AN ABSTRACT OF THE DISSERTATION OF

Michael P. Quinn for the degree of Doctor of Philosophy in Crop Science presented on October 4, 2010.

Title: Potential Impacts of Canola (*Brassica napus* L.) on *Brassica* Vegetable Seed Production in the Willamette Valley of Oregon

Abstract approved:

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Carol A. Mallory-Smith

In the Willamette Valley of Oregon, a combination of the need for rotational crops and an increased desire for biofuel production created interest in planting *Brassica napus* (canola). However, questions were raised arisen over the potential damage canola production could have on the preexisting *Brassica* vegetable seed industry. To address these concerns three studies were conducted to: 1.) Determine the potential of gene flow and hybridization via pollen from *Brassica napus* to related *Brassica* vegetable crops; 2.) Evaluate whether transgenes will be detectable in harvested *Brassica* vegetable seed; 3.) Evaluate the potential for volunteer canola to become a contaminant in the *Brassica* vegetable seed crops. Crossing experiments were conducted in 2007, 2008, and 2009 using *Brassica rapa* or *Brassica oleracea* inbred line receptor plants placed within conventional *B. napus* fields. Once seed set occurred on the receptor plants, each was harvested individually and the seed germinated in a growth chamber. Flow cytometry, morphological and molecular

analyses were performed on the seedlings. Hybridization between *B. napus* and *B. rapa* inbreds was 74% in 2007, 89% in 2008, and 15% in 2009. However, no hybridization occurred between *B. napus* and the *B. oleracea* inbred lines. Experiments were conducted using transgenic *B. napus* and the previously mentioned vegetable species, to quantify outcrossing rates in a greenhouse environment. Transgenes were detectable in both germinable and non-germinable seed produced on non-transgenic plants. Following *B. napus* harvest at the field sites, shattered canola seed was collected from both windrow and non-windrow locations. Approximately 30 days after the shatter samples were taken, canola seedling recruitment counts were made in quadrats placed immediately adjacent to the location of the seed shatter samples. Results of this volunteer assessment indicated differences in seed shatter between fields and windrow vs. non-windrow locations, but seedling recruitment only differed by fields. These studies indicate that canola, if grown in the Willamette Valley, has the potential to hybridize with related *Brassica* vegetable species grown for seed. However, when managed properly, canola volunteer persistence is unlikely to be an issue within fields in the monocot crop rotations used in the Willamette Valley.

Potential Impacts of Canola (*Brassica napus* L.) on *Brassica* Vegetable Seed Production in the Willamette Valley of Oregon

by
Michael P. Quinn

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APPROVED:

Major Professor, representing Crop Science

Head of the Department of Crop and Soil Science

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Michael P. Quinn, Author

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COUNTRIBUTION OF AUTHORS

Dr. Carol A. Mallory Smith advised all aspects of the research conducted, as well as provided feedback throughout the project. Additionally, she was actively involved in the preparation and improvement of the manuscripts. Dr. James R. Myers provided both advice and plant material for the greenhouse and field crosses and assistance with the manuscript. Dr. Andrew Hulting also was involved with the improvement and preparation of the manuscript.

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CHAPTER 1: GENERAL INTRODUCTION

Oil seed rape or canola (*Brassica napus* L.) is an allotetraploid (*2n=4x=38*, AACC) originating from an ancient hybridization between the diploids *B. rapa* (*2n=2x=20*, AA) and *B. oleracea* (*2n=2x=18*, CC) (Ford et al. 2006). This relationship was first elucidated by U (1935), who documented that *B. napus* has genomes in common with *B. oleracea* and *B. rapa*. This close species relationship between diploid and allotetraploid *Brassica* species contributes to the ease with which interspecies crossing can occur (Meyers 2006).

Brassica napus appeared as a cultivated crop in Europe sometime in the early 1300's (Tsunda 1980). Most likely it originated at multiple locations along the northern Mediterranean and western European coast where the habitats of *B. rapa* and *B. oleracea*, feral or cultivated, overlapped. Olsson (1960) suggested that *B. napus* probably arose independently several times by spontaneous hybridization of different forms of *B. rapa* and *B. oleracea* growing in medieval gardens.

The taxonomy of the *Brassica* is still not resolved completely (Rubatzky and Yamaguchi 1999). A unique aspect of many of the *Brassica* crop species is that several different crops with varying morphologies are derived from the same species. Cabbage, kohlrabi, cauliflower, broccoli, Brussels sprouts, collards and kale are

derived from *B. oleracea*, while Chinese cabbage (pak choi and pe tsai), mizuna, broccoli raab, and turnip are all *B. rapa* (Rubatzky and Yamaguchi 1999).

Within the *Brassica* species varying levels of interfertility exists between species (Rieger et al. 2001) and reports vary greatly as to the extent of hybridization that can occur between species (Hancock 2004). A distinctive feature of *Brassica* species origin and evolution is the formation of allotetraploid species from hybridization of diploid progenitors (Olsson 1960). In general, viable crosses between diploids and allotetraploids occur more readily when the diploid parent has a genome in common with the allotetraploid parent. Whether these hybrids are viable, whether they will have restored fertility in subsequent generations and whether introgression of genes occurs in subsequent generations remain as important research questions (Chevre et al. 1998; Chevre et al. 2000; Jorgenson et al. 1996). Additionally, hybridization rates can vary depending on environment (Becker et al. 1992) and distance between the plants (Hall et al. 2000; Mesquida and Renard 1982; Timmons et al. 1995). Pollen flow from canola can travel long distances. Studies in Canada have found evidence of canola pollen movement up to 3 km (Rieger et al. 2002).

Sub-species of *B. rapa* vary in their level of cross-compatibility. Crosses are common between *B. rapa* and *B. napus*, though reported levels of hybridization vary (Brown, et al. 1995; Warwick, et al. 2003; Wilkinson, et al. 2000). Hybrids have been reported to have reduced fertility and lower seed production compared to the parents (Jorgensen and Andersen 1994). *Brassica oleracea* and *B. napus* hybridization is not common (Scheffler and Dale 1994) and hybrid progeny are difficult to obtain artificially (Chiang et al. 1997). However, hybrid progeny of a *B. napus* and feral *B.*

oleracea have been found in the wild (Ford et al. 2006), but at very low frequencies. While these hybridization events may be rare, the potential does exist for them to occur under field conditions.

Western Oregon, with its mild winters and dry summers, has the ideal climate for seed production. The Willamette Valley, in particular, has a specialty seed crop industry that produces both vegetable and flower seeds. While the *Brassica* specialty seed crop growing area is small, it can be very profitable, often netting a grower more than $4,000 per hectare depending on the seed crop grown (Ehrensing 2007). In fact, western Washington and western Oregon combined produce nearly all the world supply (~90%) of European cabbage, Brussels sprouts, rutabaga and turnip seed, and a substantial portion (20 – 30 %) of radish, Chinese cabbage and other Asian *Brassica* vegetable crops (Myers 2007). This production constitutes a significant portion of the global *Brassica* vegetable seed market; particularly European and Asian markets, as 50 to 60% of the seed grown is exported to these regions.

The combination of the need for broadleaf rotational crops within the grasses grown for seed cropping systems, and an increased desire for local biofuel production has created interest among growers to plant canola in the Willamette Valley. However, the specialty seed crop growers of western Oregon and Washington voiced concern about the potential negative impact growing canola in the region could have on the industry (Myers 2006). Very few other regions of the world have the unique climate to produce high quality *Brassica* vegetable seed. The *Brassica* vegetable seed crop production could be jeopardized if contamination occurs from canola hybridizing with vegetable varieties. The risk would be even greater if the crops were contaminated

with transgenic canola. International purchasers of the vegetable seed crops have extremely low tolerances for any contamination, and some maintain a zero tolerance for transgenic contamination (Tichinin 2007).

Hybridization studies among the species related to *B. napus* have primarily focused on gene flow to either *B. rapa* or to weedy relatives (Bing et al. 1996; Brown and Brown 1996; Jorgensen and Anderson 1994; Jorgensen et al. 1996; Lefol et al. 1995; Lefol et al. 1996; Warwick et al. 2003; Williams et al. 1986). Additionally, hybridization studies have not included gene flow to the *Brassica* vegetable crops (Myers 2006). Frequently if these crops are mentioned in published studies, the authors state that the vegetable crops are harvested before they flower so gene flow is not a concern. This conclusion is true if the crops are harvested prior to flowering, such as for fresh market crops, but not if they are being grown for seed production. A compounding factor that may increase outcrossing of *B. napus* to *Brassica* vegetable crops with is that many are male sterile or self-incompatible.

Therefore to address these issues, we addressed three general objectives: 1.) Determine the potential gene flow via pollen from *Brassica napus* to related *Brassica* vegetable crops; 2.) Evaluate whether transgenes will be detectable in harvested *Brassica* vegetable seed; 3.) Evaluate the potential for volunteer canola to become a contaminant in the *Brassica* vegetable seed crops.

CHAPTER 2: ASSESSMENT OF OUTCROSSING BETWEEN CANOLA (*Brassica napus* L.) AND RELATED BRASSICA VEGETABLE SPECIES

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ABSTRACT

In Oregon's Willamette Valley, a combination of need for broadleaf rotational crops and an increased desire for local biofuel production has created interest among growers for planting *Brassica napus* (canola). However, questions have arisen over the potential damage large scale canola production could have on the existing *Brassica* vegetable seed production industry. The reputation of the *Brassica* vegetable seed production industry is based on the purity and the high quality of seed. In fact, a seed lot may be rejected if more than three outcrossed seed per 1,000 seed is found. The risk is even greater if the crops are cross pollinated with transgenic canola because some international purchasers of the vegetable seed crops have zero tolerance for transgenic contamination. While there is a great deal of information on hybridization between canola and weedy species, very few studies address hybridization between canola and related vegetable species. To address this issue, experiments were conducted in 2007, 2008, and 2009 using *Brassica rapa* and *Brassica oleracea* inbred lines as pollen receptors placed within a conventional (non GMO) *B. napus* field. Flow cytometry, morphological analysis, and molecular markers were used to identify hybridization between the species. Greenhouse crosses were conducted using either a conventionally produced imazamox resistant or a transgenic glyphosate resistant *B. napus* line as the pollen parent and either a self incompatible *B. rapa* var. *chinensis* (Chinese cabbage) or cytoplasmic male sterile (CMS) *B. oleracea* var. *italica* (broccoli) inbred lines as the maternal parent. Herbicide resistant *B. napus* lines were used because they provide a reliable selectable marker for positive identification of a cross. Results of the field experiments indicated that hybridization occurred 74% in

2007, 89% in 2008, and 15% in 2009 between *B. napus* and *B. rapa* inbred lines. However, no hybridization occurred between *B. napus* and either *B. oleracea* inbred line. Results of the greenhouse crossing experiments using *B. rapa* as the maternal parent resulted in hybridization rates which ranged from 0 to 15.3% depending on *B. rapa* var. *chinensis* inbred line, and on which herbicide resistant *B. napus* paternal parent was used in the cross. Greenhouse crosses using *B. oleracea* inbreds as the maternal parent produced no germinable seed, and none of the aborted seed tested positive for the presence of the transgene. Presence of transgenic material was detected in both germinable and non-germinable seed produced on non-transgenic *B. rapa* female plants in the greenhouse crosses. We believe this is the first documentation of transgenic material identification in non-germinable seed produced on non-transgenic plants. This research demonstrates that the potential exists for hybridization between canola and some *Brassica* vegetable species under field conditions.

Nomenclature: canola, *Brassica napus* L., *Brassica rapa*, *Brassica oleracea*

Key Words: vegetable seed, off types, outcrossing.

INTRODUCTION

As the demand for biofuels grows in the United States, there is increasing interest in producing oilseed crops. Frequently, moving production to new regions can influence the established agricultural practices in unanticipated ways. Factors such as cross contamination, transgenic or otherwise, via gene flow raise concerns for the established commodity growers. Additionally, the new crops may increase insect and disease pressure in regions where these pests were previously low. These issues can result in conflicts between producers due to either real or perceived threats to the existing industry (Goodman 2000).

Western Oregon has an ideal climate for the production of many seed crops. In the Willamette Valley of Oregon, 200,000 to 300,000 hectares of grasses grown for seed are harvested annually, and the specialty seed crop industry produces over 5,600 hectares of vegetable and flower seeds. In total, about half of the arable land in the Willamette Valley is devoted to seed production. Such a concentration of seed production can not be found anywhere else in the world (Tichinin 2007). The combination of the need for broadleaf rotational crop options with grass seed crops, and an increased desire for local biofuel production has created interest in growing *Brassica napus* (canola) for oilseed production. However, the specialty seed crop growers of Western Oregon and Washington voiced concern about potential negative impacts that growing canola in the region could have on their industry. Very few other regions of the world have the climate to produce high quality *Brassica* vegetable seed. Twenty-five hundred to 3,000 hectares of *Brassica* seed are grown in the Willamette

Valley annually, with a value of $10 to $12 million dollars (Ehrensing 2007). Fifty to 60% of the seed is exported to Europe and Asia, constituting a significant portion of the global *Brassica* vegetable seed market.

Hybridization studies among the species related to *B. napus* have concentrated on gene flow to either non-vegetable *B. rapa* sub-species or to weedy relatives (Bing et al. 1996; Brown and Brown 1996; Jorgensen and Anderson 1994; Jorgensen et al. 1996; Lefol et al. 1995; Lefol et al. 1996; Warwick et al. 2003; Williams et al. 1986). While these studies are of ecological and agricultural value, they do not address outcrossing of canola with *Brassica* vegetable species (Myers 2006). If related vegetable crops are mentioned, the authors state that are harvested before they flower so gene flow is not a concern. This is true if the crops are harvested prior to flowering, as fresh market crops, but not when being grown for seed. *Brassica* vegetable species grown for seed can be either fall planted as seed or spring planted as transplants. Depending on the method of planting, synchronization of flowering can occur with either fall or spring planted canola. Additionally, many of the vegetable crops are male sterile or self-incompatible so greater crossing would be expected to occur. In field experiments examining outcrossing in canola, hybridization rates vary depending on environment and distance between the plants (Beckie and Hall 2008).

Hybridization between *Brassica* species varies greatly (Chiang et al. 1977; Becker et al. 1992; Chevre et al. 2000). For example, *Brassica napus* is self-fertile, but outcrossing rates as high as 47% have been reported (Williams et al. 1986). Pollen flow between canola cultivars has been well documented. In Canada gene flow between transgenic lines was reported at 800 m, which was the limit of the study

(Beckie and Hall 2008). Canola volunteer plants have been identified containing transgenes for both Roundup Ready™ and Liberty Link™ traits resulting from natural pollen movement under field conditions (Scheffler and Dale 1994; Hall et al. 2000; Schafer et al. 2010). Outcrossing between adjacent canola fields with differing herbicide resistance traits has resulted in volunteer canola with both conventional and transgenic herbicide resistance (Beckie et al. 2003).

Pollen of *Brassica* species can be disseminated by insect pollinators and by wind (Mesquida and Renard 1992; Beckie et al. 2003). Typically insect pollinators, such as honey bees, are capable of moving pollen less than a few kilometers (Pasquet et al. 2008). Wind dispersed pollen moves much greater distances, in some cases tens of kilometers. Dual-vector outcrossing may explain the disparity in reported pollen dispersal from a few meters to 25 km that has been reported for canola (Timmons et al. 1995), making it difficult to predict the furthest distance that viable pollen can move. Therefore, it may not be possible to establish adequate buffer zones to prevent cross pollination of compatible *Brassica* species in the field.

The taxonomy and genetics of the *Brassica* species are complex. One of the unique aspects of the crop species is that several crops, exhibiting very different morphologies, were derived from the same species and are, therefore, highly interfertile (U 1935; Hancock 2004). Cabbage, kohlrabi, cauliflower, broccoli, Brussels sprouts, and kale originated from *B. oleracea,* while Chinese cabbage (pak choi and pe tsai), mizuna, broccoli raab, and turnip are *B. rapa* (Rubatzky and Yamaguchi 1999).

Species of *B. rapa* vary in their level of cross-compatibility (Olsson 1960). Crosses are common between *B. rapa* and *B. napus* though reported levels of hybridization vary widely (Brown, et al. 1996; Warwick, et al. 2003). Additionally, *B. rapa* x *B. napus* hybrids have been found to have reduced fertility and lower seed set compared to either parental species (Jorgensen and Andersen 1994). Hybridization of *B. oleracea* and *B. napus* is rare. However, hybrid progeny of this cross have been found in the wild (Ford et al. 2006). While these hybridization events may be rare, the potential does exist for them to occur under field conditions.

Quantification of the impact canola may have on related *Brassica* vegetable seed crops via outcrossing is of importance to the seed production sectors in the Willamette Valley. The objectives of this study were: to determine the potential for gene flow and hybridization via pollen flow from *B. napus* to related *Brassica* vegetable crops under both greenhouse and field conditions, and evaluate whether transgenes could be detected in the resulting viable and aborted seed.

MATERIALS AND METHODS

Field Experiments. Studies were conducted in 2007, 2008, and 2009 near Corvallis, OR. In each year, one field was planted with conventional *B. napus* 'Athena' at the commercial sowing rate (~ 9 kg/ha). Brassica vegetable seed inbred lines were obtained from local sources. Accession numbers and inbred parental information are propriety information for these lines; therefore, codes were used identify the inbreds

used in each cross. In 2007 a self-incompatible *B. rapa* var. chinensis (Pak choi) inbred line (BRCF) and a cytoplasmic male sterile (CMS) *B. oleracea* var. *italica* (broccoli) inbred (BOI) were grown in the greenhouse and moved into the field when the *B. napus* began flowering, and returned to greenhouse after pollination. The source of the CMS in the *B. oleracea* inbred lines was the 'Anand' cytoplasm (Cardi and Earle 1997). In 2008 and 2009, *B. oleracea* var. *capitata* (BOCF, and BOCM) and a *B. rapa* var. *pekinensis* (Pei tsai) inbred lines (BRPF) were used as receptor species in the field experiments. These plants were grown in the greenhouse and moved to the field during *B. napus* flowering. The greenhouse plants were planted sequentially to ensure synchronization of flowering with the *B. napus*. Each *B. napus* x inbred line field experiment was conducted independently to prevent cross pollination between the receptor species. Isolation was achieved by placing only one receptor species in a field at a time. Initiation and duration of flowering were recorded for each species. Receptor plants were arranged in a 4 x 4 m grid inside the perimeter of a 15 x 15 m study area with one plant located at the intersection of the grid axes. Seed of each receptor plant were harvested individually. The seed were placed into 10.2 x 10.2-cm germination boxes containing moistened blotter paper[1] and put into a germination chamber set to a 24/17 C day/night temperature regime with a 13 h photoperiod (Warman 1999). The number of germinated seedlings was recorded and germination percentages calculated for each cross. Shrunken seed produced on the *B. oleracea* var. *italica* (BOI) plants were tested for viability with a tetrazolium assay according to methods described by the Association of Official Seed analysts (AOSA 2002).

Following germination counts, seedlings were removed from the growth chamber, transplanted into commercial potting soil[2], and transferred to the greenhouse with a 20/20 C day/ night temperature and no supplemental lighting. Once the seedlings reached the five leaf stage, approximately 1 cm^2 of leaf tissue was taken from each plant, and immediately placed on ice. Tissue samples were macerated in 2 ml LB01 buffer, incubated on ice for 5 min, then filtered through a 50 μm screen. The extract was placed in a centrifuge and spun for 10 min at 1000 rpm until the DNA pelletized. The supernate was removed and the pellet was resuspended in 300 μl of a 25 μg/ ml propidium iodide solution for 10 min. Ploidy level of the plants was determined by flow cytometry with a Beckman Coulter FC 5000[3] using a forward log (FL2) scale at 639 volts. Data analysis was conducted with the Beckman Coulter CXP software package[4] using the parental inbred lines (*B. rapa* or *B. oleracea*) and *B. napus* as relative positive controls in each sample run. *B. napus* is an allotetraploid (*2n=4x=38, AACC*), while both lines of the *B. rapa* (*2n=2x=20, AA*) and *B. oleracea* (*2n=2x=18, CC*) are diploids. Therefore, hybrids of *B. napus* and the receptor species are triploid and readily distinguishable using this technique.

In addition to sampling leaf tissue from each of the seedlings, a morphological assessment was used to determine potential hybrids. Morphological descriptors such as color, shape and size of vegetative and reproductive structures have been widely used in taxonomic studies of the *Brassica* species (Gomez-Campo 1980). Seedlings were visually evaluated and rated as either the result of a self fertilization, or hybridization of the respective receptor species and *B. napus* based upon morphological characteristics defined by Musil (1950). For the progeny of each cross,

leaf shape, color, presence/absence of hair on leaves and stems, and stem shape was noted. These characteristics were then compared to those of the maternal inbred and those of *B. napus*. In the case of the *B. rapa* inbred maternal plants, self fertilizations produced progeny displaying a light green leaf color, an obelliptic shaped leaf with a prominent midrib, and no hair on either stems or leaves. These offspring appeared identical to the maternal parent. However, putative hybrid individuals produced on the *B. rapa* inbred maternal plants had a darker blue green leaf color, incised leaf with reduced midrib, and pubescence on both leaves and stems. These individuals exhibited morphological characteristics of *B. napus*. In the case of the *B. oleracea* inbred maternal plants, self fertilizations produced progeny with a dark green leaf color, a thick ovate shaped leaf, and glabrous stems and leaves. These offspring appeared identical to the maternal parent. This evaluation was conducted before cytological analysis to avoid introducing bias into the results. The results of both the flow cytometry and molecular analyses were then compared to examine the accuracy of the morphological assessment.

Hybrids between *B. napus* and either of the receptor species can be detected using molecular marker analysis. Primer pairs corresponding to the A genome of *B. napus* (Iniguez-Luy et al. 2006) were used to identify hybrid individuals in the progeny of the field crosses. We selected Primer pair 7, which amplifies the A genome from *B. napus* but not from *B. rapa* var. *chinensis* (BRCF) or *B. rapa* var. *pekinensis* (BRPF) receptor species. This marker was effective in screening for hybrids between the *B. oleracea* varieties and *B. napus* because *B. oleracea* does not have an A genome. Therefore, any progeny in which Primer 7 amplified, would be a hybrid. For

this analysis total genomic DNA was extracted from young leaves using the DNeasy 96 Plant kit[5] (Qiagen). The PCR reaction mixture (10 μL) contained 2-5 ng of genomic DNA, 0.2 μL each DNTP, 0.2 μL each primer, 1 μL 10X buffer, and 0.06 μL Taq DNA Polymerase[6] (Qiagen). The PCR program consisted of: 1 min at 95 C, followed by 35 cycles of 30 s at 95 C, 30 s at 60 C (primer specific), and 45 s at 72 C, with a final extension of 10 min at 72 C, using a C1000™ Thermal Cycler[7] (Bio-Rad). Uniformity of PCR amplification was resolved by UV fluorescence after electrophoresis on 2% agarose gel with ethidium bromide. The results of the marker screening were compared to both the cytological and morphological assays to check for discrepancies among the screening methods.

Greenhouse Experiments. *Brassica* vegetable seed inbred lines were obtained from local sources. Accession numbers and inbred parental information are propriety information for these lines, therefore codes were used identify the inbreds used in each cross. Isolated greenhouse crossing experiments were conducted using either Clearwater®, an imazamox resistant (IMI) or DKL38-25 a glyphosate resistant (RR) *B. napus* (canola) cultivar as the pollen parents, and two self-incompatible *B. rapa* var. *chinensis* (Pak choi) vegetable seed inbred lines (BRCM and BRCF) as receptor plants. Crosses were conducted with an inbred line (BOI) of cytoplasmic male sterile (CMS) *B. oleracea* var. *italica* (broccoli) as pollen receptor plants. The source of the CMS in the *B. oleracea* inbred lines was the 'Anand' cytoplasm (Cardi and Earle 1997). These species were selected because they were among the highest value *Brassica* vegetable seed produced. The imazamox resistant *B. napus* was not a

genetically modified organism (GMO) but provided a selectable marker that could be used to positively identify putative crosses. Seed from the glyphosate resistant canola, both *B. rapa* inbred lines, and leaf tissue from the *B. oleracea* inbreds was tested for the presence of the glyphosate-resistant trait with the Trait√® RUR test strip[8]. This kit detects the presence of the CP4 EPSPS protein produced by the *CP4 EPSPS* transgene which confers glyphosate-resistance. The testing was conducted both to ensure that no contamination of crossing stock existed prior to the experiment, and that the test was able to detect the protein in the transgenic canola.

Blue bottle flies (Diptera: Calliphoridae)[9] contained within 18,757 cm^3 mesh (18 x 16 mesh) cages were used to ensure pollen transfer (Currah and Ockendon 1984) and to exclude potential pollen transfer from other *Brassica* species. The *B. napus* x *B. rapa* crosses consisted of seven *B. rapa* receptor plants and three *B. napus* pollen donor plants. The *B. napus* x *B. oleracea* crosses consisted of three *B. oleracea* receptor plants and four *B. napus* pollen donor plants. The *B. oleracea* receptor plants were considerably larger than the *B. rapa* receptor plants, and thus there was only enough room to accommodate three in each mesh cage. Greenhouse conditions were set to provide a 20/20 C day night temperature with supplemental lighting to maintain a 14 h photoperiod. Both receptor plants and pollinator plants were fertilized once at the beginning of the experiment with Osmocote® 19-6-12 Smart Release® fertilizer[10], and were watered as needed.

Seed were harvested from individual receptor plants. Number of racemes, siliques, and seed per receptor plant was recorded for each cross. One hundred seed from each receptor plant, from each cross were placed in plastic germination boxes

containing moistened blotter paper[1] and placed into a germination chamber set to a 24/17 C day/night temperature regime with a 13 h photoperiod (Warman 1999). The number of germinated seed was recorded for each receptor plant, and germination percentages calculated for each cross. Non-germinating seed from the *B. napus* x *B. oleracea* crosses were examined and tested for viability with a tetrazolium assay according to methods described by the Association of Official Seed Analysts (AOSA 2000). Non-germinating seed from the glyphosate resistant *B. napus* crosses were removed from the germination boxes and evaluated for the presence of the glyphosate-resistant trait with the Trait√® RUR test strip.

Seedlings were removed from the germination boxes and transplanted into 28 x 53 cm flats filled with commercial potting soil[2] and grown in the greenhouse. At the two leaf stage seedlings from each respective cross were treated with either 440 g ai ha^{-1} glyphosate, or 183 g ai ha^{-1} imazamox plus a 90% non-ionic surfactant at 0.25% v/v using a track sprayer calibrated to deliver 216 L ha^{-1} of spray solution (Warwick et al. 2003). Plants were visually evaluated for necrosis 14 d after each herbicide application, respectively. Plants surviving the herbicide application were scored as hybrid individuals. For the glyphosate resistant crosses, surviving plants also were tested with the Trait√® RUR strip to confirm resistance.

RESULTS AND CONCLUSIONS

Field Experiments. The number of seed produced on individual receptor plants varied both by species and by year (Table 2-1). Germination also was variable among *B. rapa* var. *chinensis* (BRCF) inbreds and averaged 29 and 58% in 2007 and 2008, respectively. Viable seed were produced on the *B. rapa* var. *pekinensis* (BRPF) inbreds in both 2008 and 2009, and averaged 62 and 64% germination, respectively. Seed were only produced on the *B. oleracea* var. *italica* (BOI) plants in 2008; however, they were shrunken and failed to germinate. Seed from the 2008 *B. oleracea* var. *italica* x *B. napus* cross were tested for viability with a tetrazolium assay (AOSA 2002). Results of that assay determined that none of the seed contained viable embryos. However, self fertilized seed produced on both of the cultivars of *B. oleracea* var. *capitata* x *B. napus* were viable with an average germination of 81% for inbred BOCF and 80% for inbred BOCM (Table 2-1).

Hybrids between *B. napus* (allotetraploid) and *B. rapa* or *B. oleracea* (both diploid) were detected using flow cytometry analysis (Figure 2-1). Flow cytometry analysis on the progeny produced from the *B. rapa* var. *chinensis* (BRCF) x *B. napus* cross revealed that 74 and 89% of the offspring produced in 2007 and 2008, respectively, were hybrids. Flow cytometry analysis on the progeny produced from the *B. rapa* var. *pekinensis* (BRPF) x *B. napus* cross revealed that 17% and 15% of the offspring produced were hybrids, in 2008 and 2009 respectively (Table 2-1). Although hybridization rates varied between receptor plants (22% to 100%), there was a relatively high outcrossing potential between these species in the field. None of the

progeny produced from the *B. oleracea* var. *capitata* (BOCF,BOCM) x *B. napus* were hybrids.

Tetraploid individuals were identified by flow cytometry in *B. rapa* x *B. napus* field crosses with the exception of those in 2008. Individuals deemed tetraploid were re-sampled and a second flow analysis conducted to confirm the results. While this analysis alone does not provide paternal identity, it does allow for some insights. Several other *Brassica* species including *B. nigra* and *Sinapsis alba* were present in small numbers at the field locations. However, all of these species are diploid. Therefore, a cross of one of the *B. rapa* receptor plants and one of these individuals would produce a diploid offspring, not a tetraploid. Since these individuals were confirmed as tetraploid in a second flow cytometry analysis, and they cannot be the product of outcrossing with any of the other compatible species, another mechanism, such as self fertilization of unreduced gametes (Heyn 1977) or somatic cell doubling, could likely be a responsible for this result. These mechanisms have been previously documented in *Brassicaceae*.

Identification of hybrid individuals using morphological characteristics was in agreement with the results of the flow cytometry analysis (data not shown). Diploid progeny of both *B. rapa* and *B. oleracea* displayed leaf, stem and floral characteristics that were identical to the maternal parent. Triploid progeny displayed a mixture of characteristics of the *B. rapa* and *B. napus* parents. While their acropetal leaves were similar in shape to the diploid parent, these individuals were easily identified by the presence hairs, blue green leaf color, and the corrugated stem characteristics of *B. napus*. Tetraploid progeny displayed a unique morphology closely resembling the