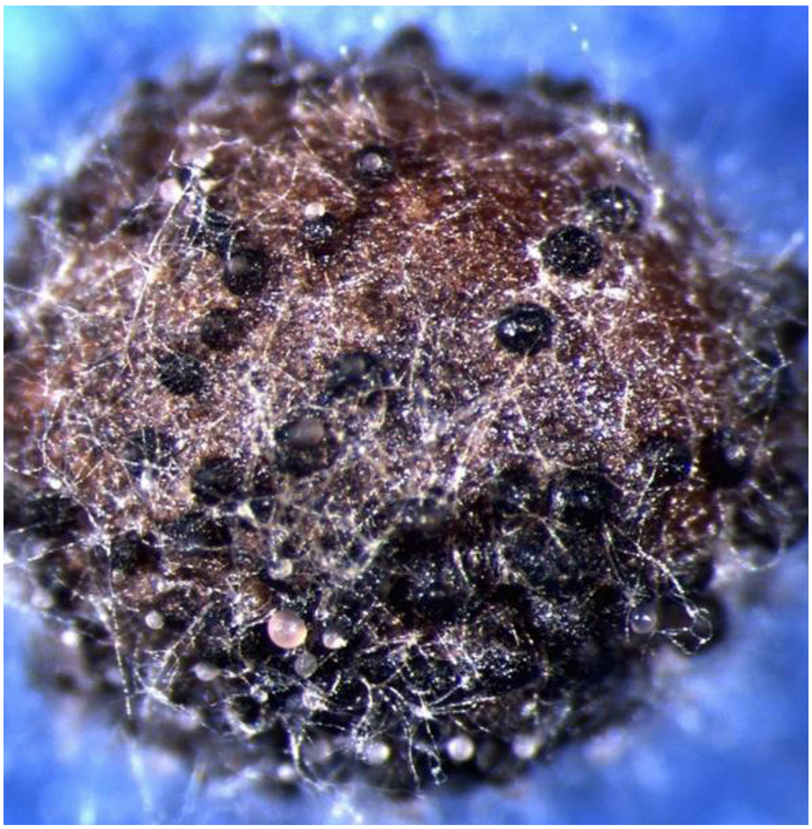
Production of Brassica Seed Crops in Washington State: A Case Study on the Complexities of Coexistence

WASHINGTON STATE UNIVERSITY EXTENSION • EM062E



Production of Brassica Seed Crops in Washington State: A Case Study on the Complexities of Coexistence

Many Brassica species and related plants in the Brassicaceae (mustard or Crucifer family) (Table 1) are grown commercially in Washington State as biofuel crops, biofumigant crops, cover crops, green manure crops, oilseed crops (edible or otherwise), and vegetable crops. Further, many of these crucifers are also grown in Washington State as seed crops (i.e., crops from which the harvested seed is sold for planting the aforementioned commercial crucifer crops).

The purpose of this publication is to inform Brassica growers, industry members, university researchers, extension specialists, and extension educators about crucial aspects of the Brassica seed industry in Washington State. The emphasis is on hybrid and open-pollinated crucifer seed crops grown in Washington State—a major vegetable seed production area of the world (Gabrielson and Pelter 1989; Schreiber and Ritchie 1995; Thomas et al. 1997). Planting crucifer crops for multiple purposes raises complex and sometimes controversial crop production and regulatory issues for which strategies of coexistence are imperative. This publication is organized as follows:

- Brassica Seed Crop Production in Washington State
- Challenges in Brassica Seed Crop Production
- Coexistence in the Brassica Seed Industry in Washington State
- Protecting Brassica Seed Crops and Commercial Brassica Crops
  - *Regulated seedborne crucifer pathogens*
  - *Canola and other crucifers as potential weeds*
  - *Managing gene flow by field isolation Herbicide-resistant transgenes*
- Effective Coexistence of Crucifer Crops in Washington State

Brassica Seed Crop Production in Washington State

The production of Brussels sprouts, cabbage, cauliflower, Chinese cabbage, Chinese mustard, collard, cress, kale, kohlrabi, mustard, rutabaga, turnip, and other crucifers as vegetable seed crops began in western Washington in the late 1800s and soon became a successful agricultural enterprise. With the advent of an extensive irrigation district in the Columbia Basin of central Washington in the 1950s, crucifer vegetable seed production was introduced east of the Cascade Mountains. In this area, seed crops of kale, mustard, radish, turnip and, more recently, canola (the latter grown both for oilseed crops and for seed crops) now comprise a second region of successful crucifer seed crop production in Washington State.

The importance of the Brassica vegetable seed industry to Washington's economy and to world food production cannot be overestimated. More than 15 species of Brassica vegetable seed crops are grown in the state with up to 1,500 acres per year at gross crop values ranging from $1,500 to >$6,500 per acre. Even though the acreage is very limited (the average size for a hybrid cabbage seed crop is 5 acres, while open-pollinated cabbage seed crops average 15 acres), the industry has a significant impact on world food production. For example, one acre of a hybrid cabbage seed crop can yield approximately 2,000 pounds of seed, which is enough seed to plant up to 10,000 acres of a head cabbage crop, which, in turn, can produce about 50 million pounds of cabbage for consumption. Brassica vegetable seed crops produced in Washington State and similar regions of Oregon have provided up to 50% of the U.S. supply and up to 25% of the world supply of seed used to grow these vegetables (Schreiber and Ritchie 1995).

The majority of commercial vegetable seed crop production in Washington State is done under bailment contracts between the seed grower (bailee) and seed company (bailor) (Thomas et al. 1997). There is almost no open market (non-contracted), small-seeded, vegetable seed production in the state, except for a very limited but growing number of people and organizations promoting open-source breeding and seed production. Many Brassica vegetable seed crops are biennial, meaning the plants only flower and produce seed during the second year of growth, following exposure to fall and winter conditions that

Table 1. Common names and Latin names of Brassica *and other vegetable, oilseed crop, weed, and ornamental species in the Brassicaceae (from Brako et al. 1997).*

Latin Name	Common name(s)
Crucifer genera and species commercially grown for seed in Washington State's Brassica vegetable seed production areas:	
Brassica chinensis	Pak choi
B. carinata	Ethiopian mustard
B. juncea	Brown mustard, oriental mustard, Chinese mustard, gai-choi, Indian mustard, kai-tsoi, karashina, leaf mustard, mustard cabbage, mustard greens, ostrich plume, southern cole, Swatow mustard
B. napus	Canola, colza, rape, rapeseed, rutabaga, Swede rape, turnip
B. napus var. *napobrassica*	Rutabaga, Swede, Swedish turnip
B. nigra	Black mustard, brown mustard, cadlock, scurvy, senvil, warlock
B. oleracea	Cabbage, kale, kohlrabi, wild cabbage
B. oleracea var. *acephala*	Borecole, braschette, cole, colewort, collards, flowering cabbage, kale
B. oleracea var. *botrytis*	Broccoli, cauliflower
B. oleracea var. *capitata*	Cabbage, savoy, savoy cabbage
B. oleracea var. *gemmifera*	Brussels sprouts
B. oleracea var. *gongylodes*	Kohlrabi
B. oleracea var. *italica*	Asparagus broccoli, Italian broccoli, sprouting broccoli
B. pekinensis	Celery cabbage, Chinese cabbage, pe-tsai, Shantung cabbage
B. rapa	Canola, bird's rape, bird's rape mustard, field mustard, turnip
B. rapa subsp. *trilocularis*	Field mustard
B. tournefortii	African mustard
Other Crucifer genera and species present in Washington (**bold font** = species sometimes grown commercially in Washington State's Brassica vegetable seed production districts)	
Alyssum spp.	Madwort, alyssum
Arabidopsis thaliana	Mouse-ear cress
Arabis spp.	Rock-cress
Armoracia rusticana	**Horseradish, red-cole**
Barbarea spp.	Winter cress, yellow-rocket
Berteroa incana	Hoary alyssum
Cakile spp.	Sea-rocket
Camelina sativa	**Camelina**
Capsella bursa-pastoris	Shepherd's-purse
Cardamine spp.	Cress, toothwort, pepperwort, bittercress, shotweed
Cheiranthus cheiri	English wallflower, wallflower
Cochlearia officinalis	Scurvy-grass
Conringia orientalis	Hare-ear mustard
Coronopus didymus	Swine-cress
Crambe spp.	Colewort, crambe, scurvy-grass, sea kale
Descurainia spp.	Tansymustard
Draba spp.	Whitlow-grass, draba
Erucastum gallicum	Dogmustard
Erysimum spp.	Wallflower, cress, mustard
Hesperis matronalis	Dame's-rocket
Iberis spp.	Candytuft
Lepidium spp.	Peppergrass, pepperwort, tongue-grass

Table 1 continued. Common names and Latin names of Brassica *and other vegetable, oilseed crop, weed, and ornamental species in the Brassicaceae (from Brako et al. 1997).*

Latin Name	Common name(s)
Lesquerella spp.	Bladderpod
Lobularia maritima	Sweet alyssum
Lunaria annua	Bolbonac, honesty-plant, money-plant, moonwort, penny-flower, silver-dollar
Matthiola spp.	Stock
Nasturtium officinale	Watercress
Parrya nudicaulis	Wallflower
Phoenicaulis cheiranthoides	Wallflower, Phoenicaulis
Raphanus raphinastrum	Jointed charlock, wild radish
R. sativus	**Radish**
R. sativus* cv. *'longipinnatus'	**Chinese radish, daikon**
Rorippa spp.	Field cress
Sibara virginica	Rock-cress, sibara
Sinapis alba	**White mustard, yellow mustard**
S. arvensis	California rape, charlock, wild mustard
Sisymbrium spp.	Mustard, tumble mustard, hedge mustard
Smelowskia spp.	False candytuft
Stanleya spp.	Prince's plume, desert plume
Streptanthus spp.	Jewelflower
Thelypodium spp.	Tumble mustard, thelypodium
Thlaspi spp.	Pennycress, stinkweed, fan weed, French weed
Thysanocarpus curvipes	Fringe-pod, lace-pod

promote vernalization and bolting (conversion from vegetative growth to reproductive growth, which is necessary for flowering and seed set). Thus, in western Washington, biennial Brassica vegetable seed crops are started by planting stock seed (the initial seed lots used to plant a seed crop) in greenhouses in June. The seedlings are then transplanted to fields in late summer (late August to early September). In contrast, annual brassica seed crops are seeded in western or central Washington in spring and harvested in the fall of the same year.

For a hybrid Brassica vegetable seed crop, rows of a designated female parent line are alternated in the field with rows of a designated pollinator or male parent line (Figure 1). Following vernalization, the overwintered plants bolt (enter the reproductive stage of growth) in the spring. A stem pushes out of each head and develops racemes (flowering branches) on which numerous flowers form (Figure 2). Brassica species cross-pollinate (i.e., pollen is moved from one plant to another, usually via honeybees and other insects). Pollinated flowers develop pods in which seeds form. Upon seed set and maturation in mid- to late summer, the plant stems are cut manually and placed in windrows, so the seeds can finish maturing and drying. The windrowed plants are then threshed mechanically using a combine. The harvested seeds are processed (cleaned and sized) by the seed company, then sold. Annual Brassica seed crops are grown similarly but over a single season, because these species bolt (flower) in response to long day length, not vernalization.

Brassica vegetable seed crop production methods vary depending on whether the crops are hybrid or open-pollinated, annual or biennial. Methods also vary depending on the region of production (e.g., non-irrigated crops are grown in the moist, maritime climate of western Washington vs. irrigated crops grown in the semi-arid Columbia Basin of central Washington). For examples, see the Cabbage Seed Crop Profile (du Toit 2007) for information on cabbage seed production in western Washington, and refer to Hinman and Pelter (1997) for information on radish seed production in central Washington. The seed from a few of the Brassica seed crops produced in Washington State are sold for purposes other than vegetable seed; for example, seed harvested from canola seed crops can be sold for planting canola oilseed crops from which oil is extracted for consump-

Figure 1. A hybrid cabbage seed crop in western Washington just prior to bloom (mid-April), with two pollinator ('male') rows alternated with two 'female' rows (photo courtesy of L. du Toit).

Figure 2. A turnip seed crop in central Washington (upper photo, courtesy of G. Pelter) and a hybrid Chinese cabbage seed crop in western Washington (lower photo, courtesy of L. du Toit).

tion by humans or animals, or the canola seed may be processed for biofuel.

Challenges in Brassica Seed Crop Production

All seed crops must meet high standards to compete successfully in the international seed market (Thomas et al. 1997). Growers are paid contract prices only when the harvested seed meets the quality standards of the bailment contract. Quality standards include genetic trueness-to-type (which is ensured by maintaining minimum isolation distances between adjacent seed crops of the same species); seed germination levels >85%; excellent seed vigor; seed free of plant pathogens; seed that is 99% clean of weed seed and other debris; and, depending on the buyers and markets, seed free of transgenes (genetically modified traits). In addition, production and labor costs associated with growing high value seed crops can have a significant influence on the number of seed contracts awarded to Washington State seed growers vs. seed growers in other states and countries.

Several challenges to the production of high quality Brassica vegetable seed crops in Washington State occurred during the early 2000s when there was state-wide expanded interest in growing winter and spring rapeseed (canola), oriental or brown mustard (*B. juncea*), and yellow mustard (*Sinapis alba* = *B. hirta*) as oilseed crops, biofuel crops, cover crops, and for other purposes (Figures 3 and 4). Acres of non-vegetable Brassica seed crops surged to meet the increased demand for seed used to produce oilseed, biofuel, and cover crops.

The first challenge related to this increased demand for non-vegetable Brassica seed crops involved two pathogens of regulatory significance to the international seed industry. *Phoma lingam* is a fungus that causes black leg (Figures 5 and 6), and *Xanthomonas campestris* pv. *campestris* is a bacterium that causes black rot of Brassicas (Figure 7). Both pathogens can be seedborne in most members of the Brassicaceae

Figure 3. A canola oilseed field east of the Cascade Mountains (photo courtesy of T. Miller).

Figure 4. Mustard cover crops in western Washington (top) and central Washington (bottom). Mowing and incorporating the crop into the soil is part of biofumigation, and can help to improve soil organic matter, tilth, and water infiltration (top photo courtesy of L. duToit; bottom photo courtesy of A. Maquire).

and are extremely threatening to a seed producer because of potential damage to Brassica seed crops and the added risk of harvesting infested seed that buyers will not purchase. Until recently, testing canola and other crucifer seeds for these two seedborne pathogens was not mandated in Washington State. However, whenever a Brassica crop is planted, there is a risk that the seed lot may be infested with one or both of these pathogens, potentially introducing the pathogens into the area. Western Washington is especially vulnerable to these two seedborne pathogens because environmental conditions in this region can be highly conducive to seed transmission of both *P. lingam* and *X. campestris* pv. *campestris*.

For many years, cooperating Brassica seed producers and seed companies operating in western Washington have addressed this challenge through self-regulation to minimize the risks presented by these seedborne pathogens. All crucifer stock seed lots are tested for these pathogens prior to using any stock seed for planting seed crops, and planting crucifer seeds for purposes other than certified vegetable seed production has been rare in western Washington. With the recent increase in demand for non-vegetable Brassicas, no mechanism was in place for routine testing of seed lots of non-vegetable *Brassica* species for *P. lingam* or *X. campestris* pv. *campestris*. In other words, no safeguards had been put in place to prevent these two pathogens from being introduced into Brassica vegetable seed production areas via Brassica seed lots used for purposes other than vegetable seed crops.

The second challenge was that canola and many crucifer species can readily become weeds. Crucifer crops produce abundant amounts of seed, and the seeds can be dispersed readily by bird predation and wind shatter. Furthermore, crucifer seed spillage is common during handling and transport of seed in trucks and railcars. Dispersed seeds may lie dormant in the soil, germinating over a protracted period of many years to produce weeds of those Brassica species (Figure 8). Resultant weeds pose a threat of cross-pollinating with Brassica vegetable seed crops that might be planted in nearby fields. However, management plans specifically to limit the inadvertent introduction of volunteer crucifer plants into vegetable seed production areas currently do not exist in Washington State.

The third challenge was related to the fact that canola is one of the world's 25 most important food crops that is also sexually compatible (Figure 9) with some related Brassica vegetable species (CAST 2007). With the recent increase in acres of canola and other crucifers grown in Washington State as well as in other areas, many Brassica seed growers are concerned that

Figure 5. Cabbage seedlings infected with Phoma lingam*. Upper left photo: Healthy seedling (back) and seedling with symptoms on cotyledon (front, arrow points to infected area of the cotyledon). Upper right photo: Close-up view of an infected cotyledon showing small black fruiting bodies (pycnidia) of the pathogen. Lower photo: Hypocotyl of an infected seedling with mature pycnidia that each produce spores in a pink, gelatinous matrix (cirrhus) (photos courtesy of L. du Toit).*

crop isolation distances might not be maintained, potentially resulting in increased risk of cross-pollination with undesirable *Brassica* species (Myers 2006).

The fourth challenge was that a majority of canola seed used to plant oilseed crops carries genetic modifications for herbicide resistance. Many vegetable seed industry members believe that canola crops with such transgenes present a significant risk of introducing herbicide-resistant transgenes into Brassica vegetable seed lots. Transgenes could be introduced by accidental cross-pollination with transgenic crops of the same species. Transgenes could also be introduced via volunteer plants in nearby fields that grow from seed left after transgenic crops have been harvested or by volunteer plants that grow from seed spilled along transportation routes (Légére 2005; Schafer et al. 2011). Currently, transgenic traits are not accepted in any of the markets in which Washington's Brassica vegetable seed lots are sold, and some buyers of Brassica vegetable seed have threatened to remove all contracts for seed crops if such transgenes are detected in Brassica vegetable seed lots produced in Washington State.

Coexistence in the Brassica Seed Industry in Washington State

Genetic purity (trueness-to-type) is one of the most essential attributes of a seed lot. To protect against pollination from undesired genetic sources and to ensure seed purity, seed crops (whether vegetable or

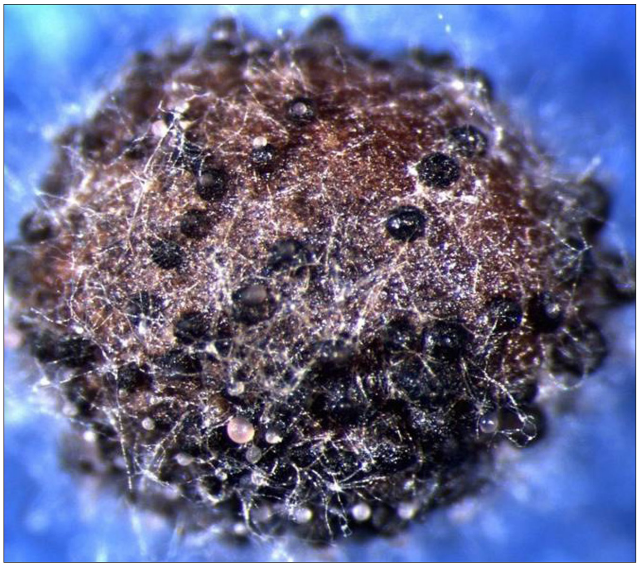


Figure 6. Cabbage seed infected with the black leg fungus, Phoma lingam. *The small black fruiting bodies (pycnidia) each produce a pink mass of spores oozing out of the open end (ostiole) when mature (photo courtesy of L. du Toit).*

Figure 7. Cabbage plants with symptoms of black rot (photos courtesy of L. du Toit).

Figure 8. Numerous volunteer canola seedlings growing in a field following seed-pod shatter (photo courtesy of T. Miller).

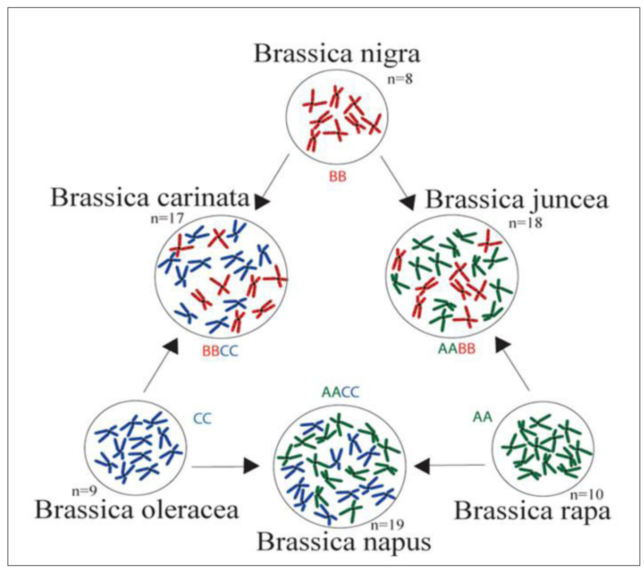


Figure 9. The triangle of U theory on the evolution and relationships among members of the genus Brassica *posits that genomes of three ancestral species combined to create three common contemporary vegetable and oilseed crop species. The diagram shows the genetic relationships among six species of* Brassica*. Chromosomes from each of the genomes A, B, and C are represented by different colors. The theory has been confirmed by DNA and protein studies (http://en.wikipedia.org/wiki/Triangle_of_U).*

other types of seed crops) are customarily isolated by a minimum distance to prevent cross-pollination among seed crops of the same species, such as canola, rutabaga, turnip, and Chinese cabbage. The isolation distances were determined by Washington seed growers and seed company specialists in the seed production areas and are enforced each season before any seed crops are planted. The minimum required isolation distance for Brassica seed crops varies depending on the species, type within species (e.g., red vs. white cultivars, smooth vs. savoy leaf cultivars, etc.), and whether the seed is grown for market seed or as stock seed. Isolation distances range from 0.25 miles to 5 miles based on the distances that insect pollinators can travel and the degree of risk from cross-pollination producing off-types in the crop planted from the harvested seed lot.

To segregate Brassica seed crops, field representatives from Washington State vegetable seed companies meet twice a year for seed crop-mapping (pinning) sessions at either the Washington State University (WSU) Mount Vernon Northwestern Washington Research and Extension Center (NWREC) (http://mtvernon.wsu.edu/index.html), or the WSU Extension office for Grant and Adams Counties (http://county.wsu.edu/grant-adams/Pages/default.aspx). At these meetings, seed crop field locations for the coming year are finalized. A random drawing is held to determine the order in which seed company field representatives choose their desired seed crop field location. Selected locations are then marked on the crop-pinning map (Figure 10). A selected field must adhere to the minimum accepted isolation distances. This time-honored system of voluntary cooperation has been in place for more than 50 years and has been a mainstay of the highly successful Brassica vegetable seed industry in Washington State. It is an excellent example of coexistence in agriculture.

Coexistence is a term sometimes applied to agriculture when conventional, organic, seed, and/or transgenic crops are grown successfully within the same geographical region. Historically, the term was used in international affairs (Wikipedia 2012) for the principles of equality, mutual benefit, non-aggression, and respect for sovereignty and territorial integrity, as well as for the concept of non-interference in internal affairs, all of which were used for peacekeeping purposes. However, whether conventional, organic, seed, and transgenic crops can coexist successfully has been a subject of much debate. Proponents of transgenic crops believe that such crops can coexist successfully with conventional crops. Many people also believe that coexistence is an economic issue rather than a safety issue, that zero tolerance thresholds for cross-contamination are not realistic for crop production systems, that more practical thresholds are viable, and that gene flow research data have inferred that breaches of established cross-pollination thresholds would be rare. Additionally, many proponents of transgenic crops believe that not all growers should have to meet the marketing standards of some growers and, ultimately, that the success of coexistence will be determined by market demand (SCIMAC 2006). In contrast, those concerned about unintentional pollen contamination of established crops by genetically modified crops point to examples where genetically engineered traits have been detected in crops of non-genetically modified plants (Légére 2005; Schafer et al. 2011). Such cases can result in a complete loss to growers if the markets to which they sell seed do not permit transgenic traits (Crossfield 2011; CAST 2007), particularly high value export markets.

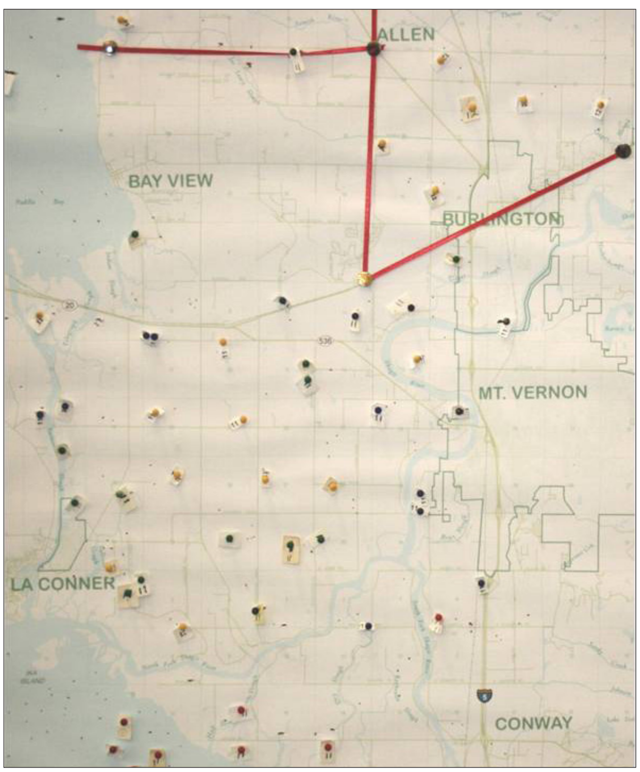


Figure 10. Vegetable seed 'crop-pinning' map showing locations of vegetable seed crops in Skagit County, Washington, that meet minimum isolation distances so as to avoid unwanted cross-pollination among neighboring seed crops. Each pin represents a seed crop. The red ribbon identifies regions acceptable for growing particular vegetables, (e.g., red vs. green cabbage cultivars, smooth vs. savoy leaf spinach cultivars, etc.) (photo courtesy of L. du Toit).

Protecting Brassica Seed Crops and Commercial Brassica Crops

Regulated seedborne crucifer pathogens. Black leg and black rot are economically significant seedborne diseases that can affect all Brassica vegetables, as well as related Brassica crops and weeds (Figures 5 and 7). *P. lingam* is a fungus that can cause severe losses as a result of transmission of seedborne inoculum after planting (Figure 6). If weather conditions and production practices are favorable, the fungus spreads via splashing water and by inoculum carried on workers' clothing and/or equipment moving through the crop. Severely affected plants can be stunted, wilted, and turn a blue-red color (hence the name black leg), and may collapse and die. Similarly, the bacterium *X. campestris* pv. *campestris* can be carried externally or internally on Brassica seed, leading to seed transmission after planting. Infected seeds will germinate and produce diseased seedlings. Infection usually moves up or down the plant's main stem, starting from infected leaves. This infection can become systemic (Figure 7), and infected plants may wilt and die. Many black rot outbreaks have been attributed to the pathogen infecting seedlings in seedbeds or nurseries, then spreading among adjacent plants after transplanting, and subsequently spreading to adjacent fields by rain and irrigation water, insects, equipment, and animals. Such rapid spread can result in severe outbreaks of black rot when temperatures are warm (80°F to 90°F).

Due to the seedborne nature of these two diseases and the need to plant pathogen-free seed to manage the diseases effectively (du Toit 2007), the Washington State Department of Agriculture (WSDA) Seed Program processed a Crucifer Quarantine request in 2005 through the rule-making process. Input was obtained from WSU Research and Extension specialists and affiliated stakeholders — the Puget Sound Seed Growers Association, Columbia Basin Vegetable Seed Association, Washington Canola Commission, vegetable seed companies, and growers, among others. A Crucifer Seed Quarantine (Table 2) for six northwestern Washington counties was subsequently implemented in January 2006 (See Chapter 16.301, Sections 490-580, Washington Administrative Code on General Seed Regulations at http://agr.wa.gov/plantsinsects/PlantQuarantines/PlantQuarantines.aspx#CRUCIFER_SEED_QUARANTINE). Washington's Crucifer Seed Quarantine includes regions of Clallam, Island, Lewis, Skagit, Snohomish, and Whatcom Counties.

Table 2 lists the various rules of this quarantine. The provisions cover crucifer seed, seedlings, roots, and/or transplants for seed, oil, and/or commercial vegetable production, as well as crucifer crop residues. Quarantine rules require that a Notice of Intent/Quarantine Compliance Form (section 16-301-530) be filed with the WSDA Seed Program before shipping, moving, or transporting crucifer seed into regulated areas. The Notice of Intent must be accompanied by a seed analysis or phytosanitary certificate from a certified laboratory that shows the seed lot tested negative for these two regulated pathogens. Another requirement is a seed analysis certificate that confirms the seed lots tested negative for dormant seed, as described in WAC 16-301-510.

To obtain a Notice of Intent/Compliance form, contact the WSDA Seed Program at 21 N 1st Ave. Suite 203, Yakima, WA 98902. Tel: (509) 249-6950, Email: VShaul@agr.wa.gov. Exemptions to the quarantine include U.S. Department of Agriculture (USDA) and university research trial grounds, prepackaged cruci-

Table 2. Chapter 16.301 Washington Administrative Code rules on Crucifer Seed Quarantine (http://apps.leg.wa.gov/WAC/default.aspx?cite=16-301).

Rule	Rule description
16-301-490	Why is the department establishing a crucifer seed quarantine?
16-301-495	What definitions are important to understanding this chapter?
16-301-500	What crucifer articles are regulated by this chapter?
16-301-505	What diseases are regulated by this chapter?
16-301-510	What seed must undergo dormancy testing?
16-301-515	What is the quarantined area for this crucifer seed quarantine?
16-301-520	What is the regulated area for this crucifer seed quarantine?
16-301-525	What are the exemptions to the crucifer seed quarantine that apply within the regulated area?
16-301-530	What requirements apply to planting crucifer seed in the regulated area?
16-301-535	What requirements apply to boxes and racks used to ship crucifer seedlings?
16-301-540	What requirements apply to crucifer transplants grown in greenhouses in the regulated area?
16-301-545	What requirements apply to crucifer seed lots that test positive for any regulated disease?
16-301-550	What protocols must be followed before the seed is planted in a regulated area, if documentation verifying that crucifer seed is free from regulated diseases is not available.
16-301-555	How are approved trial grounds established and what rules apply to them?
16-301-560	What are the inspection requirements for trial grounds?
16-301-565	What are the testing requirements for seed harvested from an approved trial ground?
16-301-570	What are the penalties for violating the crucifer seed quarantine?
16-301-575	How are diseased crucifer seeds and infected fields identified?
16-301-580	What regulations apply to diseased crucifer seeds and infected fields?

fer seed in samples of <0.5 oz (if the parent lot tested free of these two pathogens), seedlings for home garden use if free of diseases, and crucifers produced solely in greenhouses or indoors (WAC 16-301-525).

The laboratory analyses used to test Brassica seed lots for the two quarantine pathogens must meet the International Seed Federation (ISF) testing standards. (See http://www.worldseed.org/isf/ishi_vegetable.html). Detecting *P. lingam* requires a minimum sample of 400 seeds and a maximum sub-sample of 100 seeds. Detection is done by incubating seeds on a blotter and identifying the fungus microscopically based on the morphology of both pycnidia and spores. ISF seed-testing standards for *X. campestris* pv. *campestris* require a minimum sample of 30,000 seeds and a maximum sub-sample of 10,000 seeds. Viable pathogens are detected by soaking seeds and then plating the resulting seed extract on selective agar media. If a seed lot tests positive for either of the two pathogens, the seed lot must be disinfested by seed treatment, and the treated seed lot must then be retested to ensure that the lot tests negative for both pathogens. (The methods used for retesting seed are slightly different from those used for initial testing.) Retesting a seed lot is required before the seed lot can be planted in the area of northwestern Washington described in the Crucifer Quarantine. For a listing of some commercial seed-testing laboratories in the U.S., see the Pacific Northwest Vegetable Extension Group (PNW VEG) vegetable resources listed at http://mtvernon.wsu.edu/path_team/VegCropResources.htm. Some commercial seed-testing laboratories offer additional seed assays, e.g., testing for transgenes.

Canola and other oilseed crucifers as potential weeds. The Crucifer Seed Quarantine rules also require a seed analysis certificate from a seed-testing laboratory certifying that a seed lot tested negative for dormant seed. Limiting the amount of dormant seed that is planted helps minimize the number of volunteer Brassica plants that might grow as weeds in subsequent rotational crops. Volunteer Brassica plants can be a problem because pollen and/or seeds produced by these volunteer plants can be transported by wind, water, animals, and people. Also, volunteer Brassica plants compete with rotation crops and may survive to flowering, thereby producing more seed (and pollen), which can result in a field infestation that may persist for years. Fortunately, crucifer crop seeds on or near the soil surface usually germinate within a year and are, therefore, rapidly depleted in the seed bank of a field. However, buried seed can remain viable for many years. The amount of dormant crucifer seed permissible in a certified seed lot is less than 1%, so purchasing certified crucifer seed is recommended. For a listing of seed laboratories that test for dormant seed, see the PNW VEG resources listed at http://mtvernon.wsu.edu/path_team/VegCropResources.htm.

Brassica seed crops can be subject to bird predation, wind shatter (Figure 8), and spillage during transport and processing. Brassica seeds are spherical and approximately 3 mm or less in diameter, so the seeds roll and bounce and can easily contaminate farm machinery, storage bins, trucks, and train cars, and subsequently roadsides and fields during shipping and handling (Légére 2005; Schafer et al. 2011). The spread of seed can result in volunteer plants (and, hence, pollen) in undesired locations. To reduce the impact of volunteer crucifer plants in a region, good weed management practices are essential. These practices include timely control to prevent the flowering of crucifer weeds in rotational crops, along transportation routes, during seed handling, and in machine/vehicle storage areas. Some useful suggestions regarding the clean-up of oilseeds from shared farm machinery are presented by Allnutt et al. (2013) in the context of UK agriculture.

For information on managing crucifer weeds in Washington crop and non-crop areas, see the Pacific Northwest Weed Management Handbook (http://pnwhandbooks.org/weed/).

Managing gene flow by field isolation. The extent of gene flow between crop species depends on how easily cross-pollination can occur. Cross-pollination is affected by a species mode of pollination (wind-, insect-, or self-pollinated), the role of the species in the environment (food crop, weed, native plant, etc.), and the extent to which the species produce compatible male and female flower parts at the same time and location (CAST 2007). Open-pollinated species, such as Brassica species, are more susceptible to gene flow than are self-pollinated crops like wheat. In fact, contemporary vegetable and oilseed *Brassica* species, such as *B. carinata, B. juncea*, and *B. napus*, are thought to have originated when ancestral genomes combined among three species, *B. nigra, B. rapa*, and *B. oleracea* (Nagaharu 1935) (Figure 9).

Canola is an acronym for Canadian oil low in acid and is a registered name used for food-quality rapeseed oil marketed by the Western Canadian Oilseed Crushers' Association. Non-food-quality rapeseed oil, in contrast, is used as a machine lubricant or for diesel-like fuel for machinery because the concentrations of erucic acid and glucosinolates make it unsuitable for human or animal consumption. Canola is low in erucic acid and glucosinolates, making it suitable as a cooking oil and livestock feed (Bragg and Burns 2001; Hang et al. 2009). Although *B. napus* canola is a term of commerce, three species of *Brassica* are used to crush oil from seed in order to meet the consumption and quality standards of canola: *B. napus, B. rapa*, and *B. juncea*. All three species can pose challenges to crucifer seed production given how widely they are grown as oilseed crops (Table 3) and given the recent significant increase in canola production in Washington State. Canola is sexually compatible with a number of crucifer weeds (Table 4), and there are reports of canola producing fertile hybrids with *B. rapa, Sinapis arvensis, Erucastrum gallicum*, and *Raphanus raphanistrum* (Tables 1 and 4; Figure 9) (Beckie 2006; Légére 2005; Myers 2006). Furthermore, canola can readily become a weed, for the reasons discussed previously. Such risks of cross-pollination are relevant to all crucifer seed crops, not just Brassica vegetable seed crops.

Many row crop growers in Washington State have expressed interest in growing Brassica oilseed crops as an alternative rotation crop, not only for potential sales in the biofuel market, but also for high-value seed meal and as a cover crop. Research has shown that spatial isolation, pollen barriers, and other methods are effective methods for preventing cross-contamination between crop species (Devos et al. 2004; CAST 2007). In Washington State, isolating Brassica vegetable seed crops has helped prevent unwanted long distance movement of pollen for more than five decades and ensured genetic trueness-to-type in seed crop production. However, a lack of awareness regarding the implications of planting oilseed Brassica crops, such as canola and mustard, within Brassica seed production areas for purposes other than hybrid seed production can put long-

Table 3. Excerpt on canola from Production and export of major biotech crops and biological factors that may influence post-commercialization dispersal and resistance of transgenes in the U.S. and Canada. *Adapted from CAST Issue Paper No. 37, Dec. 2007.*

Characteristic	Canola
Harvested in U.S. in 2005 (millions of hectares)	0.45
Harvested in Canada in 2005 (millions of hectares)	5.51
Percentage biotech in 2005, U.S.	82
Percentage biotech in 2005, Canada	82
Percentage of yield exported in 2005, U.S.	20
Percentage of yield exported in 2005, Canada	48
Extent of outcrossing	Moderate (limited at long distance)
Sexually compatible, wild or weedy relatives in U.S.	Common
Extent of volunteers in rotational crops within agricultural fields	Common
Extent of volunteers or naturalized populations outside agricultural fields	Common

Table 4. Excerpt on rapeseed canola from World's 25 most important food crops with sexually compatible weed species. *Adapted from CAST Issue Paper No. 37, Dec. 2007* .

Rank	Crop	Scientific name	World area planted (M Ha)	World yield (MT)	Related weeds, sexually compatible with crop	Rank among world's worst weeds	Geographic distribution
11	Rapeseed (canola)	*Brassica napus* *B. ropa*	24	36	*B. napus*	>180	Europe, Argentinia, Australia, Canada, U.S.
					B. juncea	>180	Australia, Argentina, Canada, Fiji, Mexico, U.S.
					B. rapa = B. campestris	77-180	Worldwide (temperate) > 50 countries
					B. adpressa	>180	Europe, Australia, southern Africa, Argentina, U.S.
					Raphanus raphanisrum	77-180	Worldwide (temperate) > 65 countries
					Sinapis arvensis = B. kaher	77-180	Worldwide (temperate) > 52 countries

established and successful agricultural industries at risk. Despite the WSDA-implemented Crucifer Seed Quarantine, the production of canola and related Brassica species for commercial oilseed purposes within the proximity of Brassica vegetable seed crops or canola seed crops has been perceived as a threat by many seed growers. These growers are concerned that field isolation distances for Brassica vegetable seed crops may no longer be maintained effectively in Washington State.

In 2008, in response to these concerns, Brassica vegetable seed growers, canola seed growers, commercial oilseed growers, and university and government officials cooperated to create three regulated Brassica Seed Production Districts (a form of isolation district) (Table 5) (Chapter 16.326, Sections 010-060, Washington Administrative Code on Brassica Seed Production Districts http://apps.leg.wa.gov/WAC/default.aspx?cite=16-326). These WAC rules govern growing, transporting, and processing Brassica seed within the borders of the regulated areas, as well as growing Brassica seed in District 1 (parts of Clallam, Island, Skagit, Snohomish, and Whatcom Counties) and Districts 2A and 2B (parts of Grant and Adams Counties). See the Washington Brassica Seed Production Districts map on the WSDA website at http://www.arcgis.com/home/webmap/viewer.html?webmap=04941fb46ba8447e842a31d845d72a24&extent=-125.7211,44.532,-114.7897,49.6216.

Some key requirements are establishing minimum isolation distances of two miles between Brassica crops, participating during map 'pinning' sessions to ensure minimum isolation standards, and developing written agreements regarding exceptions to the rules. The rules apply to everyone, including researchers and extension educators, and were designed to avoid interfering with the production of canola or other oilseed crops outside the regulated areas. Since the districts were created, there have been no rule violations —another example of effective cooperation and coexistence. The significance of this coexistence was highlighted in 2009, when cabbage seed acreage in western Washington was the largest since 1999 (Don McMoran personal communication).

By 2008, almost 3,000 of the estimated 13,000 total acres of canola grown in dryland and irrigated areas of central and eastern Washington were certified canola seed crops, not oilseed crops (http://agr.wa.gov/Inspection/SeedInspection/docs/2011FinalProducing.pdf). Thus, at the request of stakeholders, the WSDA Seed Program Advisory Committee met in February of 2012 to discuss a proposal to amend the Washington Brassica Seed Production District Rules. This amendment would prohibit production of *B. napus* var. *biennis* (winter-type canola or rapeseed) in Brassica Seed Production District 2. The proposed amendments to WAC 16-326-040 and WAC 16-326-050 were requested by representatives of three major spring canola production companies operating in Washington State. Brassica Seed Production District 2 has developed into a premier spring canola seed production area, but when winter canola or rapeseed is grown in close proximity to a spring canola seed crop, cross-pollination can occur, resulting in off-types in the harvested seed, which can render the resulting harvested seed useless for sale as a seed lot. Prohibiting winter-type canola or rapeseed production in Brassica Seed Production District 2 provides some protection to the spring canola seed crop. This prohibition sets the stage for expanding spring canola seed production in Washington State, which seed companies and WSDA personnel have indicated "will increase business opportunities for growers and provide economic benefits to the agricultural community." Public hearings for the proposed rule-making occurred in the spring of 2012, and subsequent rule-making procedures were initiated (Victor Shaul WSDA Seed Program personal communication).

Table 5. Chapter 16.326 Washington Administrative Code rule on Brassica Seed Production District *(see http://apps.leg.wa.gov/WAC/default.aspx?cite=16-326).*

Rule	Rule description
16-326-010	What are the boundaries of the regulated areas, also called the Brassica seed production districts?
16-326-020	What are the general requirements for growing, transporting or processing Brassica seed within any Brassica seed production district?
16-326-030	What are the requirements to grow Brassica seed in Brassica seed production district 1?
16-326-040	What are the requirements for growing Brassica seed in all of Brassica seed production district 2, which is composed of two subdistricts designated districts 2A and 2B?
16-326-050	What are the differences between restrictions on Brassica seed production in Brassica seed production districts 2A and 2B?
16-326-060	What is the Brassica work group and how often does it meet?

Herbicide-resistant transgenes. Canola was among the first agricultural crops to be modified genetically for herbicide resistance. Canola cultivars with resistance to glyphosate, glufosinate, imidazolinones, and sulfonylureas (types of herbicides) are grown widely throughout the world. Such herbicide resistance offers improved weed control, higher yields, lower input costs, and greater net returns to the growers who plant herbicide-resistant crops (Devine and Buth 2001; Glick 2001). There has been some concern, however, that herbicide-resistant transgenes (e.g., Roundup Ready® or Liberty Link® in canola) may be transferred into conventional canola crops or to wild relatives of canola by pollen drift or seed dispersion. Canola pollen can travel considerable distances, but the extent of gene flow is believed to be minimal (Rieger et al. 2002). However, even minimal levels of seed dispersal can potentially result in gene flow between genetically modified and non-genetically modified plants (Knispel and McLachlan 2010). Furthermore, volunteer canola plants may be important in the spread of transgenes, which holds implications for the coexistence of genetically modified and non-genetically modified crops (Knispel and McLachlan 2010). The risk of gene flow can be seen in cases where seeds from some transgenic Brassica species have led to the adventitious presence of transgenic canola volunteers beyond agricultural fields (Hall et al. 2000; Rieger et al. 2002; Légére 2005; Saji et al. 2006; Knispel and McLachlan 2010; Schafer et al. 2011). Table 3 lists information on the worldwide production and export of canola, and Table 6 lists the biological factors that may influence dispersal and persistence of herbicide-resistant transgenes.

The economic consequences of gene flow from

*Table 6. Excerpt on canola (*Brassica napus*) from* Biology and gene flow potential of soybean, corn, and canola*. Adapted from CAST Issue Paper No. 37, Dec. 2007.*

Production	Pollen	Seeds and grain	Volunteers
• Bred for oil quality, high protein meal for animal feed, and low levels of several harmful natural products	• Primarily self-pollinating, but outcrossing between neighboring plants occurs at frequencies of 12% to 55% (Légére 2005)	• Following harvest, grain enters handling system and may be commingled before export or transport	• Seed lost during grain handling can germinate along roadways and in other disturbed areas
• Seed is third most important source of vegetable oil worldwide, about 11%	• Dispersal predominantly by insects and occasionally wind	• Seeds are small and can be lost at harvest, due to shattering or inefficient harvest	• Seeds that occur on or near soil surface usually germinate the following year and are rapidly depleted
• U.S.: grown on 0.4 M ha; 2005 value of $148 M; net importer	• Outcrossing between adjacent crops averages 1% at common border and diminishes with distance	• Grain movement and loss within handling systems are difficult to predict and provide well-known avenues for longer-distance seed transport	• Buried seed can remain viable for several years
• Canada: grown on 5.5 M ha; 2005 value of $1.85 B; exports 48% of yield, often as bulk grain	• Pollen-mediated outcrossing between herbicide-resistant biotech and nonresistant fields detected at moderate distances (several hundred meters) (Hall 2000) and long distances (several km) (Rieger 2002) from biotech pollen sources	• Well-established management practices are successfully used in Canada (Beckie 2006; Beckie et al. 2004)	• Volunteer canola currently is an important weed within crop fields and field margins in Canada. These volunteers often make a much more important contribution to adventitious presence than gene flow does, and they may require additional management practices for clean-up
• Seeds produced using both open-pollinated and hybrid variety systems	• Most open-pollinated and hybrid seed lots tested contained adventitious presence of transgenes at amounts between trace and 2% (Légére 2005)		• Herbicide-resistant volunteers that are not controlled in herbicide-resistant crops can decrease yields, so producers commonly combine herbicides and rotate herbicide-resistant crops to decrease their abundance (Beckie 2006)
• Growers rarely replant seed from hybrid varieties due to loss of vigor and crop uniformity	• When a canola crop or volunteer outcrosses, offspring may contain two or more herbicide-resistance genes (Hall 2000)		
• Three herbicide-resistant traits commercialized in 1996			
• Use of biotech canola in U.S. and Canada has not locked access to most major international markets			

genetically modified biotech (GM) crops can differ in crops produced for seed vs. crops produced for commodity uses, or in traditional vs. niche markets (CAST 2007). Managing gene flow is important for growers of genetically modified and non-genetically modified crops alike. Myers (2006) discusses the implications of growing oilseed Brassica crops in regions where there are vegetable Brassica seed crops and the need to minimize the risk of outcrossing. The University of Vermont Extension Service (Grubinger and Deziel 2003) has recommended coexistence strategies under similar circumstances that include:

- Discussing transgenic crop intentions with neighbors and considering buffer strips to mitigate potential risks of contamination,
- Knowing what is planted by checking seed labels,
- Recognizing that shared equipment, especially combines, can move seed and contribute to establishment of volunteer plants,
- Coordinating rotation plans with adjoining farms to minimize pollen drift,
- Staggering planting dates to avoid coinciding maturation dates, and
- Using physical distance to separate crops.

Effective Coexistence for Crucifer Crops

It is important that Brassica seed crop growers (vegetables and other seed crops) and commercial crucifer crop growers, researchers, and extension educators understand the significance of Washington State's vegetable seed and emerging biofuel industries. The regulatory and social implications of coexistence among the various Brassica crops in different areas are important issues. A glossary of terms and a list of suggested readings are provided in this publication to help increase the knowledge and understanding of this complex issue. In addition, all stakeholders should be aware that:

- The Crucifer Seed Quarantine requires phytosanitary testing and certification of Brassica seed lots before the seed can be planted in the following six northwestern Washington counties: Clallam, Island, Lewis, Skagit, Snohomish, and Whatcom. Testing is required to detect two seedborne pathogens, *Phoma lingam* and *Xanthomonas campestris* pv. *campestris*, and to determine the percentage of dormant seeds.
- Certified laboratory services are available for testing Brassica seed lots for the two quarantine pathogens and for seed dormancy.
- Brassica Seed Production Districts now exist in parts of Adams, Clallam, Franklin, Grant, Island, Skagit, Snohomish, and Whatcom Counties, and they govern the growing, transporting, and processing of all crucifer seeds.
- Map-pinning sessions for locating Brassica seed crops take place twice a year at the WSU Mount Vernon NWREC and the WSU Grant/Adams Area Extension office.
- Brassica seed can be dispersed readily during harvest, transport, and storage, so it is imperative to find ways of reducing inadvertent seed spillage.
- Information is readily available on crucifer disease and weed management practices that mitigate the risk of diseases developing in crucifer crops and minimize the establishment of weedy crucifer populations.
- Changes occur over time in the various Brassica crop production acreages of Washington State.
- The Seed Program within the WSDA is responsible for ensuring compliance with Washington State and U.S. seed laws and can be contacted for additional information at http://agr.wa.gov/inspection/SeedInspection/docs/Brochure.pdf, or at 866-865-6137.
- The principles of mutual understanding and cooperation can help Brassica crop production industries coexist effectively in Washington State.

Glossary of Terms:

Adventitious presence. The unwanted presence of substances which unavoidably enter the production, channeling, and marketing of crop products (CAST 2007).

Annual. A plant that takes one growing season to flower.

Biennial. A plant that takes two growing seasons to flower.

Biofuel crop. A crop from which fuel is derived from the plants.

Biofumigant crop. A Brassica or other green manure crop that can suppress some soilborne pathogens, pests, and weeds through the hydrolysis of glucosinolates or other fumigants produced by the plants, which results in a natural biofumigation effect (Kirkegaard and Matthiessen 2004).

Bolting. When plants enter the reproductive (flowering) stage of growth. Bolting can be induced by exposure of plants to an extended cold period (vernalization) or to long day length, depending on the genus and species of the plants.

Brassica. A genus of plants in the mustard family (Brassicaceae); members of this family are sometimes called crucifers, cabbages, or mustards.

Certified. Approved as meeting minimum standards set by a regulatory or certifying agency.

Cover crop. A crop planted, and sometimes incorporated into the soil, instead of being harvested as a cash crop. Cover crops are used for a range of potential benefits, such as providing nitrogen (leguminous crops), adding organic matter, improving soil structure, reducing soil erosion, providing weed control, managing nutrients, and furnishing moisture-conserving mulch (Sustainable Agriculture Network 2007).

Crucifer. A plant often referred to as a mustard, in the Brassicaceae or cabbage family. The name is in reference to the four petals on each flower, which form a cross.

Cross-pollination. When pollen is delivered to a flower from a different plant, by wind, insects, or mechanically.

Dormant seed. Seeds in which germination is delayed for various durations, even when environmental conditions for germination are optimal.

Erucic acid. A type of fatty acid present in some mustards. Erucic acid is toxic to animals, so crops with moderate to high levels of erucic acids cannot be used for animal feed, and oil from crushed seed cannot be used as vegetable oil for human consumption.

Gene flow. The successful transfer of genetic information between different individuals, populations, subsequent generations, and across spatial dimensions (CAST 2007).

Genetically modified (GM) plants. Plants with DNA that has been modified through genetic engineering (recombinant DNA) techniques. Synonymous with genetically engineered and transgenic (Byrne and Fromherz 2003).

Genetic purity. Trueness-to-type; one of the most essential attributes of a seed lot.

Glucosinolates. A group of sulfur-containing compounds, present in many cruciferous plants, that are converted enzymatically into biofumigant products toxic to many plants, fungi, bacteria, and insects.

Green manure crop. A cover crop grown and incorporated into the soil to add organic matter and nutrients to the soil.

Herbicide resistance. Herbicide resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild-type plant. In plants, resistance may be naturally occurring or induced by techniques, such as genetic engineering or selection of variants produced by tissue culture or mutagenesis (Weed Technology 1998).

Hybrid seed. Seed produced in a hybrid seed crop, resulting from pollination of the flowers of one parent line with pollen from a second parent line; the cross between parents is specific and controlled.

Isolation district. A regulated, geographic area established to prevent unwanted cross-pollination among crops.

Oilseed crop. A type of crop grown for oil extracted from the seed.

Open-pollinated seed. Seed produced when pollen is transferred (by insects, birds, wind or other natural mechanisms) between plants selected as a population for specific desired traits. Open-pollinated cultivars are more variable genetically than hybrid cultivars.

Open source. Free public access.

Outcrossing. The crossing of an organism with another organism of a different genotype; for plants, outcrossing is pollination of one plant by pollen from another plant with a different genotype.

Phoma lingam. The asexual state of a fungus that causes the disease black leg in Brassica plants and, in Washington State, is a regulated, seedborne pathogen in six counties. The fungus also has a sexual stage (teleomorph), *Leptosphaeria maculans*.

Phytosanitary certificate. A certificate issued by an inspection agency or certified laboratory to indicate that a consignment of plants, plant products, or other regulated articles meets specified phytosanitary import requirements and is in conformity with the certifying statement of the model certificate (http://www.aphis.usda.gov/import_export/plants/plant_exports/faqs.shtml#1).

Pollen drift. When pollen that is carried by wind, insects, or animals pollinates nearby crops or weeds.

Pollinator parent. A parental (proprietary) genetic line in a hybrid seed crop developed as the pollen donor for pollinating plants of the female or recipient parent of the hybrid.

Pycnidium. A cup- or flask-shaped fruiting body that produces asexual spores of certain fungi (e.g., *Phoma lingam*).

Quarantine. State and/or federal legislative law controlling the transport, import, export, and/or sale of plants, plant parts, or other material, usually to prevent spread of pathogens, insects, mites, weeds, or other pests (from Glossary of Plant-Pathological Terms).

Raceme. A simple branch on which flowers are produced on visible stalks (e.g., by crucifer plants).

Seed crop. A crop grown for seed.

Seed meal. The remains of seeds after oil has been extracted from the seed. For canola, seed meal contains about 1% residual oil and 8% to 10% moisture, and is usually granulated to a uniform consistency or pelleted (http://www.canolacouncil.org/) for subsequent uses.

Self-pollination. When pollen from a flower pollinates the same flower or other flowers of the same plant.

Small seeded. A plant species that produces small seed (usually less than 5 mm in diameter).

Spore morphology. The size and shape of a spore.

Stock seed. The seed used to plant a seed crop, that is, the generation preceding the seed that is harvested and sold to farmers to grow commercial crops.

Systemic. Spreading internally throughout the plant.

Transgene. A gene or genetic material that has been transferred naturally or by any number of genetic engineering techniques from one organism to another (Byrne and Fromherz 2003).

Vegetable crop. In the context of this publication, a crop grown to produce vegetables for human or animal consumption as opposed to a crop grown to produce seeds.

Vernalization. The induction of flowering by exposure to low temperatures for a certain period of time. The minimum temperature and duration of exposure for vernalization varies depending on the genus, species, and cultivar.

Wind shatter. The release of seeds from mature pods split by the force of wind.

***Xanthomonas campestris* pv. *campestris*.** A bacterium that causes the disease black rot in crucifers and is a regulated, seedborne pathogen of Brassica plants in six counties in northwestern Washington State.

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