaber*, *S. kaber*), wild radish (*R. raphanistrum*), sheperdspurse (*Capsella bursa-pastoris*), and bittercress (*Cardamine hirsute*) (E. Peachey, Oregon State University, personal communication).

## Brassica species relationships

Brassica species relationships are complicated by the fact that new species have arisen through the fusion of two progenitor species. The relationships among species were described in the classic work by U in 1935, in what is now termed the Triangle of U (Figure 1).

At the tips of the triangle are the species with the fewest chromosomes—*B. nigra* (N=8), *B. oleracea* (N=9), and *B. rapa* (N=10). These species are diploid, meaning they have two sets of chromosomes. N is the number of chromosomes found in either the pollen or egg, thus one-half the number found in the adult plant.

At some time in the past, the diploid species spontaneously hybridized to produce the allotetraploids. These species have four sets of chromosomes, two from each parent



**Figure 1.** Triangle of U (1935) (modified to include radish) showing genome relationships among cultivated *Brassica* species. Genomes are represented by letters (A, B, C, or R), and haploid chromosome numbers are enclosed in (). Lines represent ease with which species can be crossed. Diagram was modified to include more recent data on crossing among species, including with *Raphanus*.

species. They include *B. carinata* (N=17), *B. juncea* (N=18), and *B. napus* (N=19).

There are two ways in which fertile allotetraploids can occur. In one case, unreduced gametes join. (Unreduced gametes are pollen or eggs having the full parental number of chromosomes rather than half the number.) In the other, normal fertilization occurs, followed by doubling of the chromosomes in the hybrid plant.

Because allotetraploid *Brassica* species share a genome with their diploid parents, gene flow can continue in both directions. Of particular relevance to the potential for outcrossing between vegetable and oilseed *Brassicas* is the fact that *B. napus* was derived when *B. rapa* hybridized with *B. oleracea* (Figure 1).

Although rare, allopolyploids have occurred repeatedly in nature and have been resynthesized by plant geneticists.

## **Pollen dispersion in *Brassicas***

Most *Brassica* pollen disperses to within 10 meters (33 feet) of its source (Nieuwhof, 1963; Anonymous, 2002), although transfer has been detected up to 3 or 4 km (1.8 to 2.4 miles) away (Rieger et al., 2002; Anonymous, 2002). Pollen may be moved by wind as well as by insects. Wind-transferred pollen has been detected up to 1.5 km (0.9 mile) from the source plant (Timmons et al., 1995).

Pollen can live up to 4 or 5 days when temperatures are low and humidity is high. With warm temperatures and low humidity, survival time may drop to 1 or 2 days.

## **Probability of intraspecific crosses**

Rutabaga and Siberian kale are the same species as *B. napus* oilseed cultivars and will cross readily with *B. napus* canola. Turnip,

Chinese cabbage, and related Asian vegetables are the same species as *B. rapa* oilseed cultivars and cross readily with them.

With outcrossing rates of 10 to 50 percent in canola (Anonymous, 2002), it is very likely that gene flow would occur between canola and rutabaga or Siberian kale. The amount of gene flow would depend on many factors: number of acres planted to canola, field distance, factors affecting pollen viability, and types of pollinators present.

## **Probability of interspecific hybridization**

Many *Brassica* species show a high degree of relatedness, which allows crossing to occur across species and even genera (for example, wild radish with canola). Intercrossing occurs with varying degrees of difficulty.

Information about interspecific hybridization comes from two sources: (1) crosses attempted by plant breeders and geneticists and (2) natural hybrids found in the field.

Data from artificial hybridization are difficult to use in predicting potential for natural crossing because special techniques such as embryo rescue may be used to obtain viable offspring. However, plant breeders normally report the number of successful crosses compared to the number of attempts, as well as whether artificial measures were used. Thus, these data do provide a measure of the relative ease of hybridization.

In Figure 1, the different types of lines drawn between the species indicate the relative ease of interspecific hybridization based on available data (Anonymous, 2002; Bothmer et al., 1995; Davey, 1939; Honma and Summers, 1976; Yarnell, 1956).

## **Canola with *B. rapa* and *B. napus* vegetables**

Pollen movement from canola to related species has been detected under field conditions. *B. rapa* shares the A genome with

**Table 1. Potential for gene flow between canola (*B. napus*) and selected Brassicaceae species<sup>1</sup>**

Category	High	Moderate	Low
Artificial hybrids	Yes	Yes	Yes
Field hybrids	Yes	Yes	Not reported
Gene introgression	Yes/Likely <sup>2</sup>	Yes	Yes
Weeds	<i>B. rapa</i>	<i>R. raphanistrum</i>	—
Cultivated species	<i>B. napus</i> and <i>B. rapa</i> vegetables	—	<i>B. nigra</i> <i>B. oleracea</i> <i>R. sativus</i>

<sup>1</sup> Modified from Table 2 in Anonymous, 2002.

<sup>2</sup> Considered likely to happen over a period of time if the species are in physical proximity and have flowering synchrony.

*B. napus* (Figure 1). Several researchers have documented gene flow between these species in both directions (Bing et al., 1996; Jorgensen et al., 1996; Ellstrand et al., 1999; Wilkinson et al., 2000). Anonymous (2002) considers potential for gene flow between *B. napus* and *B. rapa* to be high (Table 1).

#### **Canola with *B. rapa* weedy mustards**

Hybridization frequencies were low (0.4 to 1.5 percent) between canola and weedy mustard plants growing just outside a field (Scott and Wilkinson, 1998). Seeds tended to be small, seedling survival was low, and fitness and fertility were reduced. With backcrosses between the hybrids and either parent, both fitness and fertility improved.

In a large-scale (15,000 km<sup>2</sup>) survey of gene flow from canola into weedy *B. rapa* in Great Britain, one hybrid was identified (Wilkinson et al., 2000). The scarcity of hybrids was due in part to the lack of overlap between cultivated canola fields and the riparian habitat preferred by *B. rapa*.

#### **Canola with *R. raphanistrum* (wild radish) and *R. sativum* (cultivated radish)**

Wild radish (*R. raphanistrum*) hybridizes spontaneously with *B. napus* to produce viable hybrids (Baranger et al., 1995). Hybrid seeds generally were smaller than normal seeds, allowing seed size to be used as a way to identify hybrids. Backcrosses to *B. napus* as the pollen parent did show a low level of fertility. Wild radish seems to cross more readily with *B. napus* than does cultivated radish (*R. sativum*) (Anonymous, 2002).

#### **Canola with *B. oleracea* vegetables (cabbage, broccoli, cauliflower, Brussels sprouts, kohlrabi, collards, kale)**

Very little information exists on potential for natural hybridization between *B. napus* and *B. oleracea*. Hybrids have been made using controlled crosses and embryo rescue (Chèvre et al., 1996; Honma and Summers, 1976; Kerlan et al., 1992 and 1993). No researcher has obtained seed from natural crossing studies or without the assistance of embryo rescue.

However, not many research programs have attempted to hybridize these two species. As such, only a limited number of different parental combinations have been tried, and it may be possible that with the right combination, natural hybridization would produce viable offspring.

Of the hybrids made through controlled crosses, two kinds of hybrids have been observed from crosses of *B. napus* and *B. oleracea*: triploids (ACC) and amphidiploids (AACCCC). These hybrids probably resulted from union of an unreduced gamete from *B. oleracea* with a reduced (normal) gamete from *B. napus*.

Chèvre et al. (1996) examined the potential for natural gene flow over two generations of backcrossing. They found increased fertility in each successive generation, particularly when *B. napus* was the maternal parent. It appears that once the F<sub>1</sub> interspecies hybrid has been made, backcrossing to either parent can produce viable and partially fertile offspring.

Wilkinson et al. (2000) also examined wild *B. oleracea* populations for proximity to canola fields and for the formation of natural hybrids. *B. oleracea* is restricted to maritime cliff habitats in Great Britain, so only one population was found to be within 50 meters (165 feet) of canola production. None of the nine new seedlings found in that population was hybrid.

There is a need for additional research to address the question of the potential for natural interspecific hybridization between *B. napus* and *B. oleracea*.

## Concerns about genetically modified canola

Genetically modified canola presents the greatest risk to vegetable crucifer seed crops. Although it is very unlikely that transgenes would persist once transferred to the seed crop, the presence of the gene would make the seed crop unsuitable for markets that have strict tolerances on GMO contamination.

Transgenes are relatively easy to detect at very low levels, so it is likely that their presence could be detected even if only a few interspecific hybrids were found in a vegetable seed lot. Contamination could still be detected even if interspecific seeds were nonviable.

## Minimizing the risk of outcrossing

Most hybrids that have been detected were from plants within the same field or closely adjacent areas. Thus, distance is a key to preventing pollen contamination of vegetable crucifer seed crops. Based on the literature, an absolute minimum distance of 2 km (1.2 miles) between canola crops and seed fields would minimize chance crossing. With pinning maps, it is relatively easy to maintain these distances.

These distances, however, do not take into account spread of canola seed along roadways after harvest. Additional measures would be needed to make sure that seed does not blow out of harvest vehicles, and seed growers would need to police roadways near their fields for volunteer canola plants.

Volunteer plants in fields previously planted to canola might be another source of contamination if a grower unknowingly plants a seed crop near a former canola field.

## Conclusions

There has been little study of gene flow between *B. napus* and *B. oleracea* or *R. sativum* under natural conditions. Further research is warranted to determine the overall risk in the Willamette Valley. Based on studies elsewhere of gene flow into weed species, we can assume interspecific hybridization will occur in certain species combinations, although probably at a low level.

Because of the dollar value per unit area and the small size of seed production fields, seed companies and growers will seek to minimize the risk of contamination. Thus, they may move their seed crops out of the valley if canola production becomes widespread. The best solution at present is to maintain canola-free zones for vegetable seed production. These zones should not allow canola production or traffic bearing seeds.

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