

# A review of *Brassica* species, cross-pollination and implications for pure seed production in New Zealand

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## Abstract

The various subspecies, forms and relationships between Brassica species and their relatives are outlined. The extent of seed set following handcrossing between them is reviewed, to determine a crossing relationship between the species. The factors affecting contamination in pure seed production are reviewed in relation to the pollen source, pollen transport by wind, insects and animals, bee behaviour, shifting of bee hives, calculation of pollination spread over distance, border zones around crops, flowering time and length of flowering period, self-incompatibility, pollen receptivity and competition on the stigma, pollination groups, seed size of resulting hybrids, germination of hybrids, morphological appearance of contaminant plants, expected standards of purity, paddock history, removing off-types and machinery cleanliness.

**Additional key words:** seed contamination, pollen. hybrids.

## Introduction

The Canterbury region of New Zealand produces many seed crops of Brassica, radish and mustard, but in contrast to many overseas production regions there is little production of oilseed Brassica crops. Historically seed production of Brassicas was dominated by the three fodder species *B. campestris*, *B. napus* and *B. oleracea*. The risks of cross-pollination among these crops were well understood with a scheme giving regional separation of seed production between Coastal Otago (thousand-headed kale), Central Otago (turnips and swedes) and inland Canterbury (rape and chou moellier) (Claridge, 1972). This also gave some separation from the worst of the wild turnip infestations in the spring cropping zone. As the range of species and types grown for seed in Canterbury has now increased, it is important to understand the risks to cultivar purity because of the many different Brassica species grown; many are hybrids demanding a much higher cultivar purity standard. Seed crops now grown include traditional fodder species such as turnips, swede, rape and kale, the traditional vegetable Brassicas, miscellaneous Asian vegetable crops, radish and mustard as well as seed multiplications of oilseed types for Europe and North America.

Knowledge of cross pollination in Brassicas has expanded in recent years as a result of the many studies done to determine the gene flow from genetically modified (GM) crops to their closely related crop and weed species.

This paper sets out the relationship among the Brassica species, radish and white mustard (*Sinapis*

*alba*), discusses the different forms within each species, and reviews the seed set when these different species are hand crossed. It then goes on to look at factors affecting cross-pollination in the field such as flowering time, isolation distance, contamination of crops by weedy Brassicas and off-types, self-incompatibility, mixed pollen competition, pollinator behaviour, topographical barriers, prevailing winds, contamination brought about by shifting bee hives and by people walking among crops. Lastly it makes some practical recommendations for isolation and practices to ensure effective pure seed production.

## Relationship among the Crop Species

The commercial Brassica species vary in chromosome number and have an interesting genetic relationship to each other. There are three diploid species with three tetraploid hybrids between them. The relationship can best be drawn as a triangle (Figure 1), which is known as the triangle of U after the Japanese-Korean Professor Nagaharu U (or Chang Choon Woo as he was known in Korea) who first unravelled it (U, 1935). This situation is further complicated by the recent development of tetraploid forms of the two diploid species, *B. campestris* and *B. oleracea*.

The three additional species grown in New Zealand, radish (*Raphanus sativus*), white mustard (*Sinapis alba*, also called *Brassica hirta*) and Rocket (*Eruca sativa*) are not included in this relationship (Prakash and Chopra, 1991). Radish has a chromosome number of 18, the same as *Brassica*

*oleracea* and can be considered to be more closely related to this than the other Brassica crop species. White mustard has a chromosome number of 24 and is quite distinct from all the Brassica crop species.

Rocket, another related crop occasionally grown has a chromosome number of 22 and is quite distinct from all the Brassica crops grown in New Zealand.

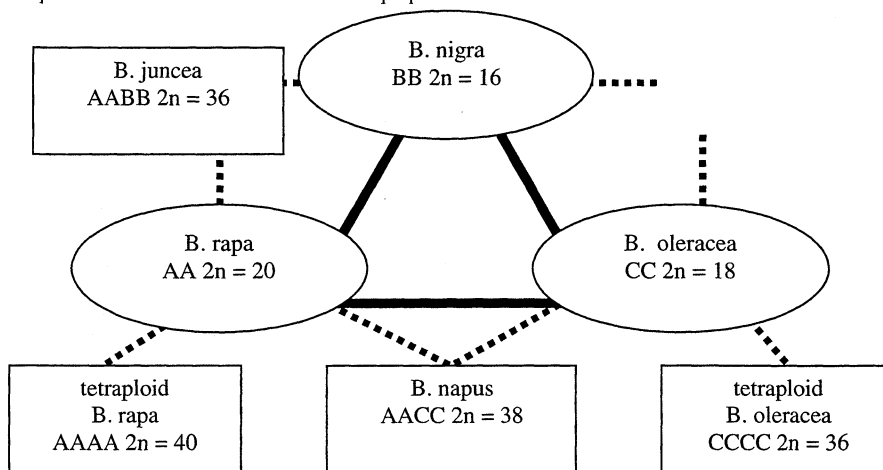


Figure 1. The relationship between the Brassica species, known as the “Triangle of U.”

#### The Forms within each Species

Over many centuries of cultivation and unconscious selection by ancient cultures the primitive annual Brassicas have developed from a simple plant form akin to our wild turnip, to a series of forms with enlarged storage organs suitable for human consumption. Types now exist with a range of enlarged organs such as the swollen hypocotyl of turnip and swede, the enlarged stem of kohlrabi, a mass of leaves as in forage kale and rape, or heart of leaves as in cabbages, enlarged petioles in some Asian vegetables, swollen stem buds as in Brussels sprouts, enlarged floral buds as in broccoli and cauliflower, and a much increased seed production as in oilseed rapeseed. Although all these forms occur within the 6 Brassica species not all these forms occur within each species. For example *Brassica oleracea* does not have a bulb form, *Brassica napus* does not have a Brussels sprout or broccoli form and *Brassica nigra* lacks almost all of these advanced forms.

Often species are mixed before use as in the case of the condiment mustards. Here seed of Black mustard (*B. nigra*), is often mixed with Brown mustard (*B. juncea*) and white mustard (*Sialba alba*). Occasionally Ethiopian mustard (*B. carinata*) and

even oilseed rape (*B. napus*) is also added, with each component giving a slightly different taste with some reacting faster and some lingering longer.

Recently, Brassicas have been used for biofumigation purposes due to their range of glucosinolates and their gaseous by-products, some of which can reduce fungi, bacteria and insect problems when they are grown in a crop rotation.

#### *Brassica rapa* (syn. *B. campestris*)

There are a wide range of plant forms within *B. rapa* as this species has two centres of origin, Europe and Asia and a long history of cultivation in both regions (Table 1). The various forms have been reviewed from differing perspectives (Sun, 1946; Li, 1980a; Cao, 1986; Batra, 1977; Prakash and Hinata 1980; Yamagishi *et al.*, 1985; Toxopeus *et al.*, 1988; Gladis and Hammer, 1992; Warwick and McDonald, 2001).

#### Ploidy differences

In nature *B. rapa* is a diploid but plant breeders have developed some tetraploid crops, notably fodder turnips and turnip rapeseed. These are often grown in Canterbury for seed.

The wild turnip is a common weed of seed production regions and can pose considerable risk

for pure seed production of many Brassicas as described later on this paper.

### ***Brassica napus***

*Brassica napus* is an allotetraploid species with a chromosome number of 38. It originates from crosses of *B. oleracea* and *B. rapa*, with some lines having *B. oleracea* as the maternal parent and others having *B. rapa* (Sarashima and Matsuzawa, 1989; Song *et al.*, 1994). There is a limited range of forms within *B. napus* as this species has a European origin tracing back only 500 - 2000 years depending on the form (Table 2). Many modern *B. napus* cultivars have been synthesised by crossing these two parent species and doubling the chromosome number (Tsunoda *et al.*, 1980). At the chromosome level these two groups can differ

slightly as the older forms of *B. napus* have undergone chromosomal rearrangements over time (Paterson *et al.*, 2000), resulting in some sterility in crosses between the two groups.

There are naturalised forms in New Zealand in coastal regions (P. Palmer pers. comm.) but for seed production purposes cultivated “escapes” pose more risk.

### ***Brassica oleracea***

There is a wide range of forms within *B. oleracea* as this species has an origin dating back to early agriculture in Europe and the Middle-East (Table 3). At the chromosome level this wide range of forms is associated with chromosomal rearrangements (Paterson *et al.*, 2000).

**Table 1. Forms within *Brassica rapa*.**

<b>Name</b>	<b>Subspecies</b>	<b>Use</b>	<b>Usual sowing time*</b>
Pak choi, Chinese mustard	<i>chinensis</i>	large petiole and leaf vegetable	Spring
Mizuna, Mibuna, Potherd mustard	<i>nipposinica</i> or <i>japonica</i>	leaf vegetable with very many small leaves	Spring
Taatsai, Taku tsai	<i>narinosa</i> or <i>rosularis</i>	Thick leaf vegetable	Spring
Komatsuna, Spinach mustard	<i>perviridis</i>	Leaf vegetable	Spring
Chinese cabbage, petsai	<i>pekinensis, glabra</i>	heart forming vegetable	Spring
Saishin, Tsai sin, mock pak choi, choy sum	<i>parachinensis</i>	loose flowering inflorescence vegetable	Spring
Broccoletto, Broccoli raab, turnip broccoli	<i>ruvo</i>	Inflorescence vegetable (Italy)	Spring
Oilseed turnip	<i>oleifera</i>	oilseed (mainly from Europe)	Autumn and/or Spring
Yu Choy, Green Choy Sum	<i>oleifera</i>	Vegetable, edible young leaves and flowering stalks	Spring
Toria	<i>toria</i>	oilseed (India)	Spring
Brown sarson	<i>dichotoma</i>	oilseed (India)	Spring
Yellow sarson	<i>trilocularis</i> or <i>sarson</i>	oilseed (India)	Spring
Seven top turnip	<i>septiceps</i>	vegetable	spring
Bulb turnips (some are tetraploid)	<i>rapa</i> or <i>rapifera</i>	Bulb or leaf fodder/vegetable	Autumn, (some spring)
Fodder turnip-rape	crosses of <i>rapa</i> and <i>chinensis</i>	Leaf fodder	Autumn
Purple stem mustard	<i>purpuraria</i>	Red multistem vegetable	spring
Wild turnip	<i>utilis, sylvestris</i>	Common weed	Autumn or Spring

\* normal sowing time for seed production in Canterbury

**Table 2. Forms within *Brassica napus***

Name	Subspecies	Use	Usual sowing time
Swede, Rutabaga	<i>napobrassica</i>	Root fodder and vegetable	Autumn
Fodder rape	<i>biennis</i>	leaf fodder	Autumn
Hungry gap kale, Siberian Kale, Ragged Jack kale	<i>pabularia</i>	leaf fodder	Autumn
Hakuran *	<i>capitata?</i>	Heart forming vegetable	Spring
Senposai **	?	Leaf vegetable	Spring
Canola, Oilseed rape	<i>oleifera, annua</i>	oilseed	Spring or autumn
Weed rape	<i>oleifera</i>	Rare weed in NZ	spring

\*Hakuran is a heart forming vegetable developed in Japan in the last 50 years from crosses of Chinese cabbage and cabbage

\*\*Senposai is a leaf vegetable developed in Japan in the last 20 years from crosses of cabbage and komatsuna

**Table 3. Forms within *Brassica oleracea***

Name	Botanical variety <sup>2</sup>	Use	Usual sowing time
Kohl rabi	<i>gongyloides, caulorapa</i>	Swollen stem vegetable	Autumn
Cabbage	<i>capitata</i>	Heart forming vegetable	Autumn
Savoy cabbage	<i>sabauda</i>	Thick crinkled leaf heart forming vegetable	Autumn
Broccoli, Calabrese	<i>italica</i>	Flowering bud vegetable (Sanders and Crisp, 1976)	Spring/Autumn
Broccoflower	<i>italica cross botrytis</i>	Cauliflower crossed with broccoli	Spring/Autumn
Cauliflower, Romanesco broccoli	<i>botrytis</i>	Flowering bud vegetable	Spring/Autumn
Broccolini <sup>1</sup> , Kalebrini	alboglabra cross <i>italica</i>	Flowering bud vegetable	Spring
Borecole	<i>selensia</i>	Flowering bud vegetable	Autumn
Brussel Sprouts	<i>gemmifera</i>	Hearting buds on stem	Autumn
Kales <sup>2</sup>	<i>acephala</i>	Fodder crop	Autumn
Jersey kale <sup>2</sup>	<i>palmifolia</i>	Fodder crop	Autumn
Marrow stem kale <sup>2</sup>	<i>medullosa</i>	Fodder crop	Autumn
Thousand head kale <sup>2</sup>	<i>millecapitata, ramosa, fruticosa</i>	Fodder crop	Autumn
Siberian kale <sup>2</sup> , Perpetual kale <sup>4</sup>	<i>ramosa, fruticosa</i>	Fodder crop	Autumn
Curly kale <sup>2</sup>	<i>fimbriata, sabellica</i>	Vegetable	Autumn
Portuguese cabbage or kale <sup>2</sup>	<i>costata, tronchuda</i>	Leaf vegetable	Autumn
Dwarf kale, Ornamental kale <sup>2</sup>	<i>nanofimbriata, fimbriata</i>	Ornamental	Autumn
Collard <sup>2</sup>	<i>sabellica, viridis</i>	Non hearting cabbage vegetable	Autumn
Kailan <sup>3</sup> , Chinese white flowered kale, Chinese broccoli	<i>alboglabra</i>	vegetable	Spring
Wild cabbage, Sea cabbages	<i>sylvestris</i>	Weed, usually by the seaside	Autumn

<sup>1</sup> Broccolini is a cross of standard broccoli with Chinese kale developed in Japan in the last 10 years, which resembles long, slender broccoli side shoots (Sakata Seed Co), also known as Kalebrini (Australia)

<sup>2</sup> The intraspecific classification names for the kales vary according to the authors and there appears to be considerable overlap of names within this less well differentiated group. Some kales such as "Hungry Gap", "Siberian" and "Ragged Jack" belong to *B. napus*.

<sup>3</sup> Probably European in origin and introduced to Asia via the silk road within the last 1000 years (Tsunoda *et al.*, 1980). Sometimes referred to as *B. alboglabra* or *B. alboflora*.

<sup>4</sup> Perpetual kale is a perennial propagated by cuttings. It was previously grown widely through northern Europe as a winter leaf vegetable; it flowers only occasionally (Zeven *et al.*, 1994)

There are naturalised weed forms in New Zealand but these are usually only found in restricted coastal areas (Palmer pers. comm.) and for seed production purposes cultivated “escapes” pose more risk.

#### *Ploidy differences*

In nature *B. oleracea* is a diploid but plant breeders have developed some tetraploid crops, notably the tetraploid fodder kales. These do not appear to have been grown in Canterbury for seed, but potentially could be.

#### ***Brassica juncea* (Brown or Indian Mustard)**

*Brassica juncea* is an allotetraploid species with a chromosome number of 36. It originates from

the cross of *B. rapa* and *B. nigra* with *B. rapa* as the maternal parent (Sarashima and Matsuzawa, 1989; Song *et al.*, 1994). There is a wide range of forms within *B. juncea* as this species originates in Asia with vegetable types predominating in China and oilseeds predominating in India (Sun, 1946; Li, 1980b; Chen, 1982; Gladis and Hammer, 1992) (Table 4). In general it is closer to *B. rapa* than *B. nigra* (Song *et al.*, 1994), possibly due to introgression from *B. rapa*.

The species is usually sown in spring for seed in Canterbury but it is not a common crop.

From a cross pollination viewpoint these forms will cross quite freely and can be considered one group.

**Table 4. Forms within *Brassica juncea*.**

Name	Subspecies*	Use
Broad leaf mustard	<i>foliosa</i>	Leaf vegetable with large entire succulent leaves
Cabbage or head mustard	<i>capitata</i> , <i>bulbifolia</i> , <i>rugosa</i>	Vegetable with succulent leaves forming a loose heart
Potherb or multishoot mustard “Green in the Snow”	<i>multiceps</i>	Leaf vegetable with very high leaf number
Curly-leaf mustard, Cut-leaf Mustard	<i>crispifolia</i> , <i>multisecta</i> , <i>integrifolia</i>	Leaf vegetable with deeply dissected carrot-like leaves
Root mustard, turnip-rooted Mustard	<i>napiformis</i> , <i>megarrhiza</i>	Root vegetable (akin to turnips)
Horned mustard, large petiole Mustard	<i>lapitata</i> , <i>strumata</i>	Leaf vegetable with succulent petioles and midribs, horned protuberance on the base of petiole
Big stem mustard	<i>tsa-tsai</i> , <i>tumida</i> , <i>linearifolia</i>	Stem vegetable with succulent tumorous growths on a swollen stem
Broccoli mustard	<i>faciliflora</i> , <i>scaposus</i>	Flowering shoot vegetable
Brussel sprout type	<i>gemmifera?</i>	Newly developed vegetable akin to Brussel sprouts (Chen, 1982)
Oilseed mustard, brown mustard, Spice mustard	<i>oleifera</i> , <i>juncea</i> , <i>spicea</i>	Oilseed crop, Seed used as a condiment

\* The intraspecific classification within *B. juncea* varies slightly between authors

#### ***Brassica nigra* (Black Mustard)**

There is a very limited range of forms within *B. nigra* as this species has largely been cultivated for seed as black mustard. It largely retains its primitive form and is a spring sown crop.

This is occasionally cultivated in New Zealand for seed of black mustard to be used as a condiment and as a result it can continue to be a weed of the immediate vicinity for a few years afterwards

***Brassica carinata* (Ethiopian mustard, Abyssinian cabbage)**

*Brassica carinata* is an allotetraploid species with a chromosome number of 34. It originates from the cross of *B. nigra* and *B. oleracea* with *B. nigra* being the maternal parent (Sarashima and Matsuzawa, 1989; Song *et al.*, 1994). There is a very limited range of forms within *B. carinata* as this species is more closely related to *B. nigra* than to *B. oleracea*. It is usually only cultivated as a leaf vegetable and oilseed in Ethiopia and India. This is rarely cultivated in New Zealand and is not known to occur as a weed. It would usually be spring sown for seed crops in Canterbury.

***Raphanus sativus* (Radish)**

Although not a Brassica it is closely related and is often placed on the Triangle of U as an offshoot from *B. oleracea* as it has the same chromosome number.

There are a number of forms within *Raphanus sativus* including leaf, bulb, edible pod and oilseed types which all have the potential to cross quite freely (Harberd, 1972). These are often known under subspecies names such as *longipinnatus* and *raphanistroides* (Chinese radish), *acanthiformis* (Japanese radish or Daikon) and *nigra* (Black or Spanish Winter radish), *rostratus* with reddish flowers, *caudatus* (syn. *mougri*) (rats tail radish, having long edible pods), *landra* (Italian radish), *oleiformis* fodder radish. The weed forms *R. raphanistrum*, *R. microcarpus* and European seaside type *R. maritimus* also cross with radish quite freely (McNaughton, 1976) and together these form one large interpollinating group. New Zealand has some weed forms particularly in the north but in seed production regions cultivated "escapes" are probably more common.

In nature *R. sativus* is a diploid but plant breeders in Europe have developed some tetraploid crops and these have been grown in Canterbury for seed. When crossed the diploid and tetraploid have a reduced seed set with some triploid hybrids with smaller seed (Nishiyama, 1952).

By and large we can ignore the differences between the types within each ploidy group as they cross quite freely.

***Sinapis alba* (White mustard)**

*Sinapis alba*, white mustard, also known as *B. hirta*, is quite limited in types with only one major form varying in degree of leafiness. It is often

cultivated for seed in Canterbury from a spring sowing and cultivated "escapes" are quite common.

In nature *S. alba* is a diploid but plant breeders in Europe have developed some tetraploid crops and these have been grown in Canterbury for seed.

***Eruca sativa* (Rocket)**

The species *Eruca sativa* known as Rocket has a chromosome number of 22. This species may occasionally be cultivated in New Zealand.

***Sinapis arvensis* (Charlock)**

*Sinapis arvensis*, charlock, also known as *B. kaber*, is a rare weed in New Zealand seed production regions. It has a chromosome number of 18, the same as *B. oleracea* (Davey, 1959).

**Synthetic potential crops**

Various combinations of Radish and Brassicas have been synthesised by plant breeders but although vigorous and promising from some aspects they have never been commercialised because of poor fertility and seed production problems (McNaughton, 1968). It is possible that with many more years of further breeding that this could be overcome in a similar manner to that of the cereal *Triticale*. However, very little work is currently being done on these crops.

***Brassico-raphanus* (sometimes called Raparadish)**

This refers to the tetraploid crop developed from crosses of turnip (*B. rapa*) (as the female parent) and radish (Davey, 1959).

***Raphano-brassica* (sometimes called Radicole)**

This refers to the tetraploid crop developed from crosses of radish (as the female parent) and kale (*B. oleracea*) (Davey, 1959).

**Napocampestris**

This refers to the hexaploid forage leaf crop developed from crosses of swede or rape (*B. napus*) and turnip (*B. rapa*).

**Factors Affecting Cross-Pollination, Seed-Set and Contamination**

**Pollination**

Although some Brassicas are self fertile many are self incompatible and require cross pollination to set seed. In these cases the seed set has been shown to be increased by the presence of bees (Korpela,

1988) and positively correlated with their activity (Steffan-Dewenter and Tschamtkke, 1999). Cross pollination usually results in many pollen parents fertilising one ovule, typically from one to four as reported by Ellstrand and Marshall (1986) for radish.

There have been many studies on gene flow and distance of pollen movement. Many of these experiments have aimed to measure quite different effects including:

- pollen moving between flowering Brassica fields, the importance of which is well understood by seed producers.
- pollen moving from isolated plants to flowering Brassica fields, an effect very relevant to seed production and sometimes overlooked by seed producers.
- pollen movement from flowering fields to scattered Brassica plants. This effect is of little importance to seed producers but is sometimes studied in pollen flow experiments, particularly those where gene flow to weed species is concerned.
- pollen movement among scattered flowering Brassica plants. This has little relevance to commercial seed production but is likely to be where the active seeking habits of bees are most relevant to pollination.
- Where crop contamination is concerned only the first two situations are relevant.

### **The pollen source**

Contaminating pollen can come from nearby fields and because large fields produce a vast quantity of pollen the size of the fields and their distance is a serious consideration, as is the level of cross fertility between the crop species and the overlap of their flowering.

However, the occasional off-type plant of cross fertile Brassicas occurring close to or within the crop is potentially at least as important as distant crops.

### **Pollen transport and distance**

Brassica pollen can travel by both wind and by insects. In studies it is often difficult to measure the contribution made by each but Brassicas should be considered as dual pollination crops.

It is clear however that much of the outcrossing in crops occurs over quite short distances. In a study with oilseed rape *B. napus* the outcrossing rate between plants was 21.0 %, between 40cm rows was 9.5 %, 80cm rows 5.6 % and 120cm rows 3.9 % (Cuthbert and McVetty, 2001).

The major difference between wind pollination and insect pollination is that the insect actively seeks out flowers to pollinate. This increases the effectiveness of pollinating scattered plants over wide areas, but may be little different to wind pollination for inter-pollination between flowering brassica fields.

### **Wind pollination**

Pollen released from Brassica plants follows a diurnal pattern peaking during the middle of the day. Daily levels vary considerably under different conditions but never reach the levels of exclusively wind pollinated species (Williams, 1984).

Most wind transported pollen is believed to be carried relatively short distances. In oilseed rape the level of pollen detected 2m from a crop was only half that at the edge (Bilsborrow *et al.*, 1994) but under some conditions the pollen detected at 360 m was 10 % of that at the field edge (Timmons *et al.*, 1995).

Where consistent wind direction occurs there is little contamination occurring upwind (Dastidar and Varma, 1998; Dastidar *et al.*, 2000).

Pollen of the tetraploid Brassica species is larger and heavier (Sun, 1946) suggesting that diploid pollen potentially poses more risk of contamination by wind pollination than tetraploid.

### **Insect and animal pollination**

Insect pollination may occur via a wide range of insects including honey bees, bumble bees, flies and numerous other insects. However honeybees would appear to be the most important pollinators (Eastham and Sweet, 2002).

People or animals walking from one flowering crop to another could potentially carry pollen and care may need to be taken where very high purity is required.

### **Bee behaviour and pollination distance**

The distance that bees forage is related to a number of factors including proximity and quality of flowers. In agricultural crops which offer a high density of flowers in close proximity the distances travelled may not be large. When there are many competing flowering crops nearby distant foraging is minimised (Eckert, 1933, Goplen *et al.*, 1973). Bees send out "scouts" over a wide range to find sources of flowers for other bees in the hive to subsequently work. Honey bees are regularly recorded as travelling 3km and even up to 10km (Eckert, 1933, Waddington *et al.*, 1994; Eastham and Sweet, 2002)

and bumble bees 600m (Osbourne *et al.*, 1999) but the vast majority do not travel these distances unless forced to by lack of suitable flowers.

It is also known that larger bee colonies forage greater distances than smaller ones and that foraging distance is related to bee body size (Waddington *et al.*, 1994, Steffan-Dewenter and Tschamntke, 1999).

Strong winds can be important for honey bees as this reduces pollination activity and any consistent wind could be expected to reduce total foraging distance (Benedek *et al.*, 1997; Vicens and Bosch, 2000). Similarly honey bees become less active and forage closer to the hive in bad weather when temperatures are below 13° C (Free, 1993) yet Brassicas continue to flower below this temperature.

Insect pollinator species can show preference for some flower colours and forms of a species over others and this may reduce cross pollination between diverse groups in some circumstances (Lee and Snow, 1988). It has been shown that bees visit the same species and even variety in successive visits and this behavioural pattern would tend to reduce

contamination between crops (Bateman, 1951; Osbourne *et al.*, 1999). However, there is pollen transfer between bees in the hive as they brush up against one another (Free, 1993) and bees foraging on a range of different species near a field of genetically modified rape had a small proportion of this pollen on them (Ramsay *et al.*, 1999).

#### Calculation of pollination and distance

The actual isolation distance required will depend on the purity required, the degree of self fertility in the crop, the amount of pollen produced by the crop itself, the border area removed and many other factors. It is well known that pollination risk decreases with distance as per the example in Figure 2, and that it decreases at, or close to an exponential rate which has been expressed mathematically by the "Exponential Decay Model" and the more complex Weibull function. This exponential rate may in fact be the sum of two different factors, one for wind pollination and one for insect pollination both decreasing at a different rate.

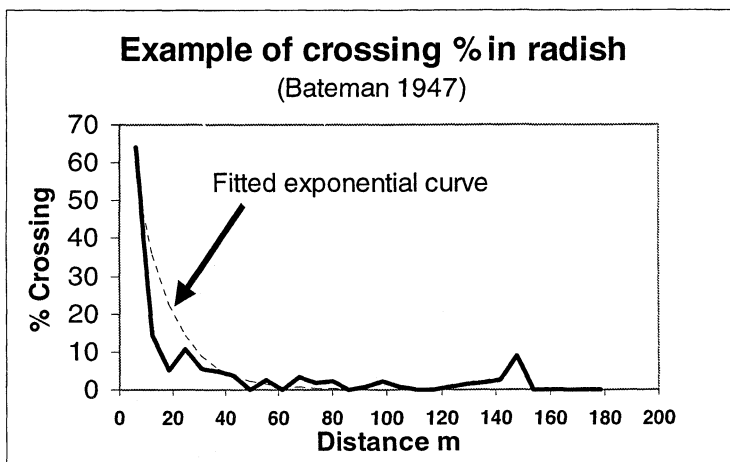


Figure 2. Cross pollination between crops as related to distance.



In this radish example it is interesting to note the variation in crossing with distance. This may partly be experimental error but it probably also represents the very real variation in cross-pollination in the field (Bateman, 1947).

Many experiments show similar exponential patterns but the actual equations vary depending on the specifics of the crop situation (Bing *et al.*, 1991).

The actual rate of crossing also depends on a number of factors including the layout of the plants. In an experiment using equal number of kale and cabbage plants in equal sized plots but varying only in layout the rates of crossing varied from 19 % to 47 % (Levin and Kerster, 1974).

The amount of crossing between adjacent plants in closely adjacent crops varies according to the crop. In radish the amount of crossing was over 80 %, in turnips 50 % (Bateman, 1947), and in hybrid association *B. napus* oilseed rapeseeds have been shown to be over 20 %. For conventional varieties of *B. napus* oilseed rape it is more commonly 1 - 4 % (Ingram, 2000; Cuthbert and McVetty, 2001).

Although the pollination risk generally decreases at an exponential rate with distance this may not adequately describe the almost random but very low level of cross pollination which can occur at distances over a few hundred metres. In a study of pollination among 63 oilseed rape fields hybrid plants were detected in seed harvested from the edge of crops up to 3km away at frequencies up to 0.07 % and this frequency was not related to distance (Reiger *et al.*, 2002).

### **Topographical barriers and hedges**

Topographical barriers such as hedges, can limit the travel of wind and insect borne pollen in certain directions, an effect difficult to predict in relation to crop contamination (Jensen and Bogh, 1941; Jones and Brooks, 1952). It does however suggest that where thick hedges are present between crops that cross contamination is likely to be reduced.

### **Shifting of bee hives**

The viability of pollen gradually declines over 4-5 days in natural conditions (Ranito-Lehtimäki, 1995) and probably does not last more than a week (Mesquida and Renard, 1982). Brassica pollen can remain viable when frozen for 3 months or more (Opena *et al.*, 1988).

An often overlooked factor leading to potential contamination is the pollen carried by bees when hives are shifted. Hives removed from a

Brassica crop will require a period away from Brassicas before they can be shifted to another Brassica crop. Many overseas companies consider that bees are not considered safe for a week after removal from a crop (E. Diaz pers. comm., 2001). It will therefore be essential to place the beehives in-situ up to 7 days before the crop flowers or else to hold bees in a Brassica free area for a week, a practice difficult to implement in seed producing regions.

### **Border zones around crops**

Pollen contamination from outside a crop decreases exponentially from the edges to the centre of a crop. For example in an oilseed rape crop more than 80 % of the pollen transported from adjacent crops was found to pollinate plants within the outside 10 m (Staniland *et al.*, 2000). For this reason it is important to distinguish between contamination within the outside border area of a crop and the contamination occurring in the total harvested crop.

The current practice of discarding seed from the edge of the field is a valuable tool in minimising any contamination as the majority of pollen transported into crops pollinates the outside few metres of the edge of the crop (Ingram, 2000).

For this reason the decision on how much border area to remove can seriously influence the contamination rate, and in many circumstances this may have more influence than further isolation (Pedersen *et al.*, 1988).

For crops desiring a particularly high standard of purity a greater border distance could be discarded, particularly where there is a market for lower purity seed from the border area. For example it should be possible for many crops to significantly reduce the contamination by increasing the border zone from 5 to 10 m.

### **Flowering time and length of flowering period**

Clearly the only time that cross pollination can occur between crops is when they are both flowering at the same time and viable pollen is transported to the flower. In particular spring and autumn sown crops may have very little overlap of flowering. However, until the flowering times of various crops have been well documented great care would be required in attempting to use different flowering times as a reliable means of preventing cross-contamination.

The length of the flowering period may also be important as crops which flower for longer periods run a greater risk of contamination. Crops

flowering rapidly in the heat of the summer are likely to offer lower contamination risks than protracted flowering spring crops.

Any seed set at the tail end of the flowering period may have insufficient time to mature prior to harvest and any overlap of flowering during this period is likely to offer less risk than seed set from the earlier flowering period. Similarly any early set seed may be lost by shattering.

### Self-incompatibility

Self-incompatibility in Brassicas is controlled by S-alleles and influenced by both the pollen and the stigma (Hodgkin, 1976). The wild diploid Brassicas have a strong incompatibility system and almost all plants are self-incompatible.

The tetraploid Brassica species have arisen from crosses of diploid species followed by spontaneous chromosome doubling, a process rare enough to lead to very few plants when self-compatibility would have been a strong selective advantage. Self-compatibility in the tetraploid may also assist in isolating the plant from the parental species (Palmer, 1962).

Many cultivated forms of the Brassicas are now largely self-compatible due to the probable loss of their S alleles through unconscious selection over many centuries in small gardens where self-compatibility has been an advantage for seed yield (Opena *et al.*, 1988).

As a result in Brassicas the wild diploid species are largely self-incompatible while the tetraploids and many of the older cultivated diploids are largely self-compatible.

The diploids *B. rapa*, *B. oleracea*, *B. nigra*, *S. alba*, *R. sativus* and *E. sativa* are largely self-incompatible requiring cross pollination for best results. However, within many varieties there can be a few self-compatible plants and in some varieties the majority of plants are self-compatible. These include *B. rapa* yellow sarson, *B. oleracea* Kailan, kohlrabi, broccoli, some cabbages and some cauliflowers (Yarnell, 1956; Davey, 1959; Watts, 1968; Olsson, 1960; Downey *et al.*, 1980; Chen *et al.*, 1999; Okuda *et al.*, 2000).

The tetraploids *B. napus*, *B. juncea*, *B. carinata* are basically self-compatible but cross pollination also occurs during seed production. However, within some varieties there can be a few self incompatible plants. Seed crops of both *B. juncea* and *B. napus* are largely self-pollinated with typically 20-33 % outcrossing (Yarnell, 1956; Davey, 1959; Olsson, 1960; Bowring and Day, 1977;

Downey *et al.*, 1980; Bhanjan *et al.*, 1991; Cuthbert and McVetty, 2001).

As there is a large variation between the incompatibility reaction of varieties information on their particular system would be required to understand its pollination requirements.

Breeders have taken advantage of this incompatibility system to breed hybrid cultivars, mainly with the vegetable and oilseed brassicas. More recently, some vegetable hybrids have been produced using cytoplasmic male sterility systems (Quiros, 2001).

In self-compatible crops, where typically less than 30 % outcrossing occurs, contamination levels resulting from outside pollen of the same species should be less than 30 % of that of a self-incompatible crop in the same circumstances.

In hybrid crops where male sterility mechanisms are used only a proportion of the plants in the crop produce pollen, resulting in an overall reduction in pollen available for pollination. In these circumstances contamination by outside pollen of the same species is likely to be higher than that of traditional crops.

### Pollen receptivity and competition on the stigma

When pollen lands on the stigma there is competition to effectively pollinate the crop. This effect is very important when pollen of a different species lands on the stigma.

In general pollen from other species will be much less competitive than pollen from the same species. In *B. napus* less than 10 % of *B. rapa*, *B. juncea*, and *B. oleracea* pollen tubes were able to enter the style, while *B. carinata* and *B. nigra* had even less success (Meng and Liu, 1987). The adhesive ability of pollen to the stigma is unrelated to self-compatibility and is influenced by the genetics of the pollen parent (Luu *et al.*, 1997).

In *B. rapa* crop contamination by adjacent crops of *B. napus*, which gives near normal seed set on handcrossing, gave only 1.3 % hybrids and similarly with *B. napus* crops adjacent to *B. juncea* only 4.7 % hybrids were found (Bing *et al.*, 1991). This suggests that the risk of contamination between species is much less than the seed set reported from hand crossing, maybe by a factor of 10-20 or more.

In male sterile hybrid seed production there will be less pollen available to pollinate the crop. In this situation stray pollen from another species may become more successful resulting in a higher contamination rate.

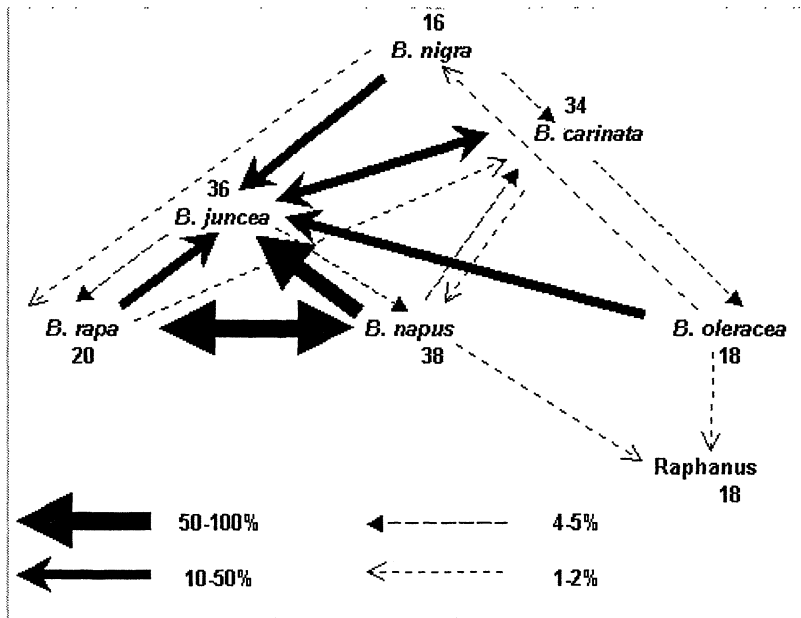


Figure 3. Seed set after handcrossing between Brassica and Radish species

#### Potential seed set when crossed

One factor influencing the potential crossing between species in the field is the seed set obtained on emasculated flowers when handcrossed. It is clear that the seed set obtained can vary enormously between individual genotypes and on occasions it is possible to find plants which cross at a much higher rate than the general population. In general the crossability among diploid species is low compared to crosses between the tetraploid species. Similarly, the crossability between diploid and tetraploid species is highest when the maternal parent is tetraploid (Nishiyama *et al.*, 1991). This means that there is greater risk of cross contamination in the tetraploid than diploid species.

It is also clear that contamination levels between crops of these species is much lower than the rate of seed set obtained by handcrossing because many other factors need to be considered such as self-compatibility, pollen competition on the stigma and overlap of flowering times.

#### Crossing relationship between species

Figure 3 summarises the seed set from handcrossing experiments. The arrows represent the

direction of pollen flow and the size of the arrow represents the seed set possible in a handcross using emasculated flowers. Arrows have not been included where the reported seed set is below 0.1 % yet all combinations have been crossed. The seed set of crosses with *S. alba*, *S. arvensis*, *E. sativa* and many other Brassica relatives are all less than 0.1 % so they are not included in this diagram. It should be noted that for the plant breeder the rate of hybrids obtained can be increased significantly by using embryo and ovule culture techniques (Takeshita *et al.*, 1980).

The resulting seed set between plants on the outside of adjacent fields may be in the order of 10-10,000 times less than that reported for handcrossing. For example crops of *B. juncea* adjacent to *B. napus* which can have near normal seed set on handcrossing will typically only have 0.1-3 % contamination or less when grown under field conditions (Bing, 1991, Jorgensen *et al.*, 1994).

Although radish can be handcrossed with *B. oleracea* or *B. napus* with a 1 % seed set there are no reports of contamination in commercial practice when radish is grown adjacent to these crops. This suggests that the arrows representing 1-2 % seed set

may pose an insignificant risk for commercial seed production.

### Pollination groups

For commercial seed production the species can be divided into pollination groups. The crossing rate between the groups is very low and usually insignificant for commercial purposes. The majority of crosses which could occur between crops occur within the outside border area of the field further reducing their significance. It is clear that when crops are grown on a field scale there is an extremely low rate of crossing between almost all the Brassica and radish species. This rate can be of concern for genetically modified crops where transfer of resistance traits to related weed species may occur (Chevre *et al.*, 1997; Reiger *et al.*, 2002). Of particular concern are the herbicide resistant traits where there the unsound practice of applying herbicides on the same area over a number of years is capable of applying the enormous selection pressures necessary for these few crosses to overcome sterility barriers and succeed.

### *B. rapa* (diploid and tetraploid) and *B. napus* crops

It is clear that these two species have a high risk of crossing in many circumstances and need to be considered as one large group for seed production purposes (Bowring and Day, 1977). On occasion seed set is reduced but it is still significant (Sutton, 1908; Calder, 1937; Davey, 1959; Palmer, 1962; Nishiyama *et al.*, 1991; Brown and Brown, 1996; Ingram, 2000; Lu and Kato, 2001). It is common for these two species to hybridise in nature and such hybrids are termed *Brassica x harmsiana* (Raybould and Gray, 1993).

From the cross-pollination viewpoint the diploid and tetraploid *B. rapa* have potential to behave slightly differently. By and large we can ignore the differences between the types within each ploidy group as they potentially cross quite freely. The exception would be the Asian oilseed crops, toria and sarson which have a reduced seed set when crossed with others *B. rapa* (Olsson, 1954). In general tetraploids of *B. rapa* appear to have a higher seed set in interspecific crosses and tetraploid seedcrops would appear to be up to twice as susceptible to cross contamination by other species than diploids (Tandon, 1952; Davey, 1959).

Seed set on crossing between diploid and tetraploid *B. rapa* is likely to be reduced to less than 3 % of normal with tetraploid crops more prone to developing hybrids than diploid crops (Nishiyama

and Inamori, 1953, 1966). It should be noted however that many tetraploid cultivars may contain a small proportion of diploid plants and these could also be important for contamination of adjacent crops.

From a cross pollination viewpoint all *B. napus* forms will cross quite freely and must be considered one group. On occasion individual cultivars may have a reduced seed set when crossed with others, notably the more recently synthesised rapes with the natural types.

Both *B. rapa* and *B. napus* are certainly a very distinct group from *B. oleracea*, radish, white mustard (*S. alba*) and *S. arvensis* with the seed set from handcrossing usually below 0.05 % and contamination rates in the field much lower than this (Sutton, 1908; Calder, 1937; Chiang *et al.*, 1977; Tsunoda *et al.*, 1980; Yamagishi and Takayanagi, 1982; Matsuzawa, 1984; Inomata, 1988; Nishiyama *et al.*, 1991; Scheffler and Dale, 1994; Chevre *et al.*, 1994, 1997, 1998; Choudhary *et al.*, 2000; Eastham and Sweet, 2002).

*B. rapa* has been handcrossed many times with *B. nigra* giving a seed set up to 2 % (Nishiyama *et al.*, 1991) but in contrast *B. napus* gave only 0.01 % in crosses with *B. nigra* (Nishiyama *et al.*, 1991) and no detectable hybrids in field co-cultivation (Bing, 1996).

*B. napus* have been handcrossed with *B. carinata* giving a seed set as high as 4 % (Roy, 1980) but in contrast *B. rapa* gave less than 0.1 % in limited crosses with *B. carinata* (Nishiyama *et al.*, 1991).

Both *B. rapa* and *B. napus* have been handcrossed with *B. juncea* giving a seed set as high as 5 % (Davey, 1959; Khanna and Chowdhury, 1974; Yamagishi and Takayanagi, 1982; Liu and Xiao, 1987; Nishiyama *et al.*, 1991; Eastham and Sweet, 2002). When grown in adjacent areas *B. napus* pollinated by *B. juncea* produced 1 % hybrids (Bing, 1996).

The species which pose some risk to *B. rapa* crops are *B. nigra* and *B. juncea* while *B. napus* crops may be at risk from *B. juncea*. Some isolation and border removal will be required where spring sown crops of *B. rapa* are grown near the occasional crop of *B. nigra* and where either *B. rapa* or *B. napus* are spring sown near the occasional *B. juncea* crop.

### *B. oleracea* (diploid and tetraploid) crops

This species is certainly a very distinct group from *B. rapa*, *B. napus*, mustard (*S. alba*), *B. juncea*, *B. nigra* and radish with the seed set from

handcrossing usually below 0.05 % and contamination rates in the field much lower than this (Calder, 1937; Yarnell, 1956; Bowring and Day, 1977; Ellerstrom, 1978; Tsunoda *et al.*, 1980; Yamagishi and Takayanagi, 1982; Matsuzawa, 1984; Nishiyama *et al.*, 1991; Chevre *et al.*, 1997).

From the cross-pollination viewpoint the diploid and tetraploid *B. oleracea* have potential to behave slightly differently. By and large we can ignore the differences between the types within each ploidy group as they cross quite freely. In general tetraploids of *B. oleracea* appear to have a higher seed set in interspecific crosses and seedcrops would appear to be up to twice as susceptible to cross contamination by other the species than diploids (Davey, 1959).

Seed set on crossing of diploid and tetraploid crops of *B. oleracea* is reduced to below 60 % in tetraploid crops and below 1 % in diploid crops (Prikhed'ko, 1976; Tsunoda *et al.*, 1980; Nishiyama *et al.*, 1991).

The species which poses some risk to *B. oleracea* crops is *B. carinata*. In hand crosses seed set was up to 5 % (Barcikowska *et al.*, 1983; Nishiyama *et al.*, 1991). Some isolation and border removal will be required where spring sown crops of *B. oleracea* are grown near the occasional crop of *B. carinata*.

### ***B. nigra* crops**

This is only occasionally cultivated in New Zealand. It forms quite a distinct group from mustard (*S. alba*), radish, *B. napus*, *B. rapa*, *B. juncea*, and *B. carinata* giving a seed set on handcrossing below 0.01 % (Davey, 1959; Nishiyama *et al.*, 1991) and no detectable hybrids in field co-cultivation (Bing, 1996).

The species which poses some risk to *B. nigra* crops is *B. oleracea*. In hand crosses seed set was up to 1 % but usually much lower (Matsuzawa, 1984; Nishiyama *et al.*, 1991; Raybould and Gray, 1993). However, in seedcrops there are no reports of contaminants and the risk appears to be acceptably low, particularly as the seed is often used for black mustard production where the requirement for purity is not exceptionally high. In addition many *B. oleracea* crops are autumn sown presenting little opportunity for crossing with spring sown *B. nigra* crops. Where a high level of purity is required some isolation and border removal will be required where the occasional crops of *B. nigra* are grown near spring sown crop of *B. oleracea*.

### ***Raphanus sativus* (diploid and tetraploid) crops**

This species is certainly a very distinct group from mustard (*S. alba*), *B. rapa*, *B. juncea*, *B. carinata*, *B. nigra*, *S. arvensis* and *E. sativa* seldom giving a seed set on handcrossing above 0.01 % (Ellerstrom, 1978; Matsuzawa and Sarashima, 1984; Dayal, 1987; Chevre *et al.*, 1997).

Seed set on crossing between diploid and tetraploid radishes is reduced with these being predominantly triploids, the tetraploid crops more prone to developing hybrids than the diploid crops (Nishiyama, 1952).

Radish has been crossed by hand with both *B. oleracea* and *B. napus* giving seed set up to 1 %, with tetraploid radishes easier to cross than diploids (Ellerstrom, 1978; Tsunoda *et al.*, 1980; Chevre *et al.*, 1998).

However, in seed production in New Zealand it has been common to grow radishes and Brassica crops nearby and there are no reports of contaminants. Therefore the risk appears to be acceptably low, particularly as many *B. oleracea* crops are autumn sown presenting little opportunity for crossing with spring sown radishes. Where hybrid radish crops are required of greater than normal purity it may be prudent to have some degree of isolation and border removal from spring sown *B. oleracea* and *B. napus*.

### **White mustard, (*Sinapis alba*) diploid and tetraploid crops**

This species is certainly a very distinct group from all the other groups. It poses very little risk for cross-contamination to or from any of the Brassica crops, radishes or *S. arvensis* (Davey, 1959; Bing *et al.*, 1991).

Seed set on crossing between diploid and tetraploid white mustard (*S. alba*) is reduced, with tetraploid crops having a higher seed set than diploid crops (Nishiyama and Inamori, 1953).

### ***Eruca sativa* crops**

This species is certainly a very distinct group from all the Brassica species, white mustard (*S. alba*) and radish.

A very low frequency of hybrids has been produced by hand pollination with both *B. rapa* (Davey, 1959) and radish (Dayal, 1987). There is little indication that in field seed production if this species will contaminate any of the Brassica crops, radishes or *S. alba*.

### ***Sinapis arvensis* (Brassica kaber)**

This is a rare weed in seed production areas of New Zealand and when present its pollen poses little risk of cross contamination into any of the commercial crop species (Davey, 1959; Bing *et al.*, 1991).

The seed set in handcrosses of *B. napus* was less than 0.001 % when pollinated by *S. arvensis* (Chevre *et al.*, 1994).

Bing *et al.*, (1991) obtained a few seeds when *B. nigra* was the pollinator of *S. arvensis* showing that gene flow can occur at very low frequency with some Brassica species, predominantly *B. nigra* and *B. oleracea* (Davey, 1959) supporting the taxonomic reclassification of this species to the Brassica genus (Mattsson, 1988).

### ***B. carinata* crops**

This species is certainly a very distinct group from mustard (*S. alba*), *B. oleracea* and *B. napus* with the seed set from handcrossing usually below 0.1 % and contamination rates in the field much lower than this (Yarnell, 1956; Nishiyama *et al.*, 1991).

The species which poses the most risk to *B. carinata* crops is *B. juncea*. In handcrosses the seed set with *B. juncea* is up to 25 % (Yarnell, 1956; Zhivotova, 1973; Nishiyama *et al.*, 1991). Crops of *B. carinata* will require some isolation and border removal from *B. juncea*.

Two further species that can pose a small risk to *B. carinata* crops are *B. nigra* and *B. rapa*. In handcrosses the seed set with *B. nigra* is up to 4 % and with *B. rapa* it is up to 2 % (Nishiyama *et al.*, 1991; Choudhary *et al.*, 2000). However, because of the self fertility of *B. carinata* the outcrossing rate to these species will be much lower in the field, potentially less than 0.1 %. Crops of *B. carinata* may require some degree of isolation or border removal from spring sown *B. rapa* crops and those occasional *B. nigra* crops when high purity is required.

### ***B. juncea* crops**

This species is certainly a very distinct group from mustard (*S. alba*) (Davey, 1959; Sharma and Singh, 1992) but is surprisingly promiscuous with the other Brassica species.

When hand pollinated by *B. napus* it can potentially have a near normal seed set (Davey, 1959; Sharma and Singh, 1992; Subudhi and Raut, 1995) and it can still give from 0.3 % to 4 % hybrids from field co-cultivation (Bing, 1991, 1996; Jorgensen *et al.*, 1994).

When handcrossed with *B. nigra*, *B. rapa*, *B. oleracea* and *B. carinata* it can potentially have a seed set from 10 % to 50 % of normal (Davey, 1959; Zhivotova, 1973; Roy, 1980; Banga and Labana, 1987; Bing *et al.*, 1991; Sharma and Singh, 1992; Nishiyama *et al.*, 1991; Subudhi and Raut, 1995; Gupta, 1997). Usually however, because of the self fertility of *B. juncea* the outcrossing rate to these species when grown in adjacent fields will be much lower, potentially less than 1 % or even 0.1 % (Bing, 1996).

This means that in practice *B. juncea* crops will require full isolation and border removal when grown near spring sown *B. napus*, and some isolation and border removal when grown near spring sown *B. nigra*, *B. rapa*, *B. oleracea* and *B. carinata*.

### **Seed size of resulting hybrids**

Often interspecific and interploidy hybrid seeds are smaller and shrivelled, so much so that breeders carrying out crosses discard the larger normal seed in order to increase their chances of obtaining hybrids (U and Nagamatsu, 1933; Nishiyama, 1952; Nishiyama and Inamori, 1953). This can allow some degree of separation during seed processing so that the larger seed resulting from size-grading may have a much lower contamination rate than field harvested seed.

### **Germination of hybrids**

Often interspecies contaminants seed is not only smaller but with a lower germination. This can lead to a lower contamination rate of plants in the resultant crop.

### **Morphological appearance of contaminant plants**

In most cases we are interested in contaminants which lower the quality of the resulting crops. In forages these may simply be those very visible annual bolters or tall off-types. In vegetables and oilseeds it could be those of inferior type, flavour or quality. The expression of these in the F1 or subsequent generation will depend on the inheritance of the trait concerned with dominant genes being expressed in the F1 and many recessive traits not being expressed.

### **Expected standards of purity**

The levels of purity considered to be acceptable vary depending on the end use of the crop and the demands of the buyer. Breeders or Basic seed used for further seed production and hybrid vegetable Brassicas used for human consumption

usually require a higher level of purity than forage Brassicas used for grazing. Often hybrid Brassicas require a level of purity such that there is less than 1 visual contaminant in 15000, which often equates to 1 contaminant per ha, while forage Brassicas may allow many more than this.

#### **Paddock history**

Brassica seeds can stay viable in the soil for over 5 years after crops have been harvested (Simard *et al.*, 2002) providing ample opportunity to germinate and contaminate new sowings.

In certain circumstances seed may stay still be a problem after 15 years. A fodder rape seed crop grown in 1987 at Ceres Research Station shattered badly in the windrow, potentially dropping 500 kg/ha or more. Seed still continues to germinate 15 years later at such a rate (averaging 7 m between plants) that pure seed production of Brassicas would not be possible.

To avoid contamination, knowledge of the cropping history of a field is important before planting.

#### **Removing off-types in and near the crop**

Any off-types in or near the crop can potentially provide greater risk of contamination than nearby crops. For example, one large off-type plant occupying 1 square metre within a field would potentially cause a 1 in 10,000 contamination rate. Crop roguing to remove the occasional off-type before flowering can reduce the contamination levels considerably.

Roguing should be carried out before flowering and expanded to include all roadsides and isolated plants of weedy Brassicas within a few hundred metres as their pollen can cause contamination.

#### **Clean machinery**

Although not concerned with cross-pollination it is always vitally important that sowing, harvesting and seed cleaning machinery is cleaned to a high standard as these provide many opportunities to contaminate the seed harvested.

#### **Improving crop purity standards**

Although isolation distance is a key factor in determining crop purity it is clear that there are a number of other factors to consider in determining crop contamination. Growers need to consider a range of factors including:

- Field history of brassica seed production and likely brassica weeds. Brassica seeds may survive 10 -15 years in the soil so a cropping history free of Brassicas will be required for a clean crop.
- Isolation distance. This appears to be well understood by growers but increasing the distance may offer less potential to improve purity than many of the other factors, particularly the size of the border area.
- The overlap of flowering time with nearby crops, off-types and weeds. As flowering time of Brassica crops is quite extended there is a large window of opportunity for overlap of flowering. However, spring and autumn sown crops do not always overlap in flowering.
- The pollination group of the nearby crops, off-types and weeds. Those in the same group offer much greater risk than those of a different group.
- The propensity of the crop to accept outside pollen. This increases from self-compatible to self-incompatible to hybrids. Furthermore those hybrids producing less pollen, such as the male sterile hybrid types, would have an even greater propensity to accept outside pollen.
- Requirement for cross-pollination by bees or wind. Bees are beneficial for hybrids and self-incompatible crop types but may offer minimal yield improvement on self-compatible cultivars.
- Cleanliness of bees transported to a flowering crop. Often overlooked as a cause of contamination are bees shifted from one area to another. Bees are not considered completely safe for 7 days after visiting Brassica crops.
- The distance of border area removed from the outside of the crop is often overlooked. Where a high standard is required this should be increased particularly when there is a market for the lower quality seed from the border area.
- The seed size of resulting hybrids. Dressing to remove small seed can often significantly reduce the contamination from other species, but is unlikely to affect the contamination caused by pollen of the same species.
- Undesirable weed or crop off-types within or nearby the crop. Removing these before flowering can significantly reduce the contamination. Those off-types in a crop will

contribute seed and pollen while those outside can contribute pollen only.

- Harvest and dressing machinery cleanliness. All machinery, seed boxes, and dressing machinery must be completely free of seed.

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