Herbicide Resistance Enriched Hybrid and Synthetic Seed Production and Performance in *Brassica rapa*

By
Todd Andrew Cutts

A Thesis
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Abstract

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Herbicide resistance enriched hybrid and synthetic seed production and performance in *Brassica rapa*

Major Professor: Dr. P.B.E. McVetty, Department of Plant Science

Transgenic, dominant herbicide resistance, such as provided by the BX gene in turnip rape (*Brassica rapa*) provides an opportunity to explore new methods of hybrid and synthetic seed production in this self-incompatible, obligate out-crossing crop. Utilization of the herbicide resistance in one parent used in the crosses to make hybrid or synthetic seed lots permits the hybrid enrichment of the seed lots at the one leaf stage via an application of the herbicide. This approach was used to produce hybrid and synthetic seed lots in *B. rapa*.

Two *B. rapa* lines, SW03375, susceptible to the herbicide and MBRR195, resistant to the herbicide, were used as parents in this study. SW03375 was used as the female parent while MBRR195 was used as the male parent. Row ratios of 1:1, 2:2 and 3:3 female : male rows were used in hybrid seed production trials grown in 6 environments over 2 years. Averaged over the six environments, the 1:1 row ratio produced the highest yield of hybrid seed (334 kg/ha) at the highest hybridity level (24%). The yields of hybrid seed and

hybridity levels were progressively lower for the 2:2 and 3:3 row ratio treatments. Averaged over the six environments, the yield of synthetic seed was 418 kg/ha with 30% resistant material.

The low proportion of resistant material was found to be due to a significant and steady decline in the proportion of MBRR195 plants during the growing season. Only 16% MBRR195 plants remained in the synthetic seed production plots by the end of flowering, down from an initial 46.3%.

In a preliminary yield trial, the enriched hybrid treatments displayed 25% high parent heterosis and 21% commercial heterosis, while the enriched synthetic treatments displayed 21% high parent heterosis and 17% commercial heterosis. The enriched hybrids and synthetics performed much better than their non-enriched counterparts.

Positive high parent heterosis for oil content was displayed for both enriched hybrid treatments and enriched synthetic treatments, averaging 2.0% and 1.7% respectively. Negative high parent heterosis for protein content was displayed for both enriched hybrid treatments and enriched synthetic treatments, averaging –7.5% and –7.4%, respectively.

No detectable levels of erucic acid were detected in the seed lots and the levels of glucosinolates fell within acceptable levels in order to attain acceptance as a canola quality product. Pure hybrid glucosinolate levels reached an average of 17.6 µmol/g of seed at 8.5% moisture while the pure synthetic seed lots reached levels of 17.7 µmol/g of seed at 8.5% moisture.

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1.0 Introduction

For some crops, domestication and selective breeding began some 10,000 years ago when ancestral man became sedentary agriculturists. Selective breeding in primitive societies for some crops usually began as retaining the seed from the plants that retained seed on the stalk to plant the next season (Knowles 1989, Poehlman et al. 1996). Today, technology has greatly advanced selective plant breeding and has increased yields and quality dramatically.

Brassica rapa, more commonly known as turnip rape or Polish oilseed rape, is an annual oilseed crop adapted to the temperate conditions of western Canada. The area sown in western Canada to canola quality oilseed rape has steadily increased since canola was first developed at the University of Manitoba in 1974 (Eskin et al. 1996). In 1986, approximately 2,592,000 hectares were sown to canola (Anonymous, 1996) while approximately 5,420,000 hectares were sown to canola quality oilseed rape in 1998 (DeClercg 1998).

In the past, the area sown with *B. rapa* and *B. napus* were approximately equal. But due to the superior yields of *B. napus* and the first availability of new herbicide tolerant cultivars in *B. napus*, this species has become the predominant one produced in western Canada in recent years. The proportion of the two species of canola sown in western Canada has shifted from 49% *B. rapa* and 51% *B. napus* in 1992 to 18% *B. rapa* and 82 % *B. napus* in 1998 (DeClercq 1998). Significant increases in yield and the availability of the new herbicide would allow new *B. rapa* cultivars to compete successfully with the new *B. napus* cultivars.

The choice of canola species to be grown is based on the relative agronomic characteristics of the two species. Traditionally, *B. napus* yields approximately 20% more than *B. rapa*, but *B. rapa* matures 10 to 15 days earlier than *B. napus* and it is more resistant to shattering and less susceptible to lodging, allowing for direct combining. In order for *B. rapa* varieties to be competitive with *B. napus* cultivars, *B. rapa* yields must increase by at least 20%.

Two techniques that could be used to rapidly improve the yield performance of *B. rapa* are the production of hybrid or synthetic populations. Hybrids exhibit a phenomenon known as hybrid vigor or heterosis, where the resulting F1 produce better yields than the average of the parents used in the cross, or better than the better parent yield used to produce the F1. Higher yielding (than conventional open pollinated populations) hybrid and synthetic populations have been developed in *B. rapa* to make this species more appealing for Canadian producers. Yield increases of as much as a 52.9 % have been reported in *B. rapa* (syn. *B. campestris*) var. Sarson (Swamy Rao 1970). Other studies have reported an increase of 18% in yield as well as an increase in relative oil content of 17%, compared to the mid parent value (Schuler et al. 1991). Synthetic *B. rapa* populations have also been reported to have an increase in yield of 23% when compared to the parents (Falk et al. 1998).

Currently, there are five methods used to produce hybrid populations. Cytoplasmic male sterility, genetic male sterility, gametocides, self-incompatibility, and manual emasculation are all methods employed by plant breeders. All of these methods have strengths and weaknesses.

Another technique to improve yield is the production of synthetic populations. A synthetic population differs from a hybrid population in that the synthetic cultivar is

a blend of male material, female material and hybrid material (Buzza 1995). The entire synthetic population comprises the new cultivar, and its agronomic performance is dependent upon the performance of the components. Hybrid material as well as male and female genotypes comprise the seed lot sold to producers.

Another factor that can improve the yield of any crop type is the elimination of competitive weeds during the vegetative and reproductive growth phases. Modern technology has facilitated the incorporation of herbicide resistance into many modern crop varieties and improved the overall yield and quality of the seed produced. Novel herbicide resistance can be used in canola to selectively eliminate unwanted weeds and improve the performance of the herbicide resistant cultivar.

Bromoxynil resistance is novel herbicide resistance currently being incorporated into canola cultivars at the University of Manitoba. Researchers at Calgene isolated the nitrilase gene from a soil borne bacterium, *Klebsiella pneumoniae* subsp. *ozanae*, capable of metabolizing the phytotoxic bromoxynil into a harmless naturally occurring benzoic acid (McBride et al. 1986). The nitrilase gene was transferred via *Agrobacterium tumefaciens* mediated transformation into *B. napus* (Stalker et al. 1988, McBride et al. 1987). Interspecific hybridization between *B. napus* and *B. rapa* and subsequent back-crossing to the *B. rapa* parent at the University of Manitoba reconstructed the *B. rapa* genome. The nitrilase gene is a dominant nuclear encoded gene (Stalker et al. 1988) that is expressed at all stages of plant development. High rates of bromoxynil (up to 17 times normal field rate) can be applied to resistant plants without apparent effects (Freyssinet et al. 1996).

B. rapa is unable to self-pollinate due the presence sporophytic self-incompatibility alleles. This condition enforces out-crossing with plants carrying

different self-incompatibility alleles within the population and promotes heterozygosity in the population.

The objectives of this research were:

1) to develop bromoxynil resistant *B. rapa* hybrid seed production protocols using two different self-incompatible *B. rapa* populations, one with bromoxynil resistance and one without.
2) to develop bromoxynil resistant *B. rapa* synthetic seed production protocols using two different self-incompatible *B. rapa* populations, one with bromoxynil resistance and one without.
3) to evaluate the performance of bromoxynil resistant *B. rapa* hybrids and synthetics with and without a bromoxynil herbicide spray in the seedling stage (i.e. to compare "enriched" hybrids and synthetics to their "non-enriched" counterparts) and compare the performance of the enriched and non-enriched materials to the performance of the better parent used in the cross.

2.0 Literature Review

2.1 Background

2.1.1 Origin and Cultivation

The Brassicaceae are thought to have originated in the Himalayan region some 60,000 years ago during the Neolithic Period (Applequivist 1974, Downey 1983, Downey et al. 1989) where early man foraged on the vegetative plants (Hyams 1971). Since then, ancestors of modern canola were grown through out the world for thousands of years and have been a recurrent food in many cultures. The oldest recorded use of a Brassica species is in India around 2000 BC (Applequivist 1974; Downey 1983) with the Japanese records indicating the introduction of a Brassica relative at about 35 BC (Applequivist 1974).

It was not until the 16th century that an undetermined Brassica species under went commercial planting in the Netherlands (Downey et al. 1989). Oil extracted from the milled seed was preferred as a fuel source for lamps due to the colorless and odorless flame emitted during combustion (Applequivist 1974; Eskin et al. 1996). The meal from crushed seeds could be fed to ruminant animals as a cheap feed supplement or it could be burned as a source of heat.

2.1.2 Evolution of Rapeseed in Canada

Prior to World War II Canada had no domestic vegetable oil supply and relied on imports from European and Asiatic countries. Blockades during this era decreased exports from these eastern countries and Canada found itself without a vegetable oil supply (White 1974, Boulter 1983).

In 1936 Fred Solovonik, a Polish producer outside of Shellbrook Saskatchewan, was the first to sow *B. rapa* in Canada (Bell 1981, White 1974, Boulter 1983). With thorough agronomic and quality testing, *B. rapa* quickly gained recognition as a valuable vegetable oil source and proved to be invaluable during the second World War (White 1974, Eskin et al. 1996).

The chemical nature of rapeseed oil allows it to adhere very well to water and steam washed surfaces and was invaluable as a lubricant in maritime vessels during the war era (Eskin et al 1996, Weis 1983, Downey 1983 b, Boulter 1983).

During the late 1950's, researchers began questioning nutritional aspects of rapeseed oil. Research found that erucic acid (C22:1) was responsible for fat deposits around the heart, adrenals and skeletal muscles of lab tested rodents (Sauer et al. 1983). It was deemed unfit for human consumption and approval for the sale of edible rapeseed oil withdrawn by the Food and Drug Directorate of the Department of National Health (Sauer et al. 1983, Eskin et al 1996).

Approximately three years later in 1959, the first *Brassica napus* line, "Liho", that was low in erucic acid was isolated. Through conventional breeding methods, Liho was crossed with agronomically adapted Canadian rapeseed varieties. In 1968, the first low erucic acid rapeseed "LEAR" *B. napus* commercial cultivar "Oro" was released. "Span", the first low erucic acid rapeseed "LEAR" *B. rapa* variety was released in 1971 (Eskin et al. 1996).

In 1970, nutritional aspects of rapeseed meal were questioned. Research had indicated that glucosinolates were responsible with the interference of iodine uptake in poultry and non-ruminant animals (Eskin et al. 1996, Sauer et al. 1983). High concentrations of glucosinolates resided in the seed meal fed to domestic animals

causing the interference upon digestion. Glucosinolates are hydrolyzed to isothiocynates (Figure 1) which interfere with the uptake of iodine by the thyroid gland. This contributes to a decrease in the size and weight gain of an animal and the development of liver disease in poultry.

In 1974, Dr. Baldur Stefansson a professor at the University of Manitoba released "Tower" the world's first *B. napus* cultivar that was low in both erucic acid in the oil and glucosinolates in the meal. In 1977, "Candle" the world's first *B. rapa* cultivar low in both erucic acid in the oil and glucosinolates in the meal was released by Dr. Keith Downey a research scientist at Agriculture Canada in Saskatoon (Eskin et al. 1996).

By 1978, there were two distinct rapeseed quality classes created within Canada. One class was suited for human consumption and/or animal feed due to the low erucic acid in the oil and low glucosinolate levels in the meal. The other class was high in erucic acid and high in glucosinolates and was suited for industrial oil uses. Clarification and differentiation between these two classes of rapeseed was necessary for orderly production, crushing and marketing of the two distinct classes. The Canadian Oilseed Crushers Association agreed on the acronym "Canola" (a variant of Canada + oil) to designate double low quality rapeseed.

Canola quality in 1978 was defined as rapeseed with less than 5% erucic acid in the oil and less than 3 mg/g of glucosinolates in the meal. By 1996, the canola definition was changed to include only *B. napus and B. rapa* cultivars with less than 1% erucic acid in the oil and less than 20 μmol/g of glucosinolates in the meal.

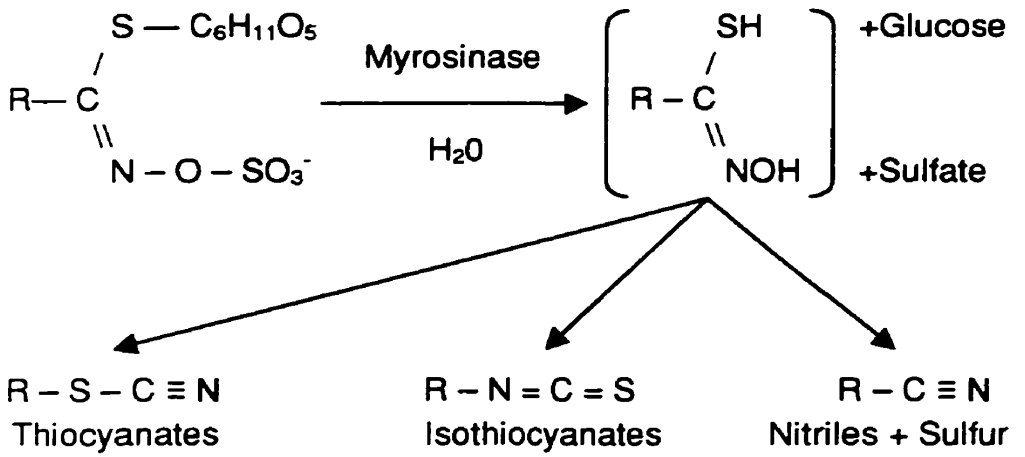


Figure 1. General Structure of glucosinolates and the enzymatic byproducts during hydrolysis (Downey 1983)

Canola has grown from humble beginnings over 50 years ago, to become Canada's most valuable crop produced in 1998. Steadily increasing world demand for vegetable oils suggests that canola production and volume could continue to increase in Canada for the foreseeable future (Statistics Canada 1999).

2.2 Brassica Taxonomy

2.2.1 Genome Composition

B.rapa (AA), *B. nigra* (BB), and *B. oleracea* (CC) are all fertile diploids (Figure 2). While. *B. napus* (AACC), *B. carinata* (BBCC), and *B. juncea* (AABB) are considered allopolyploids within the Brassica crops (U 1935, Downey et al. 1989).

Spontaneous hybridization and chromosome doubling between certain diploid species resulted in the creation of fertile alloploypoids that are critical components in modern agriculture. It has been shown through hybridization tests (Heyn 1974) that *B. napus* (AACC) originated from the union and chromosome doubling of *B. rapa* (BB) and *B. oleracea* (CC). Furthermore, *B. juncea* (AABB) originated from the hybridization and spontaneous chromosome doubling from *B. rapa* (AA) and *B. nigra* (BB) and *B. carinata* (BBCC) originated form a hybridization and chromosome doubling from the union of *B. nigra* (BB) *and B. oleracea* (CC) (U 1938, Downey et al. 1989).

Florescence *in situ* hybridization methods and computer generated condensation patterns were able to isolate the different genomes comprising the amphdiploid *B. napus* species. These particular genomes were identified and belonged to the diploid species *B. rapa* (AA) and the diploid species *B. oleracea* (CC) (Kamisugi et al., 1998).

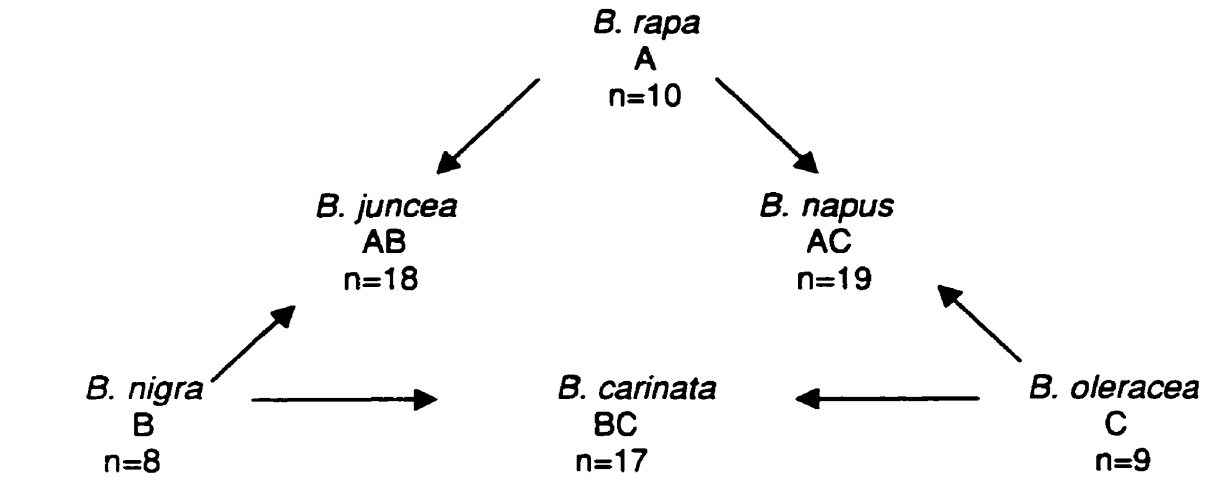


Figure 2. Triangle of U depicting the diploid and allopolyploid relationship amongst the Brassica species (Poehlman et al. 1995)

2.2 Morphology

Brassica oilseed crops possess the ability to grow at relatively low temperatures, which makes them ideal vegetable oil sources for the Canadian Prairies. The word "rape" from the word "rapeseed", as it applies to oil seed forms of both *B. rapa* and *B. napus*, arose from the Latin word "rapum" meaning root (Applequivist 1972).

Brassica rapa, more commonly known as turnip rape seems to be the most variable in morphology due to its naturally heterozygous nature (Bengston et al. 1972, Applequivist 1972). Flowering on the main raceme and subsequent side branches is indeterminate and begins on the lowest bud of the raceme (Downey et al. 1980). Flowers begin to open at approximately 8 to 9 a.m. at a rate of 5 to 8 per day for 2 to 3 weeks depending on the species and environmental conditions (Downey et al. 1983).

The floral arrangement of the Brassicacae is typically a raceme containing complete perfect flowers (Bengston et al. 1972, Downey et al. 1980). Each flower contains four petals, four sepals, six anthers, one pistil with two functional nectaries at the base of the short stamens, and two nonfunctional nectaries at the base of the long stamens (Downey et al. 1980). As a rule, *B. rapa* varieties have their buds lower on the raceme and are located below the opened flowers (Bengston et al. 1972, Downey et al. 1980). Vegetative or floral characteristic coloring is not an accurate diagnosis between species as more than 15 hues of green and 10 different hues of yellow are found in the vegetative and floral characteristics respectively (Downey et al. 1983).

The stigmatal surface is receptive to pollen three days prior to the opening of the flowers up to 3 days after opening (Downey and Robbelen 1989, Downey et al 1980). Pollen dehiscence is dependent on environmental conditions but in ideal conditions it is shed on the day the flower is opened. The pollen is approximately 15 x 30 μ in size (Downey et al. 1980). The small size of the pollen makes it ideal to be carried long distances due to eddies and wind gusts. Even at 366 m isolation distance, a 1 to 2 % contamination/out-crossing is evident between *B. rapa* plots (Stringham and Downey 1978).

Upon successful fertilization of compatible Brassica species, a silique is formed. The silique is composed of 2 carpels, which are further divided by a false septum (Bengston et al. 1972, Applequivist 1972). On either side of the septum lies the maturing seed, the actual number of seeds per pod is variable between species but modern varieties consist of 15 to 40 seeds per silique. (Bengston et al. 1972, Downey 1980).

2.3 Self-Incompatibility

Incompatibility is essentially infertility resulting from the failure of otherwise normal pollen or ovules to set seed caused by a physiological hindrance (Poehlman et al. 1995). Self-incompatibility (SI), as the name implies, is the result of the pollens' inability to penetrate the upper papillar cells on the stigma and fertilize ovules upon the same plant upon which it was borne. Sporophytic SI is the system in place within *B. rapa* that prevents self-pollination and facilitates obligate out-crossing to compose

a readily heterogeneous population. This property was an important mechanism in the evolution of *B. rapa* in the past (Hinata et al. 1994).

Sporophytic SI, is a one-locus (S-locus) system with a large number of S alleles present within a population. Over 150 different alleles have been estimated to occur in *B. rapa* to date (Nou et al. 1993). Sporophytic SI differs from gametophytic SI by having a dominance relationship of the S alleles in place within the SI mechanism and the site at which the pollen interacts with the stigma. The haploid pollen grains outer exine has an S-factor deposited into it by the parental tissues' anther tapetal layer (Goring and Rothstein 1992). It is this S-factor that exhibits the dominance relationship and how it will interact in the S-factors in the female reproductive tissues. In short, the plant upon which the pollen was borne determines the dominance relationship.

Typically, sporophtyic systems prevent pollen tube penetration into the style by forming a cuticle around the pollen grain (Kanno and Hinata 1968). Non structural polysaccharides form lenticules around the "self-pollen" grain in the papillae cells (O'Neil et al. 1984). Further research suggested that plants that do not exhibit the SI response are able to produce the enzyme cutinase that is able to digest the cuticle barrier (Linskens et al. 1962 as cited in Kanno and Hinata 1968).

Electron microscopy studies in 1968 revealed physical barriers existing between the upper stigmatal surface and the haploid pollen tube preventing self-fertilization (Kanno and Hinata 1968). Some recognition event is present in order for the stigmatal papillae to interact with the exine coat of the pollen grain.

The exact mechanism by which *B. rapa* recognizes self-pollen is still much debated (Nasrallah and Nasrallah 1993). Two closely linked genes, S Locus

Glycoprotein (GLG) and S locus Receptor Kinase (SRK) have been identified and are inseparable from one another. Further analyses have depicted that the two genes are separated by approximately 200 kb of DNA (Boyes et al. 1993) with a great deal of homogeneity between the two amino acid sequences (Nasrallah and Nasrallah 1993). The SLG gene encodes for a 400 amino acid signal protein (Nasrallah and Nasrallah 1993) that is abundantly secreted (Nasrallah et al. 1987)

The SRK gene, is predicted to encode for a membrane associated protein kinase (Stein et al. 1991 as quoted in Nasrallah et al. 1993). The SRK gene encodes for a protein that spans the extracellular domain, a transmembrane region, and the cytoplasmic domain.

There is approximately 98% homogeneity exists between the SLG and SRK domain gene sequence at the nucleotide level (Watanabe et al. 1994).

Later, Goring and Rothstein (1992) isolated a SRK gene cDNA fragment from a self-incompatible *B. napus* variety and found that the SRK gene encodes for a functional serine/threonine receptor kinase within the cytopalsmic domain.

SLG accumulates mainly in the upper pappillar of the stigmatal surface. There is an interaction between self-pollen and the stigmatal surface, the SLG then becomes modified and is able to bind with the SRK. It is the SRK that becomes activated when self-pollen is introduced and the SI reaction is evident. If there is no recognition of self-pollen, then the SLG protein is not modified and the SRK does promote SI (Nasrallah et al.1993)

The SLG and SRK genes share a high degree of homogeneity suggesting that at one point in evolution that there was selection pressure for both genes and

the two evolved into a complicated SI system requiring both factors in place (Nasrallah and Nasrallah 1993, Watanabe et al. 1994).

The act of selfing leads to homozygosity over time. The amount of homozygosity that can be tolerated in a cross pollinated species varies with the species (Forsberg et al. 1980). SI alleles are in a population to facilitate outcrossing between individuals within a population and promote heterozygosity. By self-pollinating cross-pollinated plants, inbreeding depression usually occurs. This is where there is a lack of vigor, low levels of seed set and/or abnormalities (Knowles 1989).

2.3.1 Mechanisms of Overcoming Self-Incompatibility

2.3.1.1 Carbon Dioxide

One common practice to increase the amount of self-pollinated seed in *B. rapa* is to increase the carbon dioxide concentration in the area immediately surrounding the plant. Air containing 3% carbon dioxide was found to promote more pollen tube penetration and an increase in pollen tube length into the upper papillae when compared to ambient air (0.035% carbon dioxide) (O'Neill et al. 1984, Dhaliwal et al. 1980). The best timing of CO_2 treatment is approximately 150 min to 240 min after pollination for a 2 treatment of CO_2 (Nakanishi et al. 1973).

Carbon dioxide has been suggested to promote pollen tube growth by interacting with the respiratory process within the styles (Dhaliwal et al. 1980) thereby promoting an increase in the metabolic activity further promoting an increase in pollen tube growth (Nakanishi and Sawano 1989).

The primary action of carbon dioxide in overcoming SI is to prevent the blockage of the pollen tube by removing the callose rejection response. Another alternative mechanism is the actual disruption of communication between the pollen tube and the stigmatic papillae (O'Neill et al. 1984). By introducing carbon dioxide into a system, certain conformational changes could take place in the glycoproteins responsible for SI that prevents the stigma from recognizing self-pollen pollen (O'Neill et al. 1984).

2.3.1.2 Mechanical Treatment

Mechanical treatment has shown much promise in the way of overcoming self incompatibility although much of the efforts are labor intensive and time consuming. Bud pollination is a relatively effective method of overcoming the SI response. Two to three days prior to anthesis, the SI response is not yet manifest within the stigma of the plant. Self-pollen can then be applied to flowers and viable seed can be obtained.

Other methods of circumventing the SI response are: abrasion of the stigmatal surface with a wire brush (Roggen et al. 1972), the application of heat (Roggen et al. 1976) and the use of salts (Fu et al. 1992).

2.3.1.3 Sodium Chloride

Sodium chloride has been demonstrated to inhibit SI. The optimum concentration of NaCl to overcome SI in *B. rapa* has been demonstrated to be 5% (Fu et al. 1992). In order to overcome SI, spraying both the stamens and the stigmas have been found to have the greatest impact on the compatibility index

(Compatibility index = total number of seeds set / total number of flowers pollinated) (Fu et al. 1992).

Other research has indicated that the time of application of NaCl is very important. If NaCl is applied too long before pollination, then the plant is able to recover and the SI response is not overcome. If NaCl is applied after pollination, the amount of set seed from self-pollination is decreased indicating that the SI response is active and can not be overcome post-pollination (Monteiro et al. 1988).

The mode of action of NaCl spray is still unclear but it is evident that it NaCl does interfere with the pollen stigma recognition process. NaCl treatment has been found to produce low levels of callose on the upper papillae of the stigma after self-pollen was applied to plants (Monteiro et al. 1988). The exact mechanism is still unclear but it has been suggested that either the NaCl spray is able to immediately breakdown the callose barrier (Fu et al. 1992) or the NaCl spray is able to interfere with the actual signaling response between the pollen and stigma (Monteiro et al. 1988).

2.4 Bromoxynil Herbicide

Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) is a selective contact folliar broadleaf herbicide used extensively in monocotyledonous crops in order to control dicotyledonous weeds (Annonymous, WSSA 1994 Herbicide Handbook). Bromoxynil was first synthesized in 1886, but it was not used as a herbicide until 1966 (Arnold 1990).

Bromoxynil is an acidic phenol that is insoluble in water and only moderately soluble in most organic solutes. The physical state is a light creamy crystal that has

a high melting point of 190 °C (Anonymous, WSSA 1994 Herbicide Handbook).

2.4.1 Mode of Action

Bromoxynil is a foliar applied herbicide that translocates poorly within the plant (Arnold 1990). Due to the poorly translocated nature of the herbicide, Stalker (1988) deemed that the crop products are relatively safe due to the reduced risk of herbicide residues and the residues can be metabolized by soil borne pathogens (Comai et al 1984).

The chemical grouping of bromoxynil is a hydroxybenzonitrile, and is most active on dicotyledonous plants when they are young, generally before the plants reach the 3-leaf stage. Exposed areas of the plant receiving adequate active ingredient develop necrotic patches approximately 24 hours after exposure. Monocots typically posses a nitrilase gene responsible of metabolizing the phytotoxic compound into a harmless bi-product conferring a naturally occurring herbicide resistance (Stalker et al. 1996, Buckland et al. 1973).

The herbicide acts both as an electron transport inhibitor and as a de-coupler of oxidative phosphorolation. Bromoxynil destroys the energy producing centers of the plant by blocking electron transport by binding to the quinone-binding protein complex in Photosystem II (Freyssinet et al. 1995, Stalker et al. 1996). This leads to the production of singlet oxygen and non-specific bleaching, lipid peroxidation, and membrane disruption (Arnold 1990). With the energy production centers destroyed, the plant is unable to convert sunlight into carbohydrates and staves to death within three days.

2.4.2 Bromoxynil Resistance

Bromoxynil readily breaks down after exposure to the soil. The short residual nature of bromoxynil can be attributed to the soil matrix absorbing the molecules or the activity of soil microbes (Freyssinet et al. 1995). Nitrilase is the enzyme in plants responsible for metabolism of bromoxynil to a harmless benzoic acid that does not have herbicidal activity (McBride, Kenny, and Stalker 1986).

A bromoxynil resistance gene (BXN) was isolated from the soil borne bacterium; *Klebsiella pneumonia* subsp. *ozanae* (Stalker and McBride 1987) after saturating the soil with bromoxynil and obtaining surviving bacterium. *K. pneumonia* subsp. *ozanae* was found to confer bromoxynil resistance in transformed *E. coli* cells due to the production of a nitrilase enzyme (Stalker and McBride 1987).

A single dominant nuclear encoded gene that behaves in a Mendelian fashion (Stalker et al. 1988) controls bromoxynil resistance. Similar to the microorganisms, plants at any stage of development containing the nitrilase gene are able to convert the phytotoxic compound to a harmless polar molecule. Transformed cotton plants are capable of withstanding 20 times normal field application (11.2 kg active ingredient per hectare) (Freyssinet et al. 1996, Stalker et al. 1996).

A number of economically important crops have been transformed with the BXN gene. Such crops include oil seed rape, potato, cotton, and tobacco (Stalker et al. 1996, Freyssinet et al. 1996).

Experiments have been conducted on yield reduction in both winter and spring varieties of *B. napus* oil seed rape. These studies have shown that there is no yield penalty for either the treatment or the introduction of the BXN gene (Freysinet et al. 1996).

2.5 Hybrid and Synthetic Plant Breeding

The initial step in any hybrid or synthetic breeding program is the assembly of desirable germplasm containing the genetic material fitting the breeding objectives of a particular program. Selection of parental material is critical in both determining short term effects and long term effects and defines much of the boundaries of the breeding program (Forsberg et al. 1980). Traditional breeding procedures in *B. rapa* promote the out-crossing between desirable germplasms. Breeding through mass selection, recurrent selection, synthetic and hybrid breeding is done to improve agronomic and/or overall quality characteristics of crops. Breeding procedures in cross-pollinated crops are based largely on increasing the genetic frequency of desired genes within a population and moving that proportion of genes to homozygosity, thus making them true breeding.

Mass selection as outlined by Poehlman and Slepper (1995), is designed to promote a movement to homozygosity of desirable phenotypes in a cross pollinated population. It involves the visual assessment of superior individuals and advancing those individuals to the next generation (Knowles 1989). Individuals are selected solely on the phenotypic assessment and performance within a field season (Falconer and Mackay 1996). Seed from selected parents is bulked after propagation in order to construct the progeny to be grown in the next generation. Traits with low hertiability are often difficult to advance and the progress of such selection criteria is often slow if not impossible. In addition to the ineffectiveness of selection and the slow progress for improvement of traits with low heritabiltiy, the inability to control the pollen makes advancement slow and at the best difficult to predict the genetic recombination of selected parents (Poehlman et al. 1995).

In contrast to mass selection, recurrent selection involves the selection of superior individuals based on the performance of the progeny through next generation testing. Like mass selection, it is designed to promote homozygosity in a readily crossable population. This allows greater control of the pollen donor and superior advancement through genetic recombination at the consequence of slower advancement due to next generation testing (Poehlman and Slepper 1995). Two years are required in order to evaluate the progeny from a particular parental cross in order to ascertain which parents are ideal for the criteria of a breeding program.

Mass selection and recurrent selection can only advance a particular breeding regime if the parental varieties are not related. *B. rapa* does not tolerate inbreeding as the resulting vigor and yield is greatly reduced (Sun 1937, Downey 1989).

Consequently, *B. rapa* genotypes from genetically diverse geographical regions exhibit a higher degree of heterosis when crossed when compared to crosses of similar geographical regions. Schuler et al. (1991) reported increases in yield and relative oil content of 18% and 17% respectively, in F1's derived from crosses of *B. rapa* cultivars from Germany, Canada, Sweden, and Europe.

2.5.1 Hybrid Seed Production

Commercial production of hybrids and synthetic populations requires a means of controlling pollen movement that limits the degree of self-pollination and promotes out-crossing. *B. rapa* is predominately cross-pollinated and the need to control selfed individuals is not necessary. But with the estimated number of different SI alleles present in the *B. rapa* population exceeding 150 (Nou et al. 1993), there is an inherent concern for siblings of a particular *B. rapa* hybrid line to inter-pollinate one

another within the row. A means of controlling pollen movement and/or production is necessary to ensure purity of hybrid seed and to be economically viable. By promoting out-crossing between unrelated genotypes, a diverse assortment of favorable genes is available and heterosis can be exploited.

Five pollen control mechanisms are available to ensure purity of hybrid populations. Cytoplasmic male sterility, genetic male sterility, self-incompatibility, manual emasculation, and gametocides are mechanisms available to breeders which are able to control the direction of pollen. Each mechanism has benefits associated with it as well as constraints and hindrances.

2.5.1.1 Cytoplasmic Male Sterility

Perhaps one of the most accepted forms of producing hybrid seed is the use of cytoplasmic male sterility (CMS). Sterile plants produce functional flowers but have non-functional anthers or pollen. CMS is defined as having two unique components that interact and promote male sterility. A sterile cytoplasmic component designated as (S) and fertility restorer gene component designated as (R_f) in the nucleus. Due to the fact that the cytoplasmic component is located in the mitochondria of the cytoplasm, CMS can only be inherited through the female reproductive genome (Poehlman et al. 1995, Forsberg et al. 1980, Knowles, 1989).

An interaction between the recessive nuclear maintainer genes (*rf rf*) and the sterile cytoplasm (S) within a plant cell cause cytoplasmic male sterility (CMS). CMS plants can be fertile if the cytoplasm designation is fertile (N) and if the nuclear complementation is *rf rf* or *Rf rf* or *Rf Rf*. Plants can also produce normal pollen if the cytoplasm is sterile (S) but there is homozygous dominant (*Rf Rf*) or

heterozygous (*Rf rf*) nuclear gene complementation (Poehlman et al. 1995, Forsberg et al. 1980, Knowles et al. 1989).

Microsporogenesis is an energy intensive process that demands high levels of energy or plant resources. CMS seems to result from the mistimed or insufficient supply of energy or nutrients that are essential for pollen production (McVetty 1996).

In order to produce and maintain CMS lines, a three stage breeding regime is necessary in order to promote, carry out and maintain the CMS component in a particular variety. A sterile female (S *rf rf*) is crossed with a fertile male (S or N *Rf Rf*). The resulting progeny is fertile (S *Rf rf*) and can be sold to producers as hybrid seed. To maintain the sterile female side, a fertility maintainer line is needed in order to ensure that the sterile cytoplasm and recessive fertility genes are maintained in the population. This particular line has recessive nuclear genes but the cytoplasm is normal, thereby producing functional pollen. The recessive haploid pollen fertilizes the recessive haploid ovules and the sterile cytoplasm is transferred into the next generation (Figure 3).

2.5.1.2 Genetic Male Sterility

Like CMS, genetic male sterility (GMS) is able to produce fertile receptive female plants without the production of functional pollen through genetics. Pollen control is effected through the nuclear genome of the plants and is predominately a single gene (Poehlman et al. 1995, Sawhney 1996). Homozygous recessive individuals (msms) are typically male sterile. The exact method of how a plant is able to prevent pollen synthesis is not clearly defined. Some research suggests that any disruption of critical hormones during pollen generation would be sufficient to prevent

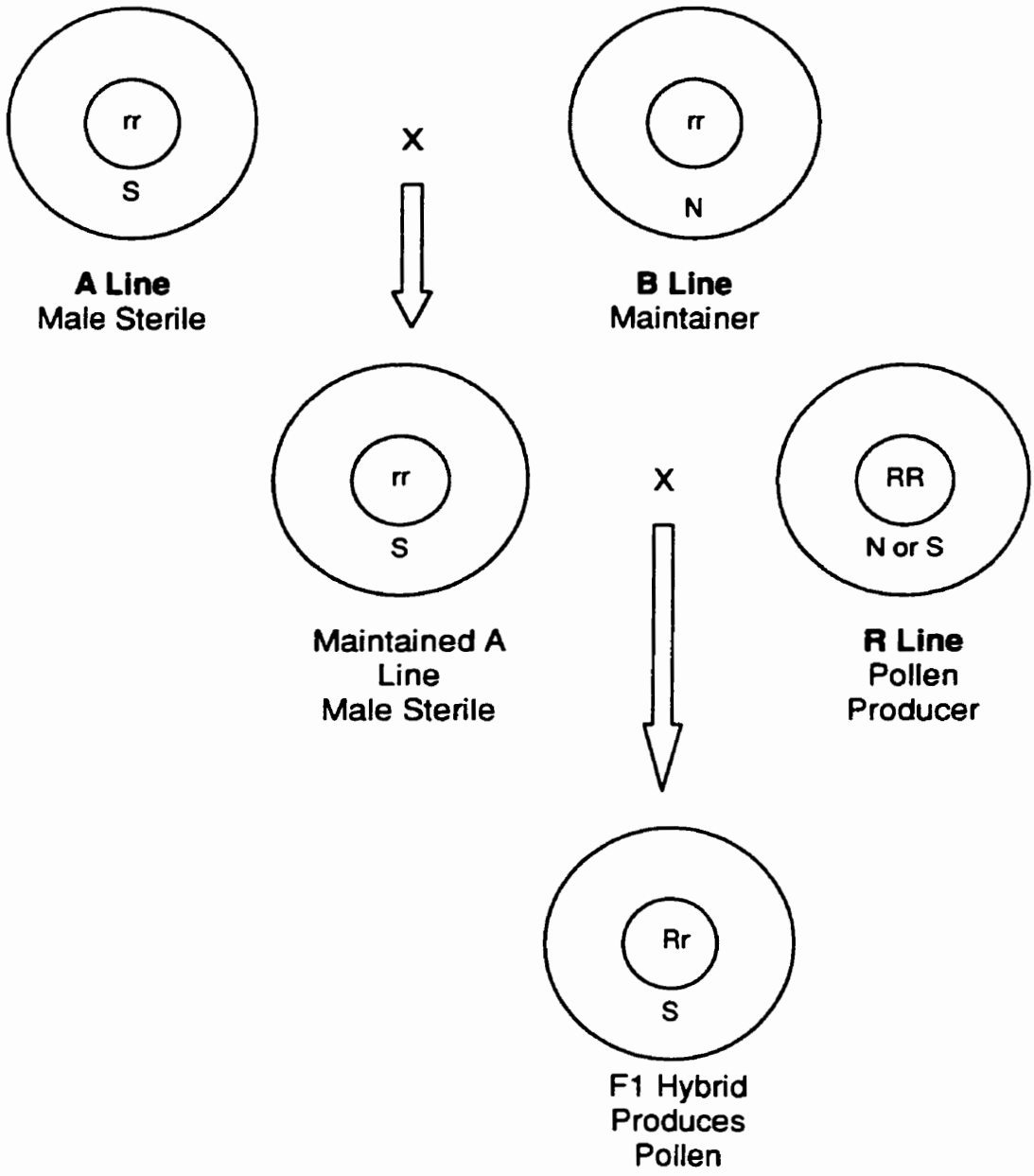


Figure 3. Diagrammatic representation of CMS pollination control strategy and the Production of F1 Hybrid Seed

microsporogenesis.

The advantage GMS has over CMS is the fact that no special care is needed to select for the male lines and no special cytoplasm is transferred to the hybrid (Rao et al. 1990).

The disadvantage GMS has as opposed to CMS is that 100% male sterile females are not obtained. Upon crossing male sterile females (*ms ms*) with heterozygous fertile males (*Ms ms*) the progeny results in 50% fertile (*MS ms*) plants and 50% sterile (ms ms) plants (Poehlman et al. 1995). Intensive rouging efforts are required to eliminate the fertile female plants. Unfortunately, male sterility is only identifiable during flowering and this can result in unwanted pollination within female rows.

Plant breeders can counteract this by linking morphological markers to sterile plants (Galinat 1975 as reported by Rao et al. 1990). White endosperm was linked to the male sterile gene (5% recombination), researchers could selectively plant white endosperm seed and be 95% confident that the seeds would develop into sterile plants.

2.5.1.3 Gametocides

Unlike CMS or GMS that rely on genetics to limit the amount of pollen produced, gametocides are chemicals that limit or terminate pollen production in selected individuals. Compounds have been known to cause feminization of functional male florets, destruction of anther development, interference with tapetal layer, interference with microsporogenesis and interfere with pollen germination (Cross et al. 1997).

Chemical induction of male sterility in some instances is considered desirable to plant breeding programs. Elite stocks of material can be selected and induced to become male sterile thereby eliminating intensive breeding programs to try and incorporate sterile germplasms into those lines.

Gametocides face a number of environmental, biological, and economical challenges. Prolonged flowering after the application of such chemicals can increase the number of male fertile flowers. Environmental conditions (such as rain and heat) can eliminate efforts to try and induce sterility. Economical cost and unreliable efficiency make gametocides an inefficient mechanism for controlling male sterility. (Rao 1990, Cross et al. 1996)

Gametocidic chemical classifications range from natural occurring hormones, to synthetic hormones, to metabolic inhibitors, to phytotoxic compounds. The overall end result is the failure of the plant to produce functional pollen by disrupting the delicate balance of microsporogenesis.

2.5.1.4 Manual Emasculation

Manual emasculation is a time consuming labor intensive process that can be used to produce hybrid seed. Through the physical removal of the anthers in selected individuals, researchers/plant breeders are able to limit pollen production (Fehr 1980). Physical removal of the anthers is labor intensive and highly uneconomical, however (Rao et al. 1990, Wright 1980).

Emasculation procedures involve the removal of the outer sepals, and immature petals with sharp scissors or fine pointed forceps. Once the stamen and pistil are exposed, the anthers can be removed (Downey et al. 1980) in order to

make the florets male sterile.

2.5.1.5 Self-Incompatibility

This method does not eliminate production of male gametes, but rather it is able to control the direction of pollen flow. By having hybrid lines containing identical SI alleles, female lines cannot accept pollen from within a row or from itself and the result is a high level of outcrossing. In a sense, the pollen must come form an alternative row namely the male donor. The result is hybrid production between rows of different self-incompatibility alleles. This method controls the direction of pollen movement and not per say the synthesis or dispersal of male gametes. This application in B. rapa has proven to be impractical (Downey 1989).

2.5.2 Synthetic Seed Production

Unlike hybrid seed production where distinctive male and female rows are defined and individually harvested, synthetic populations are a blend of male and female material. Synthetic populations produce seed from two or more uniform inbred lines mixed in equal proportions (Buzza 1995). Synthetic seed can be described or regarded as the intermediate between conventional cultivars and pure hybrid F_1 material.

Synthetics of *B. rapa* are usually composed of two and at most three parental lines. Due to the self-incompatibility nature of *B. rapa*, no selfed seed is obtained and the overall pollination is more estimateable. For a "Synthetic 1" population composed of equal proportions of male and female material, the expected progeny is predicted to be 25% female material, 50% hybrid material, and 25% male material (Poehlman

et al. 1995, Falk et al. 1998). These ratios are assumed to be true if the pollination was random and there was no directional movement of pollen (Buzza 1995).

Becker (1989, as reported by Falk et al. 1998) distinguishes a synthetic population from an open pollinated population on the basis of three distinct characteristics:

1) Number of parents that construct a synthetic population is small.
2) Parental components are reared in isolation and the synthetics are reconstructed from pure parental seed.
3) The base population is entirely constructed of selected plants.

2.6 Heterosis

By definition, heterosis (or hybrid vigour) is the increase in size, yield, growth, and or vigour of a hybrid resulting from a cross from genetically unlike organisms (Poehlman et al. 1995). Shull (1908) first observed heterosis where the inbreeding of corn resulted in a decreased stature of the inbred lines but upon reconstituting the original population, size and vigor was restored.

Two theories on the explanation of heterosis have been around for quite some time. The dominance theory, states that a large number of deleterious recessive characteristics accumulate within an isolated population (Poehlman et al. 1995). These recessive characteristics are masked by their dominant counterpart and upon inbreeding these recessive characteristics manifest themselves resulting in decreased vigour, size etc. (Poehlman et al. 1995). Hybrid vigor results from the bringing together of favorable dominant genes.

The contrary theory to explain heterosis is the overdominance hypothesis. This hypothesis assumes that contrasting alleles contribute more to productivity than loci that are homozygous. Each pair of contrasting allele produces a favorable product to the plant and the combination of contrasting alleles produces a product that is beneficial to the plant more so than having similar favorable alleles (Poehlman et al. 1995).

Quantitative representation of increased yield or size is usually in comparison to the mid parent value. This is the increase in yield of the F_1 as compared to the average of the two parents and is called "mid-parent heterosis".

In contrast, a quantitative representation of heterosis can also be performed where the hybrid F_1 is compared to the better (high) parent performance. This is entitled "high-parent heterosis".

2.6.1 Heterosis in *B. rapa*

Researchers have observed a number of physiological benefits to agronomically important plants exhibiting hybrid vigour. Days to flower, days to maturity, rosette vigour, plant height, seed size, silique number per plant, silique length, 1000 seed weight, number of primary and secondary branches per plant, and yield per plant can be enhanced through hybrid vigor.

Duhoon and Basu (1981) published a paper on the observed agronomically important field characteristics. Six crosses were made between a wide range of economic characters. All possible combinations were made and resulted in 15 F_1 hybrids. These hybrids were compared to mid parent value and high-parent values of the hybrid crosses. Primary and secondary branches increased in 15 and 12 of

the hybrids respectively when compared to the mid parent value. Number of siliques, and silique length increased in all 15 of the hybrids when compared to the mid parent value. There was an expression of earlier maturity for 10 out of the 15 hybrids when compared to the mid parent value. One thousand seed weight and overall yield per plant increased when expressed to the mid parent value. The number of seeds per pod decreased, there was only an increase in the number of seeds per pod in 4 out of the 15 crosses when compared to the mid parent value.

In other studies, seed number per pod was found to increase in hybrid plants (Labana et al. 1978). But plant height, row yields, pods per plant, and pods on the main shoot were observed to increase in all hybrid crosses when compared to the better parent in *B. rapa* (syn. *B. campestris* L.) var. *Yellow Sarson* (Labana et al. 1978).

Swamy Rao (1970) observed a large variation in heterosis of both the mid parent and the better parent value when examining the degree of oil expression. These heterotic values ranged from –125% to +52.9% for mid parent value *in B. rapa* (syn. *B. campestris* L.) var. *Sarson.*

Schuler et al. (1991) also reported an increase in yield by 18%, an increase in oil content by 17%, fewer days to maturity (-0.7%), and a larger average plant height (+7.0%) in *B. rapa* F1 cultivars derived from crosses of parents of diverse origin including Germany, Canada, Sweden, and Europe.

Hutcheson et al. (1981) observed yield increased as much as 40% over the better parent when Canadian and Indian *B. rapa* cultivars were crossed to generate F1's.

Heterosis for yield not only occurs in pure hybrid material, but also for synthetic populations. For example, *B. rapa* synthetic populations have been reported to yield 23% more than the better parent used to make the synthetic (Falk et al. 1998).

3.0 Methods and Materials

3.1 Seed Populations

3.1.1 Female Population

The *B. rapa* cultivar "Foothills" (SW03375), registered in Canada in 1997, was used as the female parent for the hybrid and synthetic seed production trials. The cultivar Foothills was developed in Sweden in 1989 by crossing the registered variety Indus with an experimental variety SV03616. The resulting seed constituted the F1 and was then multiplied as an open pollinated population to constitute the F2. The F2 generation was screened for *Albugo candidia* race 7a resistance. The surviving plants that set seed then constituted the F3 where tests for glucosinolates, yield, oil, protein, lodging, and maturity were conducted.

3.1.2 Male Population

The male parent for the hybrid and synthetic seed production trials was a *B. rapa* population designated as MBRR195. This population is a fifty BC4F4 family intercross of pure breeding bromoxynil resistant lines derived from Reward. The nitrilase gene responsible for metabolizing bromoxynil was introduced via an interspecific hybridization between an experimental bromoxynil resistant *B. napus* line and bromoxynil sensitive *B. rapa* Reward. Four generations of subsequent backcrossing of the *B. rapa* interspecific hybrid was conducted to the female Reward parent. This was done to re-incorporate the *B. rapa* genome and to select for resistance to bromoxynil. The result was a Reward *B. rapa* population that was capable of resisting high levels of bromoxynil.

Reward had a lower disease incidence to *Albugo candida* Race 7, matured earlier, higher oil content, higher protein content when compared to the check variety Tobin. Glucosinolates, height and lodging were all similar to Tobin (Scarth et al. 1992).

3.2 1997 and 1998 Hybrid Seed Production Trials

Hybrid seed production trials were established in 1997 and 1998 at the University of Manitoba Research Farm at Carman, The University of Manitoba Research Station at Glenlea, and on the campus at the University of Manitoba in Winnipeg, Manitoba.

The hybrid seed production plot sites were seeded with a Hefty-G belt-cone nursery seeder at a rate of 1g of parental material per 5-m row (approximately 5 kg/ha). A granular insecticide, Carbofuran (10% granules), was banded with the seed at an approximate rate of 11 kg a.i./ha to control flee beetles (*Phyllotreta cruciferae* Goeze and *P. striolata F.*).

The Carman hybrid seed production trial was seeded on May 15, 1997 and on May 11, 1998, while the Glenlea hybrid seed production trial was seeded on May 29, 1997 and on May 21, 1998. The Winnipeg hybrid seed production trial was seeded on May 27, 1997 and on June 4, 1998.

The total size of the hybrid seed production trials at each location was 12 m x 17 m. Each hybrid production trial was divided into two replicates. Replicates at each site were 5 m x 17 m separated by a 2m wide pathway. Each replicate containing 39 rows with each row separated by a distance of 40 cm. The individual rows were arranged to produce three male to female row-planting ratios: 1:1, 2:2,

and 3:3 (Figure 4). The 1:1 row ratio treatment consisted of 1 row of susceptible female parental population planted beside 1 row of resistant male parental population repeated 3 times in each replicate for a total of 6 rows. The 2:2 row ratio treatment consisted of 2 rows of susceptible female parental populations planted beside 2 rows of resistant male parental populations repeated 3 times in each replicate for a total of 12 rows. The 3:3 row ratio treatment consisted of 3 rows of susceptible female parental populations planted beside 3 rows of male parental populations repeated 3 times in each replicate for a total of 18 rows.

3.2.1 Crop Measurements

Eight agronomic observations were taken for each row within the hybrid seed production trials including days to emergence, vigor, days to first flower, plant height, lodging, maturity, total seed yield and hybrid seed yield.

The Harper Burkenkamp stage key is an illustrative guide depicting the critical stages of development of *B. napus* and *B. rapa* (Harper Burkenkamp 1975). Numerical classification of the crititical stages helps researchers and producers to accurately depict the growth stages of canola (Figure 5).

Emergence was assessed as the time (days) after sowing to the date when 50% of the plot had emerged at the cotyledon stage. Days to flower were assessed as the days after sowing to the time when 50% of the plants showed at least one open flower (Burkenkamp stage 4.1). Plant height was measured by talking five plants within a row and taking the average height of those plants in centimeters at maturity (Burkenkamp stage 4.4). Lodging was rated on a scale from 1 to 5 where the 1 indicates that the plot is standing completely erect, where 5 indicates that the

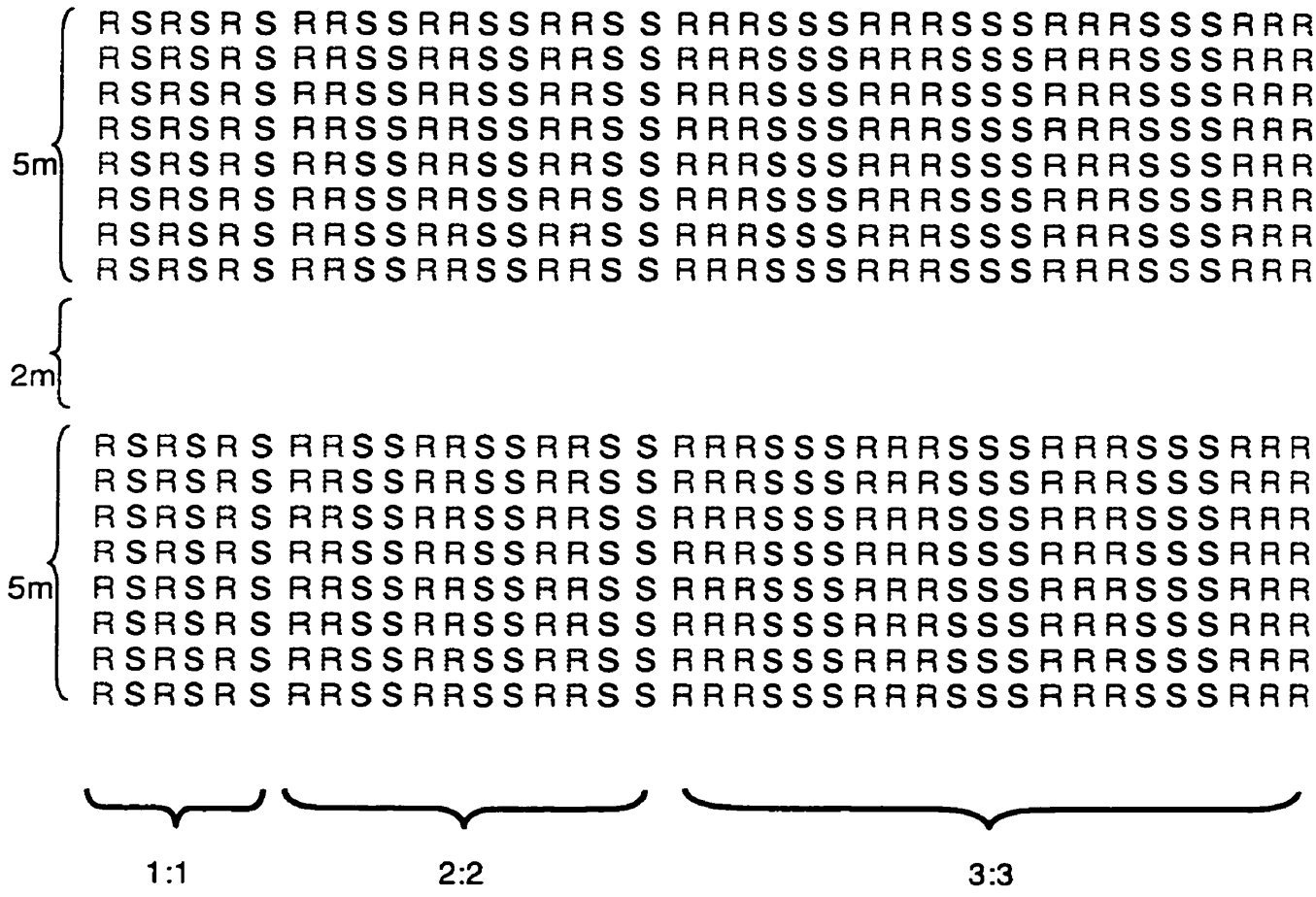
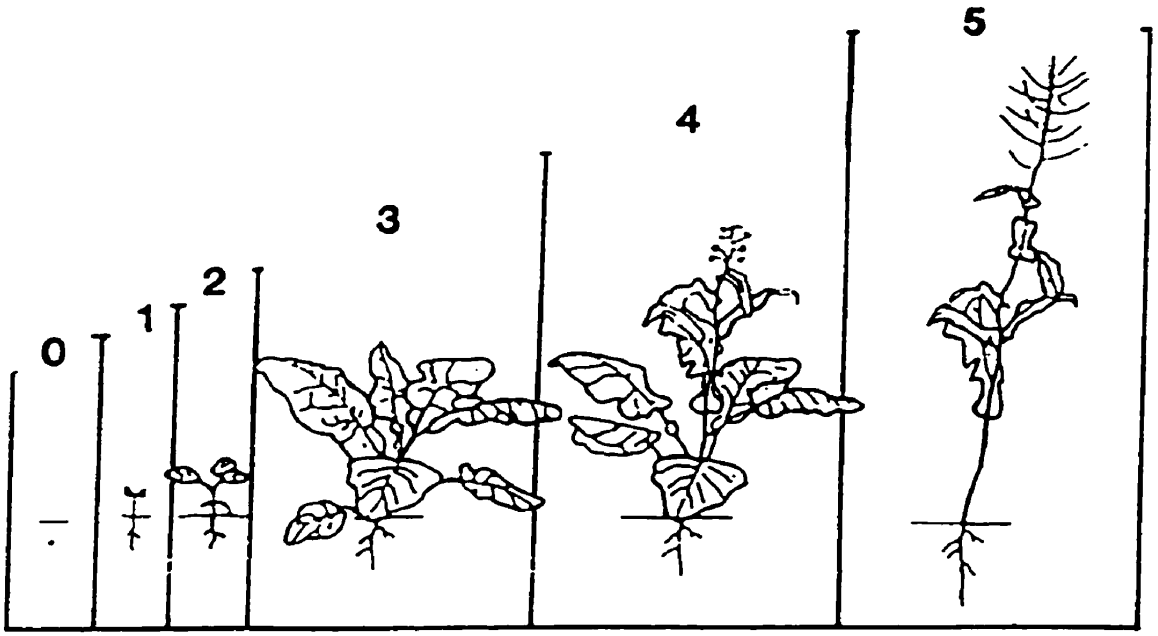


Figure 4. Diagrammatic scheme of hybrid seed production trials in the 1997 and 1998 field seasons representing the different planting ratios in both replicates



Descriptions are based on the main stem

Stage

0 Seed

1 Seedling
- 1.1 First true leaf (senesces).
- 1.2 Second true leaf (senesces).

2 Rosette
- 2.1 Third true leaf expanded
- 2.2 Fourth true leaf expanded
- (add 0.1 for each additional leaf)

3 Bud
- 3.1 Inflorescence visible at center of rosette
- 3.2 Inflorescence raised above level of inflorescence
- 3.3 Lower buds yellowing

4 Flower
- 4.1 First flower opens
- 4.2 Many flowers opened, lower pods elongating
- 4.3 Raceme still flowering, lower pods starting to fill
- 4.4 Flowering complete, seeds enlarging in lower pod

5 Ripening
- 5.1 Seeds in lower pods full size, translucent
- 5.2 Seeds in lower pods deep green
- 5.3 Seeds in lower pods green-brown mottled
- 5.4 Seeds in lower pods brown
- 5.5 Seeds in all pods brown, plant senescent

Figure 5. A growth stage key for rapeseed (*B. campestris* and *B. napus*) (Harper and Burkenkamp 1975)

plot is lying flat on the ground. Maturity was assessed when the lowest most seeds were brown and yellow indicating sufficient maturity (Burkenkamp stage 5.4). Total seed yield was measured in g/plot but was converted to kg/ha. Total Hybrid seed yield was calculated after the hybridity of the seed lot was established during the indoor screening trials.

Total hybrid seed yield is a measurement of the total amount of hybrid seed existing in a row at the time of harvest and is also expressed in kg/ha.

Weed control at all locations in all years was done by hand weeding. Grasshopper outbreaks in Glenlea in 1997 and 1998 and Winnipeg in 1997 required control with the insecticide, Decis, applied using a backpack sprayer equipped with a standard flat fan nozzle at a rate of 7.41 g a.i./ha approximately once every 2 weeks throughout the growing season.

Fertilizer (20-0-0-24) was applied to all hybrid seed production trials at an approximate rate of 112 kg of actual N per hectare.

The hybrid seed production plots were harvested at physiological maturity (Berkenkamp scale stage 5.4) (Figure 5). Male and female rows were cut individually using a serrated hand sickle. Individual rows were placed on a flexible plastic sheet where the row could be bundled up and tightly squeezed together. Rolled packages where then placed in a burlap bag and individually labeled. Burlap bags were transported back to Winnipeg, where they were placed on an outdoor rack to dry.

After adequate dryness, a Kinkaid stationary thresher was used to thresh individual bags. Seed was collected from the individual rows to determine total seed yield and provide seed samples in order to assess seed quality and seed lot hybridity.

Approximately 27g of seed from each row at each location from each year was submitted to the canola quality laboratory for oil content, protein content, erucic acid content and glucosinolate content was analyzed. Oil content was expressed as a percent of the total dry weight of the seed at 0% moisture and was quantified by using an Oxford 4000 Nuclear Magnetic Resonance Analyzer. Protein content was determined using a LEKO FP 428 protein analyzer and was expressed as a percentage of the seed total dry weight at 0% moisture.

Fatty acids and glucosinolates were analyzed by Varian 3350 gas chromatographs. Oil was dissolved in heptane and petroleum ether in order to extract fatty acids. Fatty acids were express as a percentage of the oil. Glucosinolates were analyzed much in the same way as the fatty acids only the meal was subjected to the solvent puradine in order to extract the glucosinolates. Amount of glucosinolates in the meal is expressed as total glucosinolates μmol/g of seed at 8.5% moisture.

3.3 1997 and 1998 Synthetic Seed Production Trials

Synthetic seed production trials were established in 1997 and 1998 at the University of Manitoba Research Farm at Carman, University of Manitoba Research Station at Glenlea, and on campus at the University of Manitoba in Winnipeg Manitoba. The synthetic seed production trials were located at approximately 240 m away from hybrid seed production trials at all locations in all years to minimize pollen transfer between the two types of seed production plots.

The synthetic seed production plots were seeded with a Hefty-G belt-cone nursery seeder at a rate of 1g of parental material per 5 m row (approximate seeding

rate of 5 kg/ha). A granular insecticide, Carbofuran (10% granules), was banded with the seed at an approximate rate of 11 kg a.i./ha to control flea beetles (*Phyllotreta cruciferae* Goeze and *P. striolata* F.).

The Carman synthetic seed production trial was seeded on May 15, 1997 and on May 11, 1998, while the Glenlea synthetic seed production trial was seeded on May 29, 1997 and on May 21, 1998. The Winnipeg synthetic seed production trial was seeded on May 27, 1997 and on June 4, 1998.

The total size of the synthetic seed production plots at each location was 12 m x 17 m. Each synthetic production plot was divided into two replicates. Replicates at each site were 5 m x 17 m separated by a 2m wide pathway. Each replicate containing 39 rows with each row separated by a distance of 40 cm. Each row was planted to 50% MBRR195 seed and 50% Foothills (SW 03375) seed mixture (Figure 6).

3.3.1 Crop Measurements

Eight agronomic observations were taken for each row within the hybrid seed production trials including days to emergence, vigor, days to first flower, plant height, lodging, maturity, total seed yield and hybrid seed yield.

The Harper Burkenkamp stage key (as described in section 3.2.1) is an illustrative guide depicting the critical stages of development of *B. napus* and *B. rapa* (Harper Burkenkamp 1975). Numerical classification of the crititical stages helps researchers and producers to accurately depict the growth stages of canola (Figure 5).

Figure 6. Diagrammatic scheme of synthetic seed production trials in the 1997 and 1998 field seasons

Emergence was assessed as the time (days) after sowing to the date when 50% of the plot had emerged at the cotyledon stage. Days to flower were assessed as the days after sowing to the time when 50% of the plants showed at least one open flower (Burkenkamp stage 4.1). Plant height was measured by taking five plants within a row and taking the average height of those plants in centimeters at maturity (Burkenkamp stage 4.4). Lodging was rated on a scale from 1 to 5 where the 1 indicates that the plot is standing completely erect, where 5 indicates that the plot is lying flat on the ground. Maturity was assessed when the lowest most seeds were brown and yellow indicating sufficient maturity (Burkenkamp stage 5.4). Total seed yield was measured in g/plot but was converted to kg/ha. Total resistant seed yield was calculated after the resistance level of the seed lot was established during the indoor screening trials.

Total resistant seed yield is a measurement of the total amount of resistant seed existing in a row at the time of harvest and is also expressed in kg/ha.

Weed control at all locations in all years was done by hand weeding. Grasshopper outbreaks in Glenlea in 1997 and 1998 and Winnipeg in 1997 required control with an insecticide. Decis, was applied using a backpack sprayer equipped with a standard flat fan nozzle at a rate of 7.41 g a.i./ha approximately once every 2 weeks throughout the growing season.

Fertilizer (20-0-0-24) was applied to all synthetic seed production trials at an approximate rate of 112 kg of actual N per hectare just prior to bolting (Bukenkamp stage 3.1) (Figure 5).

The synthetic seed production plots were harvested at physiological maturity (Berkenkamp scale stage 5.4) (Figure 5). Synthetic rows consisting of 50% female

parental populations and 50% male parental population within a row were cut individually using a serrated hand sickle. Individual rows were placed on a flexible plastic sheet where the row could be bundled up and tightly squeezed together. Rolled packages where then placed in a burlap bag and individually labeled. Burlap bags were transported back to Winnipeg, where they were placed on an outdoor rack to dry.

After adequate dryness, a Kinkaid stationary thresher was used to thresh individual bags. Seed was collected from the individual rows to determine total seed yield and provide seed samples in order to assess seed quality and seed lot hybridity.

Approximately 27g of seed from each row at each location from each year was submitted to the canola quality laboratory for oil content, protein content, erucic acid content and glucosinolate content was analyzed. Oil content was expressed as a percent of the total dry weight of the seed at 0% moisture and was quantified by using an Oxford 4000 Nuclear Magnetic Resonance Analyzer. Protein content was determined using a LEKO FP 428 protein analyzer and was expressed as a percentage of the seed total dry weight at 0% moisture.

Fatty acids and glucosinolates were analyzed by Varian 3350 gas chromatographs. Oil was dissolved in heptane and petroleum ether in order to extract fatty acids. Fatty acids were express as a percentage of the oil. Glucosinolates were analyzed much in the same way as the fatty acids only the meal was subjected to the solvent puradine in order to extract the glucosinolates. Amount of glucosinolates in the meal is expressed as total glucosinolates μmol/g of seed at 8.5% moisture.

3.4 Determining Hybridity Levels in Hybrid Seed and Herbicide Resistant Levels in Synthetic Seed Lots Produced in 1997 and 1998

3.4.1 Indoor Screening Trials

The level of hybridity in the hybrid seed lots derived from the different hybrid seed production trial row ratios and the herbicide resistant levels in the synthetic seed lots was determined in growth room studies at the University of Manitoba in the fall of 1997 and 1998. All female rows harvested from the hybrid seed production plots grown in the 1997 and 1998 field seasons were screened using a minimum family size of 48 plants. Selected rows from the synthetic seed production trials grown in 1997 and 1998 were screened using a minimum family size of 96 plants.

Hybrid seed production plots at each location in each year had a total of 36 female rows to be screened (1:1, 2:2, 3:3 planting ratios in each of two replicates). All three locations had a total of 108 individual rows to be screened in each year.

The synthetic seed production trials had 10 selected rows from each location in each year entered into the screening trial (5 entries from each of two replicates). All three locations for the synthetic seed production trials had a total of 30 individual rows to be screened in each year (Table 1).

A 288-plug tray was used to grow the seedlings. Each plug tray had a width of 12 plugs and a length of 24 plugs with each plug having the dimensions of 2 cm x 2 cm x 3 cm. The bottoms of the plug trays were covered with duct tape in order to prevent the metro mix from falling out of large holes in bottom of plug tray. Holes were punched through the duct tape in order to aid in drainage.

Measuring the dimensions of the plugs and calculating the exact center of each of the plugs generated the potential to create a "drain hole stamp". A 9-cm x 24-cm x 1-cm plywood sheet was cut and nails pushed through the sheet corresponding with the exact center of the plug. By generating a drain hole stamp, less time was used in order to prepare the plug trays (Figure 7).

Table 1. Amount of seed screened indoors for the screening trials for the 1997 and 1998 hybrid and synthetic seed production trail seed samples

Trial Type	Ratio	No. of Locations Per Year	No. of Rows Screened at each Location	No. of Indoor Screening Trials	Min. Family Size	Total Amount of Seed Planted
97 Hybrid	1:1	3	6	2	48	1728
	2:1	3	12	2	48	3456
	3:3	3	18	2	48	5184
97 Hybrid Total						**10368**
97 Synthetic	50%:50%	3	5	2	96	5760
97 Combined Total						**16128**
98 Hybrid	1:1	3	6	2	48	1728
	2:1	3	12	2	48	3456
	3:3	3	18	2	48	5184
98 Hybrid Total						**10368**
98 Synthetic	50%:50%	3	5	2	96	5760
98 Combined Total						**16128**
Grand Total						**32256**

note: Trial = type of seed production trial
# of Locations per year = Number of Locations producing hybrid and synthetic seed material in one year
# of Rows screened at each Location= Number of Rows within a sub-sample screened at each location in one year
# of Indoor Screen Trials = Number of screening trials done in one year for one location
Min. Family Size = Calculated minimum family size needed

Plug trays were then filled with standard metromix and excess metromix brushed off. Dimensions needed to generate the "drain hole stamp" were also used to generate a "seed hole stamp" (Figure 8). A 9-cm x 24-cm x 1-cm plywood sheet was cut and forty-eight 0.25-cm diameter-doweling stamps were glued onto the plywood corresponding with the center of the plug. Each hole generated would be approximately 1.75 cm in depth and be of adequate depth as to not impede plant growth.

One seed was to be placed in one hole. A total of 16,168 seeds needed to be planted for one year. This translated into many hours of monotonous seed placement. To efficiently overcome the time needed to plant the abundance of seed, a seeding device was created in order to speed up the time required to plant all 16,168 seeds. A 9-cm x 24-cm Plexiglas sheet was cut out and 48 5-mm holes drilled through the sheet. The position of the holes corresponded with the position of the seed holes generated by the "seed hole stamp" in the metromix.

Three smaller 1 cm strips were glued around the outside of the drilled Plexiglas. This acted as a spacer for another Plexiglas sheet described later on. On top of the 1cm spacers another three 1.5cm strips of Plexiglas were glued on. These acted as supports to prevent the second Plexiglas sheet from moving around.

Another sheet of Plexiglas was overlaid on top of the drilled Plexiglas sheet. Holes were placed in this sheet as well only the diameter of the holes in the upper sheet was approximately 1mm in size. This was of adequate size as to accommodate only one seed per hole. A total of 48 holes were drilled into the Plexiglas sheet as to accommodate the 48 holes in the metromix.

When the top sheet was overlaid with the bottom sheet, the spacer provided just enough space to allow the two to glide over one another. The upper support guides kept the bottom and top Plexiglas sheet in close contact. The holes could be manipulated as to be open (i.e. the two holes aligning with one another) or closed (the 2 hole not aligned with one another and seed would only fill the smaller hole). The idea behind this device was that when the seeding device was closed, seed could be placed on top and shaken around. The diameter of the upper sheet holes would only accommodate one seed and the excess would be brushed off. The upper sheet would then be slid over the bottom sheet and the seeds would fall through the larger bottom holes. The position of the holes corresponded with the holes in the metromix, and the end result was one seed falling into each hole. Forty-eight seeds could be planted at one time, thus decreasing the amount of time required to produce screening flats (Figure 9).

Metromix was placed on top of the flat to fill up the holes generated by the seed hole stamp and the excess was brushed off. Flats were watered daily and 20-20-20 fertilizer was applied every second day at a rate of 4 ml/l to ensure healthy plants at time of spraying. Bromoxynil was applied at a rate of 560 g of a.i./ha when the seedlings were at the 2-leaf stage, approximately 17 days after planting.

Plants were scored after applying bromoxynil as either live or dead approximately three days after applying the herbicide. Hybridization between herbicide resistant male and female plants resulted in a surviving (live) hybrid progeny, while female x female intra-row pollination resulted in herbicide sensitive female sibs that were killed by the herbicide spray. Hybridity of the hybrid seed lots was therefore measured as the number of surviving (live) plants over the number of

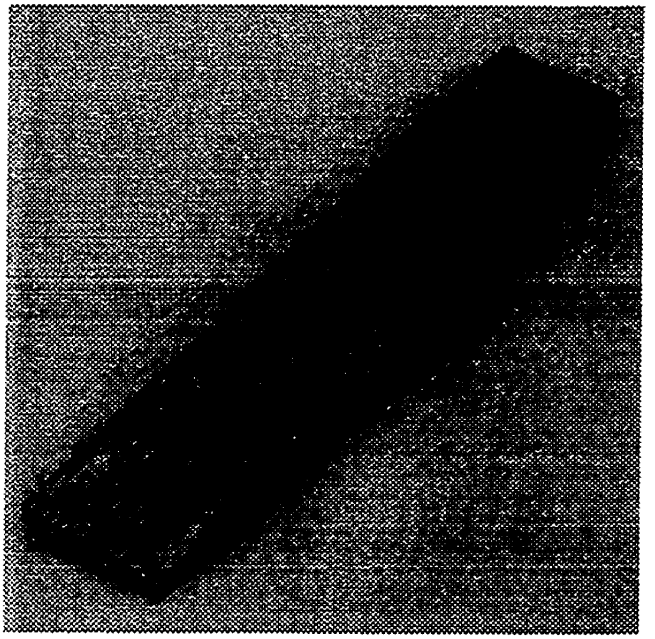


Figure 7. Photo of drain hole punch used to generate drainage holes in plug trays

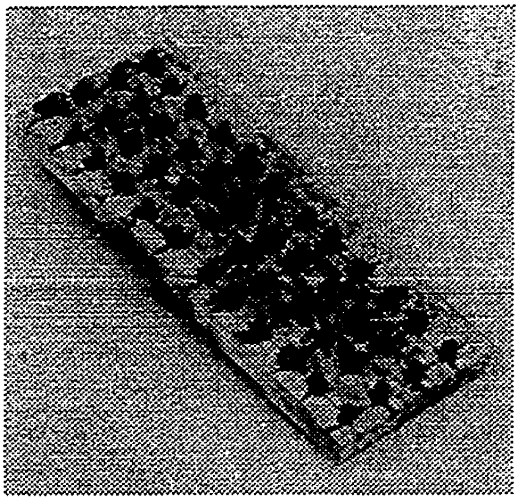


Figure 8. Photo of seed hole stamp used to produce holes for seed in plug trays

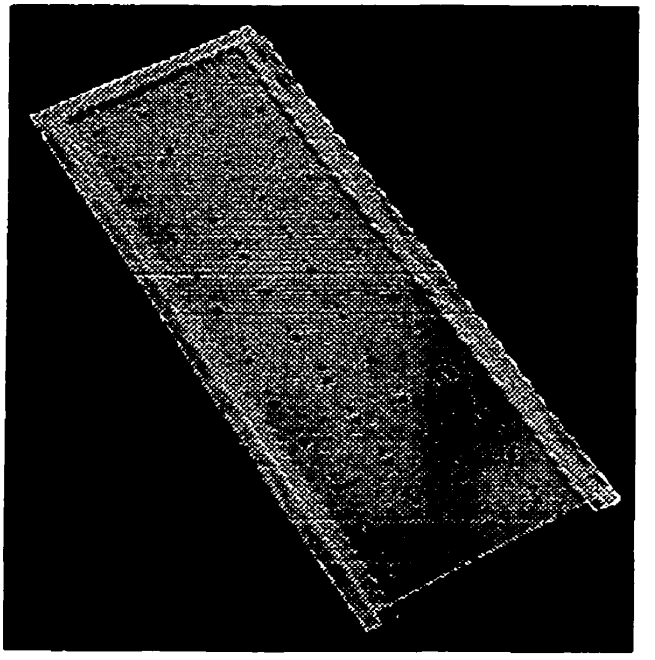


Figure 9. Photo of seeder used to seed indoor screening trials

total plants counted before the spray treatment for each seed sample.

The synthetic seed production trials seed lots were handled in a similar fashion to the hybrid seed production seed lots, however, 96 seeds were planted and screened for each synthetic seed production trial seed lot.

3.4.2 Outdoor Screening Trial

All Carman, Winnipeg, and Glenlea hybrid and synthetic seed production entries from the 1997 field season were screened in Carman during the 1998 field season. The dimensions of the outdoor screening trials were 40 m x 58 m divided into 8 ranges. All material from the 1997 field season was replicated twice within the field. Individual plots had the size of 3 m x 1.4 m with a 2 m pathway in-between ranges.

The outdoor screening trial was seeded on May 12, 1998 with a Hege belt-cone small plot, 6 row seeder at a rate of 6 kg / ha. An insecticide (Carbofuran 10% granules) was banded with the seed at a rate of 11 kg a.i /ha in order to control flea beetle (*Phyllotreta cruciferae* Goeze and *P. striola*ta F.). 1997 hybrid and synthetic seed production material went into each plot.

Emergence began on May 21, 1998 and plant stands were counted on approximately one third of the plots, chosen as outlined below, at the 2 to 3 leaf stage. The plots to be counted were determined by walking a "zig-zag" formation over the field covering all ranges. This was done to decrease the variations in the field and to ensure an accurate assessment of plant counts in the field. Two representative rows were selected and counted in each plot. This two-row stand

count was averaged and then multiplied by 6 to estimate the plant stand number for the selected plots.

Average stand counts were obtained on May 26, 1998 on all selected plots and these were then were then averaged. A total of 41 plots were counted. The average plant count per row over all selected plots was 191.77 with a standard deviation of 17.4. This selected plot stand count was 1150.6 with a standard deviation of 104.4. This plot stand count number was then used as the estimate of the number of plants in each plot before herbicide spraying.

Bromoxynil was applied on May 29, 1998 with a 3-point hitch sprayer at a rate of 560 g a.i./ha. Three days after spraying surviving (live) plants in all lots were counted and this number then divided by the estimated plant stand counts for all plots. This was done to obtain the hybridity of the hybrid seed lots from the hybrid seed production trials and the herbicide resistant levels from the synthetic seed lots from the synthetic seed production trials.

The values for hybridity and resistant plant levels obtained from the outdoor screening were used to verify the indoor screening results.

3.5 1998 Synthetic Inter-Genotype Competition Trial

To establish the relative competitive abilities of the two genotypes used in the synthetic seed production trials, an inter-genotype competition experiment was established at the Carman Research Farm in 1998. The inter-genotype competition trial consisted of 16 plots, each with each plot planted with 50% MBRR195 seed and 50% Foothills (SW 03375) seed. The plots were seeded with a Hege belt-cone 3-point hitch small plot seeder. The individual plots were 3m long and contained 6

rows within each plot spaced 20 cm apart. The distance between each plot was 40 cm within one range. A total of 8 ranges were established and a 2-m pathway was maintained between ranges.

The thinning experiment commenced when the plants within the plots reached the 2 to 3 leaf stage. Two plots were randomly chosen and plants in the plots counted prior to the application of herbicide. Initial plant numbers were recorded and then bromoxynil was applied at a rate of 560 g a.i./ha using a standard backpack sprayer equipped with a flat fan nozzle.

Approximately 3 days later, the surviving plants were counted and compared to the initial plant numbers. Counts of initial plant stands prior to the application of herbicide followed by spraying and counting of survivors continued for 8 consecutive weeks until the end of flowering.

3.6 1998 Preliminary Yield Trials

Samples which expressed the highest levels of resistance, established in the 1997 indoor screening trails, from the Carman, Winnipeg, and Glenlea 1997 hybrid and synthetic seed production trials were selected for entry into Preliminary Yield Trials. The samples chosen from each location in the 1997 hybrid and synthetic seed production trials are listed in Table 2.

Two Preliminary Yield Trials were planted at Carman and Glenlea. The Carman trail was planted on May 12, 1998. The Glenlea trial was planted May 15, 1998. At both locations, the Preliminary Yield Trials consisted of 4 replicates over 8 ranges with 18 entries in each replicate separated by a 2m pathway in between

ranges. Each entry consisted of 6 rows of material spaced 20 cm apart over a plot length of 3m separated by 60 cm between plots.

Selected plots were to be sprayed to determine the yield performance of pure hybrid seed lots, and herbicide resistant level enriched synthetic seed lots produced in the previous year. Plots which were not to be sprayed were seeded at a rate of 5 kg/ha (3g/6m^2), while plots which were to be sprayed plots were seeded at a rate of 10 kg/ha (6g/6m^2). The difference in seeding rate was required to establish similar plant densities in all plots after the application of herbicide to the sprayed plots (Table 2).

All plots were seeded with a Hege belt-cone 3-point hitch small plot 6 row seeder. An granular insecticide, Carbofuran (10% granules), was banded with the seed at an approximate rate of 11 kg a.i./ha to control flee beetles (*Phyllotreta cruciferae* Goeze and *P. striola*ta F.).

A tank mixture of Poast at 300-g a.i./ha, Muster at 22-g a.i./ha and Lontrel at field rates of 300-g a.i./ha was applied, with a three-point hitch sprayer, post crop emergence to control annual and perennial grasses, Canada thistle and wild mustard.

Bromoxynil was applied to selected plots at a rate of 560 g a.i./ha using a portable bicycle wheel plot sprayer equipped with flat fan nozzles delivering 108 L/ha at 275 kpa. Two wind-boards flanked either side of selected plots in order to prevent herbicide drift onto neighboring unsprayed plots. Commercial cultivars and experimental populations sensitive to bromoxynil such as Reward, Parkland, Mavrick, Foothills (SW 03375) were left unsprayed. Selected entries that were resistant to bromoxynil and selected hybrid and synthetic plots were also left

Table 2. Entries comprising the 1998 Preliminary Yield Trials of hybrid and synthetic seed production trial seed lots produced in 1997 including parents used in the crosses and registered check cultivars seeded in 4 replicates

Entry 1997 Row	Row Ratio Description	Percent Resistant	Sprayed	Seed Quantity(g/plot)
9045	97 Carman Hybrid 1:1	30.95	Yes	6
9045	97 Carman Hybrid 1:1	30.95	No	3
9245	97 Winnipeg Hybrid 1:1	32.29	Yes	6
9245	97 Winnipeg Hybrid 1:1	32.29	No	3
9402	97 Glenlea Hybrid 1:1	31.88	Yes	6
9402	97 Glenlea Hybrid 1:1	31.88	No	3
9175	97 Carman Synthetic	31.78	Yes	6
9175	97 Carman Synthetic	31.78	No	3
9368	97 Winnipeg Synthetic	33.68	Yes	6
9368	97 Winnipeg Synthetic	33.68	No	3
9568	97 Glenlea Synthetic	38.74	Yes	6
9568	97 Glenlea Synthetic	38.74	No	3
MBRR 195	Male Check	100	Yes	3
MBRR 195	Male Check	100	No	3
SW 03375	Female Check	0	No	3
Parkland	Commercial Check	0	No	3
Mavrick	Commercial Check	0	No	3
Reward	Commercial Check	0	No	3

unsprayed (Table 2).

Fertilizer (20-0-0-24) was applied at an approximate rate of 112 kg of actual N per hectare with a Cyclone 3-point hitch spreader just prior to bolting (Burkenkamp stage 3.1 (Figure 5).

3.7 Statistical Analysis

Statistical analysis was done using SAS system for windows v 6.21 (SAS Institute Inc. Cary, North Carolina, U.S.A.).

Hybrid seed production agronomic, seed quality, and hybridity for the 1997 and 1998 seed production trials were analyzed using a split plot design. Rows within each planting ratio in each replicate represented a sub-sample. The 1:1 row ratio treatment had 3 rows with the average hybridity for these three rows averaged together to produce one value for this ratio in each replicate at each location in each year. This approach was used for all treatments in each replicate at each location and each year.

A total of 36 treatments were generated representing all planting row ratios at all locations in all years. Locations and years were classified as separate environments, therefore there were 6 separate environments studied in this research.

In the split-plot analyses, environments were the main effect and treatments (i.e. row ratios and/or synthetic seed lot production environment) were the sub-plots.

A test for homogeneity of error variance was used between the different environments as outlined by Gomez and Gomez (1984). Test for homogeneity of error variance was determined on all agronomic traits measured and the hybridity

between the six different environments (Carman, Winnipeg and Glenlea in 1997 and 1998).

All rows within a synthetic seed production trial were identical within replicates. A replicate generated a single value for all agronomic characteristics, quality characteristics and herbicide resistant level, with a standard deviation around the mean generated by comparing replicate values. This was done for each location in each year and combined over the 6 environments.

The 1998 synthetic thinning trial was analyzed using a standard completely randomized design model.

The 1998 Preliminary Yield Trials were analyzed using a standard Randomized Complete Block Design. This was done for all agronomic and seed quality characteristics. An LSD test was conducted between the different entries to detect significant differences

4.0 Results and Discussion

4.1 Hybrid Seed Production Trials

4.1.1 Hybrid Seed Production Trials in Six Environments

Individual results for the six different environments (three locations over two years) are presented in Tables 3 to 8.

There were no significant differences among the treatments for days to emergence in any environment (Tables 3 to 8). Days to emergence ranged from 8 to 11 days over the six environments.

There were no significant differences in vigor among treatments for five out of the six environments (Tables 3 to 8). In one environment (Carman 1997) there were significant differences among the treatments for vigor. The 1:1 row ratio treatment in this environment had a lower vigor rating than the other treatments because of the proximity of a willow tree shelter belt located approximately 3 m away from the 1:1 row ratio treatment.

There were no significant differences among the treatments for days to flower in any environment (Tables 3 to 8). The range of the days to first flower was 34 to 39 days for all treatments over all environments.

There were no significant differences among the treatments for lodging in any environment. All treatments over all environments had a lodging score ranging from 1 to 4.

There were no significant differences among the treatments for plant height in any environment (Tables 3 to 8). All treatments over all environments had a plant height ranging from 88 to 133 cm (Tables 3 to 8).

There were no significant differences among the different treatments in any environment (Tables 3 to 8). Days to maturity ranged from 71 to 79 days.

Total seed yield (kg/ha) displayed no significant differences between the different treatments in any environment (Tables 3 to 8). Total seed yield ranged from 968 to 2736 kg/ha.

There were no significant differences among treatments for total hybrid seed yield (kg/ha) for five out of the six environments (Tables 3 to 8). In one environment (Winnipeg 1998) there were significant differences among the treatments for total hybrid seed yield. The 1:1 row ratio treatment in this environment had a higher total hybrid seed yield than the 2:2 or 3:3 treatments. The 2:2 row ratio treatment was also higher yielding than the 3:3 row ratio treatment.

Since the seed quality traits were assessed on a single bulk of the two replicates, no statistical comparisons can be made among the different treatments for oil content, protein content, erucic acid content, or glucosinolate content for individual environments. These assessments were conducted to verify that the seed quality of the hybrid seed lots was in the normal range for *B. rapa* and that it met canola standards. The oil percentage ranged form 39.7% to 50.3% over all treatments over all environments. The protein percentages ranged from 20% to 29.3%. There were no detectable levels of erucic acid in any of the treatments over all environments. Glucosinolate content ranged from 7.4 to 27 μmol total glucosinolates per gram of seed at 8.5% moisture (Tables 3 to 8).

Hybrid populations grown in the 1997 field season were assessed for hybridity screened both indoors and outdoors. There were no differences in hybridity for three out of the six environments when screened indoors. Environment 3 (Table 5)

showed the 1:1 ratio having a higher percent hybrid material the other two treatments. Environment 5 (Table 7) showed significant differences between all three treatments, the 1:1 treatment expressing the highest level of hybridity followed by the 2:2 and then the 3:3. Environment 6 (Table 8) showed significant differences between the 1:1 and 3:3 row ratio treatments with the 1:1 expressing the highest level of hybridity. The 2:2 was non-significantly different from either treatment (Tables 3 to 8).

The outdoor screening trial showed significant differences in hybridity among the different treatments. Both environment 1 (Table 3) and environment 3 (Table 5) produced the 1:1 row ratio treatment with the highest level of hybridity which was statistically different form the 2:2 and 3:3 row ratio treatments. The 2:2 and 3:3 were non-significant from one another (Table3 and Table 5). All treatments in Environment 2 (Table 4) were significantly different from one another. The 1:1 ratio expressed the highest level of hybridity followed by the 2:2 treatment then the 3:3 treatment.

Table 3. Days to emergence, vigor, days to flower, lodging, height, days to maturity, total seed yields yield, hybrid seed yield, oil, protein erucic acid, glucosinolates, and hybridity for each row ratio treatment for the hybrid seed production trial grown at Carman in 1997

Row Ratio	Emergence (days)	Vigor (1-5)	Days to Flower	Lodging (1-5)	Height (cm)	Days to Maturity	Total Seed Yield (kg/ha)	Total Hybrid Seed (kg/ha)	Oil (%)	Protein (%)	Erucic Acid (%)	Glucs (umol/g)	Hybridity Indoor (%)	Hybridity Outdoor (%)
1:1	11 (a)	1.7 (c)	36.7 (a)	2.8 (a)	100 (a)	72 (a)	1011 (a)	199 (a)	42.8	26.6	0.0	6.5	20 (a)	18 (a)
2:2	11 (a)	3.3 (b)	36.5 (a)	2.7 (a)	115 (a)	72 (a)	1237 (a)	213 (a)	42.3	27.0	0.0	7.8	17 (a)	13 (b)
3:3	11 (a)	4.1 (a)	36.1 (a)	2.6 (a)	121 (a)	72 (a)	1430 (a)	176 (a)	41.2	28.0	0.0	8.8	12 (a)	11 (b)
CV	0	5.905	1.07	4.51	7.81	0	8.17	18.67					16.003	7.94
LSD	0	0.8	1.7	0.5	37.64	0	431	157					11	5
MSE	0	0.031	0.15	0.02	76.52	0	10016.2	1337.91					6.848	1.26
R^2	0	0.99	0.668	0.94	0.756	0	0.918	0.49					0.794	0.96
Alpha	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05					0.05	0.05

Table 4. Days to emergence, vigor, days to flower, lodging, height, days to maturity, total seed yields yield, hybrid seed yield, oil, protein erucic acid, glucosinolates, and hybridity for each row ratio treatment for the hybrid seed production trial grown at Winnipeg in 1997

Row Ratio	Emergence days	Vigor (1-5)	Days to Flower	Lodging (1-5)	Height (cm)	Days to Maturity	Total Seed Yield (kg/ha)	Total Hybrid Seed (kg/ha)	Oil (%)	Protein (%)	Erucic Acid (%)	Glucs (umol/g)	Hybridity Indoor (%)	Hybridity Outdoor (%)
1:1	10 (a)	2 (a)	41.8 (a)	1 (a)	113 (a)	73 (a)	1177 (a)	274 (a)	48.6	21.1	0.0	10.1	23 (a)	22 (a)
2:2	10 (a)	3 (a)	41.6 (a)	1.1 (a)	115 (a)	73 (a)	1438 (a)	231 (a)	49.1	20.7	0.0	8.5	16(a)	14 (b)
3:3	10 (a)	3 (a)	41.8 (a)	1.1 (a)	113 (a)	73 (a)	1181 (a)	137 (a)	47.3	22.1	0.0	7.4	12(a)	11 (c)
CV	0	30.61	1.14	7.62	10.63	0	7.87	31.02					25.12	2.52
LSD	0	3.51	2.1	0.4	52	0	428.6	286					18	2
MSE	0	0.67	0.23	0.01	146.5	0	9923.9	4405.1					18.01	0.15
R^2	0	0.6	0.76	0.74	0.17	0	0.82	0.7					0.79	0.99
Alpha	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05					0.05	0.05

Table 5. Days to emergence, vigor, days to flower, lodging, height, days to maturity, total seed yields yield, hybrid seed yield, oil, protein erucic acid, glucosinolates, and hybridity for each row ratio treatment for the hybrid seed production trial grown at Glenlea in 1997

Ratio	Emergence Days	Vigor (1-5)	Days to Flower	Lodging (1-5)	Height (cm)	Days to Maturity	Total Seed Yield (kg/ha)	Total Hybrid Seed (kg/ha)	Oil (%)	Protein (%)	Erucic Acid (%)	Glucs (umol/g)	Hybridity Indoor %	Hybridity Outdoor %
1:1	8 (a)	4.0 (a)	37.8 (a)	2.2 (a)	128 (a)	71 (a)	968 (a)	265 (a)	39.7	28.6	0.0	17.0	27 (a)	20 (a)
2:2	8 (a)	4.1 (a)	38.3 (a)	1.8 (a)	130 (a)	71 (a)	1121 (a)	198 (a)	40.6	28.3	0.0	11.2	18 (b)	15 (b)
3:3	8 (a)	4.0 (a)	38.1 (a)	2.4 (a)	133 (a)	71 (a)	1172 (a)	169 (a)	40.1	28.6	0.0	9.1	14 (b)	12 (b)
CV	0	1.72	0.47	12.81	3.57	0	5.65	13.1					7.65	4.26
LSD	0	0.3	0.8	1.2	20	0	264	119					7	3
MSE	0	0.04	0.03	0.07	21.64	0	3774.22	762.97					2.29	0.43
R^2	0	0.6	0.74	0.84	0.64	0	0.9	0.88					0.975	0.98
Alpha	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05					0.05	0.05

Table 6. Days to emergence, vigor, days to flower, lodging, height, days to maturity, total seed yields yield, hybrid seed yield, oil, protein erucic acid, glucosinolates, and hybridity for each row ratio treatment for the hybrid seed production trial grown at Carman in 1998

Ratio	Emergence (days)	Vigor (1-5)	Days to Flower	Lodging (1-5)	Height (cm)	Days to Maturity	Total Seed Yield (kg/ha)	Total Hybrid Seed (kg/ha)	Oil (%)	Protein (%)	Erucic Acid (%)	Glucs (umol/g)	Hybridity %
1:1	9 (a)	4.8 (a)	39 (a)	1 (a)	110 (a)	79 (a)	1221 (a)	319 (a)	50.0	20.0	0.0	10.9	25 (a)
2:2	9 (a)	4.2 (a)	39 (a)	1 (a)	108 (a)	79 (a)	1030 (a)	148 (a)	50.3	20.7	0.0	13.7	14 (a)
3:3	9 (a)	4 (a)	39 (a)	1.5 (a)	116 (a)	79 (a)	1131 (a)	148 (a)	49.1	21.4	0.0	15.4	14 (a)
CV	0	14.39	0	34.99	10.51	0	15.01	34.54					25.38
LSD	0	2.7	0	1.8	50.2	0	728	304					19
MSE	0	4.3	0	0.17	136.5	0	28607.36	4998.7					19.29
R^2	0	0.75	0	0.6	0.79	0	0.96	0.91					0.818
Alpha	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05					0.05

Table 7. Days to emergence, vigor, days to flower, lodging, height, days to maturity, total seed yields yield, hybrid seed yield, oil, protein erucic acid, glucosinolates, and hybridity for each row ratio treatment for the hybrid seed production trial grown at Winnipeg in 1998

Ratio	Emergence (days)	Vigor (1-50	Days to Flower	Lodging (1-5)	Height (cm)	Days to Maturity	Total Seed Yield (kg/ha)	Total Hybrid Seed (kg/ha)	Oil (%)	Protein (%)	Erucic Acid (%)	Glucs (umol/g)	Hybridity %
1:1	9 (a)	5 (a)	34 (a)	3.2 (a)	91 (a)	69 (a)	1342 (a)	308 (a)	39.0	28.5	0.0	16.0	23 (a)
2:2	9 (a)	5 (a)	34 (a)	2.9 (a)	88 (a)	69 (a)	1424 (a)	187 (b)	39.6	28.2	0.0	15.6	13 (b)
3:3	9 (a)	5 (a)	34 (a)	3.1 (a)	92 (a)	69 (a)	1246 (a)	121 (c)	39.4	28.0	0.0	16.9	10 (c)
CV	0	0.91	0	6.32	4.21	0	7.07	1.41					3.5
LSD	0	0.2	0	0.8	16.3	0	407	12					2
MSE	0	0.002	0	0.04	14.35	0	8940.6	8.36					0.28
R^2	0	0.6	0	0.46	0.63	0	0.65	0.999					0.997
Alpha	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05					0.05

Table 8. Days to emergence, vigor, days to flower, lodging, height, days to maturity, total seed yields yield, hybrid seed yield, oil, protein erucic acid, glucosinolates, and hybridity for each row ratio treatment for the hybrid seed production trial grown at Glenlea in 1998

Ratio	Emergence (days)	Vigor (1-5)	Days to Flower	Lodging (1-5)	Height (cm)	Days to Maturity	Total Seed Yield (kg/ha)	Total Hybrid Seed (kg/ha)	Oil (%)	Protein (%)	Erucic Acid (%)	Glucs (umol/g)	Hybridity %
1:1	8 (a)	2.7 (a)	38 (a)	4 (a)	94 (a)	78 (a)	2326 (a)	639 (a)	40.6	29.1	0.0	25.5	27 (a)
2:2	8 (a)	3.8 (a)	38 (a)	4 (a)	99 (a)	78 (a)	2736 (a)	422 (a)	41.6	29.3	0.0	22.2	15 (ab)
3:3	8 (a)	3.1 (a)	38 (a)	4 (a)	85 (a)	78 (a)	2649 (a)	333 (a)	42.6	28.3	0.0	25.2	13 (b)
CV	0	17.41	0	0	4.87	0	10.89	32.01					15.21
LSD	0	2.4	0	0	19.5	0	1204	640					11
MSE	0	0.31	0	0	20.43	0	78339.25	22103.29					7.68
R^2	0	0.67	0	0	0.84	0	0.786	0.77					0.94
Alpha	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05					0.05

4.1.2 Hybrid Seed Production Trials Combined Over all Environments

A test for the homogeneity of error variance was conducted on all agronomic characteristics measured throughout the six environments. Error variances for all characteristics (emergence, vigor, days to flower, lodging, plant height, maturity, yield, hybrid yield, and hybridity) were homogenous.

Results of analysis of variance tests for vigor, days to flower, lodging, height, total seed yield, total hybrid seed yield, and hybridity are shown in Appendix Tables 1 to 7.

All row ratio treatments, averaged over 6 environments (Carman 1997 and 1998, Winnipeg 1997 and 1998, and Glenlea 1997 and 1998) emerged 9 days after seeding with no significant differences among treatments (Table 9).

There were significant differences in vigor among the row ratio treatments averaged over the 6 environments. The 1:1 row ratio treatment had a significantly lower vigor rating than the other two row ratio treatments (Table 9). The lower vigor rating for the 1:1 row ratio treatment in Carman in 1997 and the lower vigor rating for the 1:1 row ratio treatment in Glenlea in 1998 resulted in the lower vigor rating for the 1:1 row ratio treatment averaged over 6 environments.

Days to flower exhibited no significant difference among the treatments averaged over all environments (Table 9).

There were no significant differences for lodging among the row ratio treatments averaged over all environments (Table 9).

Table 9. Days to emergence, vigor, days to flower, lodging, height, days to maturity, total seed yields yield, hybrid seed yield, oil, protein erucic acid, glucosinolates, and hybridity for each row ratio treatment for the hybrid seed production trial grown in Carman, Winnipeg and Glenlea in 1997 and 1998

Ratio	Emergence (days)	Vigor (1-5)	Days to Flower	Lodging (1-5)	Height (cm)	Days to Maturity	Seed Yield (kg/ha)	Hybrid Seed (kg/ha)	Oil (%)	Protein (%)	Erucic Acid (%)	Glucs (umol/g)	Hybridity %
1:1	9.2 (a)	3.4 (b)	37.9 (a)	2.4 (a)	106 (a)	73.7 (a)	1498 (a)	334 (a)	43.5 (a)	25.6 (a)	0	14.3 (a)	24 (a)
2:2	9.2 (a)	3.9 (a)	37.9 (a)	2.4 (a)	110 (a)	73.7 (a)	1468 (ab)	233 (b)	43.9 (a)	25.7 (a)	0	13.2 (a)	16 (b)
3:3	9.2 (a)	3.9 (a)	37.8 (a)	2.3 (a)	109 (a)	73.7 (a)	1341 (b)	181(b)	43.3 (a)	26.1 (a)	0	13.8 (a)	12 (c)
CV	0	13.07	0.69	9.51	7.68	0	10.62	30.04	1.63	2.17	0	16.86	17.4
LSD	0	0.4	0.2	0.2	7.4	0	136	67	0.9	0.7	0	3	2.7
MSE	0	0.233	0.07	0.05	69.32	0	23266.92	5602.72	0.5	0.31	0	5.39	9.07
R^2	1	0.927	0.996	0.98	0.91	1	0.977	0.901	0.98	0.98	0	0.91	0.906
Alpha	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

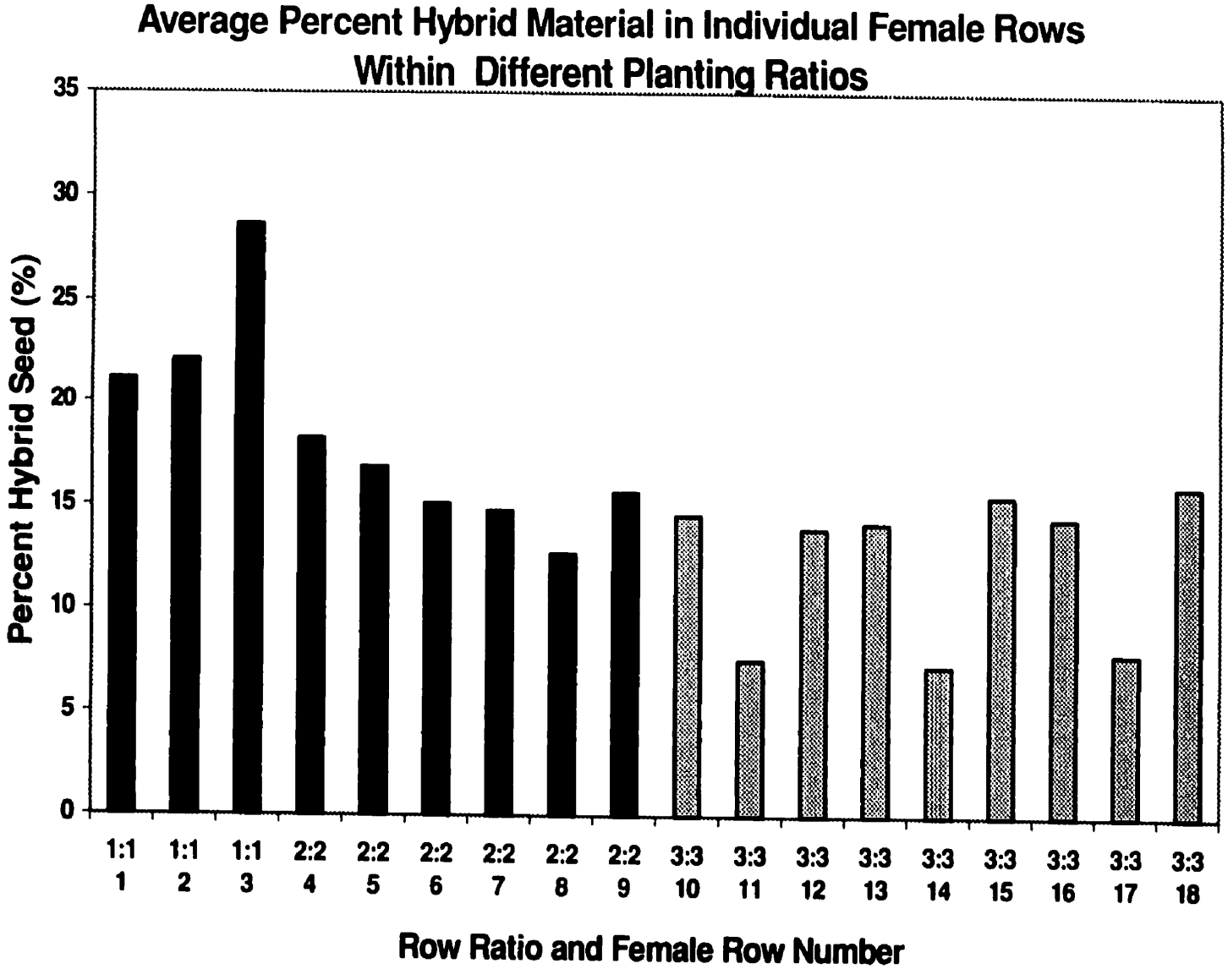


Figure 10. Average percent hybrid material expressed in each female row within each 1:1, 2:2, and 3:3 row ratio treatments averaged over all environments

There were no significant differences among the different row ratio treatments for height over all environments (Table 9).

Days to maturity also displayed no significant differences among the treatments (Table 9). All row ratio treatments matured 73.7 days after emergence.

There were significant differences in the total seed yield among the row ratio treatments. The 1:1 row ratio treatment yielded significantly more than the 3:3 row ratio treatment (Table 9). This could be explained by the different plant heights were observed between the two parental genotypes. The female plants in the 1:1 row ratio treatment were planted beside shorter males. The taller nature of the female plant allowed them to gather more light. The female rows growing in the 3:3 tow ratio treatment were in competition with one another because their canopies were at the same height. The inter-competition between rows could have resulted in a decrease and a significant difference in the row ratio treatment. Another possible explanation for the higher yield of the 1:1 row ratio treatment could be the placement of the 1:1 and 3:3 row ratio treatments in the field. More available nutrients and better field conditions for the 1:1 row ratio treatment could have given rise to a higher yield.

Statistical comparisons are possible for treatment effects for oil content, protein content, erucic acid content, and glucosinolate content for the trials combined over environments. There were no significant differences among row ratio treatments for oil content averaged over environments (Table 9). Excellent oil content was observed for all treatments averaged over all environments.

Similarly, there were no significant differences among the row ratio treatments for protein content averaged over all environments (Table 9). All protein values were good to excellent.

All treatments in all environments had non-detectable levels of erucic acid in the seed (Table 9).

Similarly, there were no significant differences among the row ratio treatments for glucosinolate levels averaged over all environments (Table 9). The glucosinolate contents for all treatments ranged from 13 to 15 µmol/gram of seed at 8.5% moisture (Table 9). All glucosinolate levels were well within the canola standard.

In summary, the seed quality for all treatments averaged over six seed production environments was fully acceptable for all quality traits.

The hybridity of the all row ratio treatments was determined indoors in the fall of 1998 at the University of Manitoba. Although two hybridity estimates (indoor and outdoor) were obtained for the 1997 seed production year, only indoor hybridity estimates are available for the 1998 seed production year. Only the averaged indoor values are reported in Table 9 since this provides a valid comparison of hybridity between the two seed production years (Table 9).

Significant differences in hybridity were observed among the row ratio treatments combined over environments. The 1:1 row ratio treatment had a significantly higher hybridity than the other two row ratio treatments for the combined trials (Table 9). The hybridity of all row ratio treatment are statistically significant from one another for the trails combined over environments. Hybridity decreased as row ratio number increased.

Pollen can travel for considerable distances in wind cross pollinated crop such as *B. rapa* (Stringam et al. 1978). Depending on wind direction and speed, pollen from one plant can pollinate another plant at various distances from the pollen producing plant. The likelihood of pollination is greater for plants that are closer to

the pollen source than for plants that are farther away from the pollen source. The likelihood of cross pollination declines exponentially with distance. Closer pollen sources, such as generated in the 1:1 row ratio treatment are expected therefore, to have higher hybridity than the larger row ratio treatments (i.e. 2:2 and the 3:3 row ratio treatments), even in a wind pollinated crop.

A 50% hybridity level was expected for the 1:1 row ratio treatment. The actual hybridity for the 1:1 treatment was only 24%, approximately half of what was expected. The 2:2 row ratio treatment had lower hybridity, while the 3:3 row ratio treatment had the lowest hybridity averaged over all environments. The lower than expected hybridities may have occurred because the parent populations used to make up the hybrids share one or more self incompatible alleles in common, thus reducing the degree of cross-pollination. Alternatively, pollen movement may have been very limited. The 1:1 row ratio treatment, with male rows adjacent to every female row, had the highest hybridities. The reduced hybridity levels for the 2:2 row ratio treatment and the 3:3 row ratio treatment indicates that most of the pollen present on the female flowers comes from the nearest possible source, i.e. adjacent plants within female row and plants in the adjacent male or female rows. The exceptionally low hybridities in the center row of the 3:3 row ratio treatment (Figure 10) supports the idea that the vast majority of pollen on the flowers in the female row comes from the immediately adjacent rows and/or within the row.

There is some male plant pollen moving into these inner female rows from the nearest male rows located 80 cm and 120cm away, but the two flanking female rows located 40 cm away contribute to the level of hybridity to a much greater extent.

Approximate hybridity levels as seen in this bar chart indicate the outside rows in the row ratio 3:3 treatment are approximately 15% averaged over all environments, while the middle row is approximately 7.5%, half of the outside row values (Figure 10). The outside row values for the 3:3 row ratio treatment are comparable to the values expressed in the 2:2 row ratio treatment.

There are a number of possible explanations to account for the lower hybridities than expected. Primarily there is the spatial relationship between the hybridity and the distance to the pollen sources. If this holds true, then a significant amount of pollen must originate from within the row as well as from the immediately adjacent rows. The lower than expected levels of hybridity seen in this study could be attributed from sib-matings within the row. Plants within a row were approximately 6 cm apart at flowering. Due to the branching nature of the *B. rapa* plants, a very close orientation of flowers on adjacent plants is likely. This close arrangement would make neighboring sib flowers more accessible to sib pollen. Wind movements within the canopy could brush neighboring sib flowers against one another therefore facilitating the exchange of pollen between siblings and thus increase the amount of non-hybrid seed generated.

To imagine this another way, the greatest concentration of pollen is present at the point of dehiscence immediately surrounding the flower. If a neighboring flower is close at hand, then the area of overlap will produce the greatest likelihood of pollination. The result of susceptible sib plant cross-pollination would be a decrease in the amount of resistant progeny harvested from the female row.

Another possibility is that pollen production by the female plant was greater than the pollen production of the male plants. With a predominance of female plant

originated pollen, the majority of the pollinations in the female rows would be non-hybrids – i.e. sibing instead of crossing.

Yet another causal agent for the decrease in hybridity observed, is the presence of similar self-incompatibility alleles between the male and female populations. Nou (1993) estimated that the amount of SI alleles in the *B. rapa* genome to be approximately 150. Even though this number is high, and the likelihood of like alleles existing within a population, having like SI alleles within the male and female populations could produce lower fertilization rates between the two parent populations. The net result would be unsuccessful fertilization due to the interaction of SI alleles and a lower degree of resistant material (i.e. hybrid) from the female row.

There were significant differences between the amount of hybrid seed produced among the different row ratio treatments. The 1:1 row ratio treatment, produced 334 kg/ha of hybrid seed, the 2:2 row ratio treatment produced 233 kg/ha of hybrid seed, while the 3:3 row ratio treatment produced 181 kg/ha. These are all low amounts of actual hybrid seed. The total amount of hybrid seed generated from the average of the row ratios indicates the relative cost effectiveness of generating hybrid seed based on the study at hand. If this method of hybrid seed production were to be implemented, the 1:1 row ratio would generate the most hybrid seed. Given average level of hybridity across all environments and the average yields, the 1:1 row ratio would generate 334 kg/ha of hybrid seed, the 2:2 row ratio would yield approximately 30% less hybrid seed than the 1:1 row ratio. The 3:3 row ratio would yield 55% less than the 1:1 row ratio. The 2:2 and 3:3 row ratio treatments probably do not produce enough hybrid seed to be economically viable. The amount of hybrid

seed produced by the best row ratio treatment (the 1:1 row ratio) is also low and more research is needed to determine the optimum conditions required to produce large quantities of hybrid seed using the approach used in this study.

4.2 Synthetic Seed Production Trials

The synthetic seed production trials consisted of two replicates of a single treatment in each environment. All rows were composed of (50% male plants and 50% female plants as measured at emergence). A mean and standard error for each trait in each environment is presented in the following Table. Similarly, a mean and standard error for each treatment averaged over all six synthetic seed production environments is presented in the following Table. A comparison of the treatment mean for each trait with a hypothesis of zero difference is possible.

4.2.1 Synthetic Seed Production Trials in Six Environments

Emergence in all environments ranged from 8 days after planting to 11 days after planting with no variability observed at each environment. Vigor over all environments ranged form 2.49 to 4.36 (Table 10). The lodging rating ranged from 2 to 4 over all environments (Table 10). The height of the plots ranged form 104 cm to 127 cm amongst the environments while the plots maturity ranged form 69 days after emergence to 80 days after emergence (Table 10). Synthetic plot total seed yield (kg/ha) ranged form 688 kg/ha to 2053 kg/ha with a percent resistant seed in each environment ranging from 18% resistant material to 35% resistant material (Table 10). These two factors combined gave a total amount of resistant seed at each location ranging from 240 kg/ha of resistant seed to 642 kg/ha of resistant seed (Table 10).

Seed quality characteristics such as oil percentages, protein percentages, erucic acid content, and glucosinolates were analyzed at the University of Manitoba. These assessments were conducted to verify that the seed quality of the synthetic seed lots was in the normal range for *B. rapa* and that it met canola standards.

Oil percentages ranged from 40.5% oil to 49.2% oil over all environments while protein ranged form 20% protein to 28% protein (Table 10). As oil increased in percentage the protein percent content decreased. This trend is evident over all environments. There were no detectable levels of erucic acid in the oil for any environment. Glucosinolate content ranged from 7.4 µmol total glucosinolates per gram of seed at 8.5% moisture to 22.8 µmol total glucosinolates per gram of seed at 8.5% moisture (Table 10).

4.2.2 Synthetic Seed Production Trials Averaged Over All Environments

The synthetic seed production trials were averaged over all environments (Carman 1997 and 1998, Winnipeg 1997 and 1998, and Glenlea 1997 and 1998) and a standard error of the mean determined. The average date of emergence of the synthetic seed production trials over all environments was 9.17 (Table 10).

Vigor had a mean rating of 3.18 (Table 10) averaged over all environments. The synthetic seed production rows began to flower on average in 35.33 (Table 10) when combined over all environments. The average lodging score was 2.67 over all environments. The synthetic seed production plots began to mature 73 days after emergence on average over the six environments. The total seed yield for the synthetic seed production plots averaged over all environments was 1418 kg/ha (Table 10). This is an excellent yield for *B. rapa*.

The most surprising result in the performance of the synthetic seed production trials was the level of resistant material within the harvested rows. Over all environments, the average percent of resistant material was only 30.13 (Table 10). This is much lower than the 75% resistant material initially anticipated.

In a large randomly mating population with no selection, mutation or migration, gene frequencies will remain constant from one generation to the next as outlined by the *Hardy-Weiberg* law (Falconer et al. 1996). The proportion of individuals within a population can be represented by the equation: $p^2 + 2pq + q^2$, where p^2 represents the frequency of the genotype of parent 1, q^2 represents the frequency of individuals of parent 2, and 2pq represents the frequency of individuals resulting form the union of parent 1 and parent 2 gametes.

This equation can be used to predict the anticipated proportions of genes in the next generation from the synthetic seed production trials. A certain proportion of the synthetic seed production plot seed was expected to be of male-male mating (represented by p^2), a certain proportion was expected to be of female-female matings (represented by q^2). And a certain proportion was expected to be a hybrid between the male and female genomes (represented by 2pq).

Based on the initial concentrations of 50% male material and 50% female material, the expected genetic frequency of individuals in the first synthetic generation was as follows: 0.25 RR (matings between male parents), 0.50 Rr (hybrid individuals from male-female matings or from female-male matings), and rr (matings between female parents). Based on these values, a total of 75% resistant individuals were expected or anticipated to occur in the synthetic population assuming the *Hardy-Weinberg* law was operational.

Instead, an average of only 30.13 percent resistant plants was observed within the synthetic seed lots produced in these trials when averaged overall environments. Therefore, some assumption of the *Hardy-Weinberg* law was being violated.

An inter-genotypic competition trial was established at Carman in 1998 to determine the cause of the decrease in resistant plant proportion in the synthetic seed production plots (Table 11 and Figure 11). Initially, concentrations of resistant parental individuals were 46.3% at 17 days after seeding. This was initially close to the 50% parental frequencies which was initially expected. Twenty-four days after seeding, the proportion of resistant individuals dropped to 40%. This was significantly lower than the initial 46% value (Appendix Table 8). Thirty-four days after seeding, the proportion of resistant individuals in the plots dropped to 33.9%. Again this was significantly lower than the previous 40% observed at 24 days. Forty-one days after seeding, the proportion of resistant pollen donors droped to 23.1% again significantly lower than the previous sample date value of 33.9%. At fifty days, fifty-seven days, and sixty-six days after seeding, the proportion of resistant plants in the plots stabilized in the range of 16.5 to 18%. This is in contrast to the 50% resistant material that was expected to occur in these plots at all sample dates.

The proportion of resistant plants decreased dramatically over time. The herbicide susceptible female plant type Foothills (SW03375) had a more competitive plant type and growth habit than MBRR195, resulting in Foothills increasing in frequency over time and the herbicide resistant male parent (MBRR195) decreasing in frequency over time. The proportion of resistant plants present during the flowering period (16-34%), and the probable reduced seed set on the MBRR195

plants due to the effects of competition explains, in part, the reduced percent resistant plants obtained in the synthetic seed production seed lots produced in this study.

The possession of identical self-incompatibility alleles in both *B. rapa* populations used in this study could have limited the degree of successful matings between male and female in the synthetic parent population. An experiment designed to determine the SI allelic makeup of these two populations was unsuccessful.

Seed quality assessments consisted of oil percentages, protein percentages, erucic acid percentage, and glucosinolates measured at µmol/gram of seed at 8.5% moisture. The average oil percentage of synthetic seed production trial seed lots (Table 10) over all environments was 44.3 percent oil. Protein averaged 24.8 percent protein. There were no detectable levels of erucic acid in the seed samples. The average glucosinolate content of the synthetic seed production plots was 13.6 µmol/gram of seed at 8.5% moisture. Seed quality for the synthetic seed production trial seed lots was excellent.

Table 10. Days to emergence, vigor, days to flower, lodging, plant height, days to maturity, total seed yield, total synthetic seed yield, oil, protein erucic acid, glucosinolates, and percent resistant synthetic seed for the synthetic seed production trials grown at Carman, Winnipeg and Glenlea in 1997 and 1998

Location	Emergence (days)	Vigor (1-5)	Days to Flower	Lodging (1-5)	Height (cm)	Days to Maturity	Seed Yield (kg/ha)	Resistant Seed (kg/ha)	Oil (%)	Protein (%)	ER (%)	Glucs (umol)	Indoor % Resistant	Outdoor % Resistant
Carman 97	11	2.49	37	2	113	75	1400	257	40.5	26.8	0	7.5	18	17
S.E.	0	0.42	0	0.5	8	0	221	71	0.6	0.9	0	1.1	0.95	0.7
Winnipeg 97	10	2.88	38	2	121	71	1855	526	44.1	25.1	0	7.4	29	27
S.E.	0	0.34	0	0.35	5	0	233	84	1.3	1.2	0	1.4	2.79	1.4
Glenlea 97	8	2.70	37	3	104	70	976	329	41.1	27.8	0	13.4	34	31
S.E.	0	0.42	0	0.35	0	0	168	68	0.6	0.6	0	2.1	1.69	0.7
Carman 98	9	4.36	29	4	127	69	2053	642	48.4	21.3	0	13.6	32	
S.E.	0	0.37	0	0.43	9	0	261	79	0.6	0.8	0	1.1	1.85	
Winnipeg 98	9	3.27	33	4	107	80	688	240	49.2	20.0	0	17.0	34	
S.E.	0	0.69	0	0.28	8	0	218	85	0.7	0.5	0	1.4	0.83	
Glenlea 98	8	3.38	38	4	116	75	1536	518	42.4	28.0	0	22.8	35	
S.E.	0	0.47	0	0	5	0	261	79	0.6	0.5	0	2.0	0.53	
Combined Mean	**9.17**	**3.18**	**35.33**	**2.67**	**114.50**	**73.33**	**1417.91**	**418.67**	**44.25**	**24.84**	**0.00**	**13.62**	**30.13**	
S.E.	0.83	0.47	2.56	0.83	6.00	2.92	365.54	117.37	2.63	2.41	0.00	4.14	4.41	

Table 11. Average percent resistant plants at selected time intervals after emergence for synthetic seed production plots grown at Carman in 1998

Sample Time (Days after emergence)	Average % Resistant Plants
17	46.3 (a)
24	40.0 (b)
34 (beginning of flowering)	33.9 (c)
41	23.1 (d)
50	18.0 (e)
57 (end of flowering)	16.7 (e)
66	16.5 (e)
CV	3.04
LSD	2.0673
MSE	0.844
R^2	0.997
Alpha	0.05

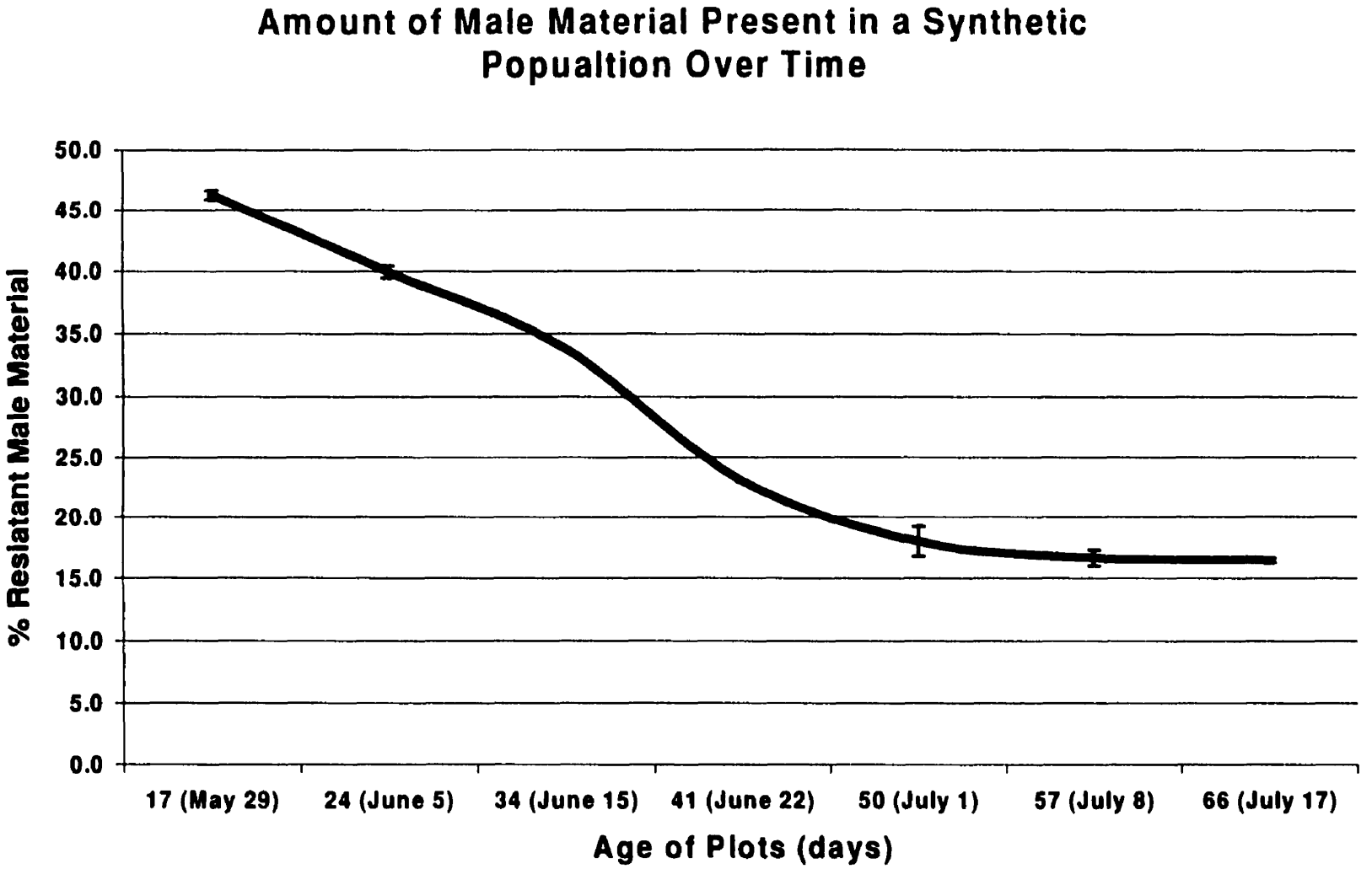


Figure 11. Percent of resistant *B. rapa* MBRR195 parental material over time within a synthetic population grown at Carman

4.3 Preliminary Yield Trials

Preliminary yield trials were planted at Carman and Glenlea in 1998. Unfortunately, heavy rainfall at Glenlea just after seeding produced very thin plant stands at emergence and the trial was therefore abandoned. The Carman trial grew well throughout the entire growing season, however, and valid treatment comparisons for all traits could be determined for this trial.

There were no significant differences in the days to emergence for the Carman preliminary yield trial. All plots in the preliminary yield trial emerged 9 days after seeding (Table 12).

There were significant differences among the treatments for vigor observed in this trial (Table 12). In general, there was not much evidence of growth vigor displayed by either the hybrids or synthetics compared to the female parent used in the seed lots, namely Foothills or SW03337. This population is very vigorous, especially under the ideal growing conditions present at Carman in 1998. Spraying of the hybrid and synthetic plots in the seedling stage to enrich the proportion of hybrid plants appeared to reduce the vigor since plant stands were reduced dramatically by the herbicide spray and plant stands is an important visual component of apparent vigor. This would explain the apparent decrease in vigor of the hybrid and synthetic plots as compared to the checks when they were expected to be more vigorous.

There were no significant differences in days to first flower (Table 12).

In general, there were no significant differences among treatments in the preliminary yield trial for lodging (Table 12). The single exception to this to this was the sprayed Glenlea hybrid treatment which was better for lodging than Mavrick.

There were some statistically significant differences in height among the entries. However, these were within a relatively narrow range of 106 cm to 128 cm and are of little, if any, biological significance (Table 12).

There were no significant differences in days to maturity. All plots began to mature 89 days after seeding (Table 12).

There were significant differences among treatments in the preliminary yield trial for yield (Table 12). The enriched (sprayed) hybrid treatments yielded the most followed by the enriched (sprayed) synthetics, followed by the unsprayed hybrids and followed lastly by the unsprayed synthetics (Table 12).

Enrichment of the proportion of hybrids in both the hybrid seed lots and the synthetic seed lots provided numerical increases in yield, although this was not a statistically significant increase when compared to the respective hybrid or synthetic seed lots without spraying. The differences in estimated high parent heterosis averages between enriched (sprayed) and non-enriched (unsprayed) treatments were dramatic for both hybrid and synthetic seed lots, even though these are not statistically significant.

The highest yield increase was displayed by the sprayed Carman hybrid seed lots where 23.8% yield advantage over the average of the commercial checks was obtained. This is an excellent increase in commercial heterosis and would be a tremendous benefit to Polish canola growers in Canada. This translated into +28.2 % high parent heterosis when compared to the parent SW03375. The average of the sprayed hybrids resulted in 20.8% yield increase over the average of the commercial checks, or an average of +25% high parent heterosis. This is similar to the findings of Hutcheson et al. (1981) where a naturally occurring hybrid yielded 146% more

than the cultivar Candle. The results obtained in this study were higher than results reported by Falk et al. (1994) who reported a +13% heterotic effect for yield increases in *B. rapa* crosses in Saskatchewan. In addition to this, Falk et al. (1998) reported a yield increase of +25% more than the average of the parents for yield.

Schuler et al. (1990) reported high parent heterosis of 12%, while Duhoon et al. (1979) demonstrated as high as 102% high parent heterosis for yield on a per plant basis for *B. rapa* hybrids. Labana et al (1978) also demonstrated an increase in yield associated with producing *B. rapa* hybrids with high parent heterosis values of 62.72% reported.

Unsprayed hybrid entries yielded an average of 11% yield increases over the average of the commercial varieties, or this translates into a high parent heterosis of +15 % (Table 12).

Sprayed (enriched) synthetic plots resulted in an average yield increase of 16.8 over the average of the commercial varieties. This further translates into a high parent heterosis of +21%. These results confirm those reported by Falk in 1998, where a yield increase of +23% was observed for synthetic populations when compared to the average of the parents (Table 12).

Unsprayed (non-enriched) synthetic plots resulted in an increased yield of 10% as compared to the average of the commercial plots. This can be viewed as a high parent heterosis of +13.9% (Table 12).

The sprayed (enriched) hybrid and synthetic treatments, as groups, displayed significant higher oil content than the mean of the check cultivars. The sprayed hybrid and synthetic treatments were significantly higher in oil content than their respective unsprayed treatments. Again the benefit of enriching the plots for both

hybrid and synthetic treatments is readily apparent. There is evidence of high parent heterosis for oil content for both the enriched hybrid and enriched synthetic treatments. An unusual result has been obtained in this study, simultaneous high parent heterosis for seed yield and oil content! There are no previous reports in the literature of this occurring. Confirmation of this result will be required.

Both sprayed hybrid and synthetic treatments resulted in an average of 46.1 and 46% oil respectively, that is a +2.0% and a +1.7% high parent heterosis (Table 13). Unsprayed plots resulted in an average of 44.7% and 45.1% oil respectively. Commercial and parental oil percentages averaged 44.9% oil. Hutcheson et al. (1981) observed a negative percent oil for hybrids when compared to the checks. This finding was also similar to Falk et al. (1993) where 7 out of 12 hybrids exhibited lower oil content than the better parent used in the cross.

Both sprayed hybrid and synthetic entries resulted in an average of 25.4 protein content. This amounted to a high parent heterosis of –7.5, and –7.4 respectively. Unsprayed plots resulted in an average protein percentage of 26.3 and 26.1 percent protein respectively. This amounted to a high parent heterosis of –4.3 and –4.7 respectively. Commercial and parental protein percentages averaged 26 % protein.

Enriched hybrids and enriched synthetic treatments therefore displayed lower but not statistically significant lower, protein content. Unsprayed hybrids and synthetic treatments are not significantly different from mean of commercial cultivars (Table 13).

No detectable levels of erucic acid were evident in any of the treatments (Table 13). The hybrid and synthetic seed treatments ,both sprayed and unsprayed,

displayed 0% erucic acid followed by the commercial cultivars and parental checks displaying 0% erucic acid. This meets the commercial standard for canola quality oil.

There were few significant differences in glucosinolate content among the treatments (Table 13). The hybrids and synthetics both enriched (sprayed) and non-enriched (unsprayed) treatments had low glucosinolate levels, lower than the mean of the check cultivars. Reward displayed considerably higher glucosinolate levels, raising the check mean substantially. All hybrid and synthetic treatments easily met the canola standard. In contrast to popular expectation, high yields and high glucosinolate levels do no have to go together in *B. rapa* hybrid and synthetics.

Falk et al. in 1998, reported increased yield, oil%, and protein% associated with the development of hybrid and synthetic varieties in *B. rapa* cultivars but alluded to the fact that a pollination control strategy must be in place in order to efficiently produce hybrid and synthetic material. The utilization of a dominant herbicide resistant gene in one parent in *B. rapa* hybrid and synthetic seed production plot trials is another way to produce seed lots which exploit high parent heterosis in *B. rapa*.

Table 12. Emergence, vigor, days to flower, lodging, height, days to maturity, total seed yields yield, relative yield and high parent heterosis for the preliminary yield trials of hybrid and synthetic seed lots from the 1997 field season grown in Carman in 1998

Entry	Spray	Emergence (days)	Vigor (1-5)	Days to Flower	Lodging (1-5)	Height (cm)	Days to Maturity	Yield (kg/ha)	Relative Yield (%)	High Parent Yield Heterosis (%)
F_1 Carman Hybrid	Yes	9 (a)	3.5 (bcd)	39 (a)	2.8 (ab)	114 (abc)	89 (a)	3561 (a)	123.8	28.2
F_1 Winnipeg Hybrid	Yes	9 (a)	3 (de)	39 (a)	2.8 (ab)	115 (abc)	89 (a)	3468 (ab)	120.6	24.9
F_1 Glenlea Hybrid	Yes	9 (a)	2.3 (e)	39 (a)	2.5 (b)	111 (bc)	89 (a)	3389 (abcd)	117.8	22.0
Average									**120.8**	**25.0**
F_1 Carman Hybrid	No	9 (a)	3.5 (bcd)	39 (a)	2.8 (ab)	116 (abc)	89 (a)	3346 (abcd)	116.3	20.5
F_1 Winnipeg Hybrid	No	9 (a)	4.5 (ab)	39 (a)	3.3 (ab)	115 (abc)	89 (a)	3082 (bcdef)	107.2	10.9
F_1 Glenlea Hybrid	No	9 (a)	4.3 (abc)	39 (a)	2.8 (ab)	121 (ab)	89 (a)	3153 (abcde)	109.6	13.5
Average									**111.0**	**15.0**
F_1 Carman Synthetic	Yes	9 (a)	3.3 (cd)	39 (a)	2.8 (ab)	114 (abc)	89 (a)	3285 (abcd)	114.2	18.3
F_1 Winnipeg Synthetic	Yes	9 (a)	3.3 (cd)	39 (a)	2.8 (ab)	106 (c)	89 (a)	3449 (abc)	119.9	24.2
F_1 Glenlea Synthetic	Yes	9 (a)	3.3 (cd)	39 (a)	2.8 (ab)	119 (abc)	89 (a)	3347 (abcd)	116.4	20.5
Average									**116.8**	**21.0**
F_1 Carman Synthetic	No	9 (a)	4.3 (abc)	39 (a)	3.3 (ab)	119 (abc)	89 (a)	3157 (bcde)	109.8	13.7
F_1 Winnipeg Synthetic	No	9 (a)	4.8 (a)	39 (a)	2.8 (ab)	115 (abc)	89 (a)	3023 (cdef)	105.1	8.8
F_1 Glenlea Synthetic	No	9 (a)	4.5 (ab)	39 (a)	3 (ab)	116 (abc)	89 (a)	3312 (abcd)	115.2	19.2
Average									**110.0**	**13.9**
Mavrick	No	9 (a)	4.5 (ab)	39 (a)	3.5 (a)	115 (abc)	89 (a)	2977 (cdef)		
Parkland	No	9 (a)	5 (a)	39 (a)	2.8 (ab)	124 (ab)	89 (a)	2981 (cdef)		
Reward	No	9 (a)	3 (de)	39 (a)	3 (ab)	120 (abc)	89 (a)	2962 (def)		
MBRR195	No	9 (a)	3.5 (bcd)	39 (a)	2.8 (ab)	111 (bc)	89 (a)	2690 (f)		
MBRR195	Yes	9 (a)	3.8 (bcd)	39 (a)	3 (ab)	116 (abc)	89 (a)	2673 (f)		
SW03375	No	9 (a)	4.3 (abc)	39 (a)	3 (ab)	128 (a)	89 (a)	2778 (ef)		
CV		0	15.87	0	18.7	7.092	0	8.94		
MSE		0	0.36	0	0.29	68.13	0	79.18		
R^2		0	0.7	0	0.355	0.369	0	0.57		
Alpha		0.05	0.05	0.05	0.05	0.05	0.05	0.05		

Note: Relative yield percent is the increase in yield compared to the average of the commercial varieties and parents

Table 13. Oil, oil high parent heterosis, protein, protein high parent heterosis, erucic acid and glucosinolates for the preliminary yield trials of hybrid and synthetic seed lots from the 1997 field season grown in Carman in 1998

Entry	Spray	Oil (%)	High Parent Heterosis (Oil %)	Protein (%)	High Parent Heterosis (Protein %)	ER (%)	Glucs (umol/g)
F_1 Carman Hybrid	Yes	46 (abc)	1.8	25.3 (cde)	-7.7	0 (a)	18.2 (cd)
F_1 Winnipeg Hybrid	Yes	45.9 (abcd)	1.5	25.6 (bcde)	-6.6	0 (a)	17.5 (cd)
F_1 Glenlea Hybrid	Yes	46.4 (a)	2.7	25.2 (de)	-8.2	0 (a)	17.0 (cd)
Average		**46.1**	**2.0**	**25.4**	**-7.5**		**17.6**
F_1 Carman Hybrid	No	44.5 (fgh)	-1.5	26.3 (bcd)	-4.0	0 (a)	17.3 (cd)
F_1 Winnipeg Hybrid	No	44.2 (gh)	-2.2	26.5 (ab)	-3.5	0 (a)	16.3 (cd)
F_1 Glenlea Hybrid	No	45.4 (bcdef)	0.4	25.9 (bcde)	-5.4	0 (a)	18.4 (cd)
Average		**44.7**	**-1.1**	**26.3**	**-4.3**		**17.3**
F_1 Carman Synthetic	Yes	46.3 (ab)	2.4	25.2 (de)	-8.0	0 (a)	17.6 (cd)
F_1 Winnipeg Synthetic	Yes	45.6 (abcde)	0.9	25.3 (cde)	-7.7	0 (a)	18.1 (cd)
F_1 Glenlea Synthetic	Yes	46.0 (abc)	1.8	25.7 (bcde)	-6.4	0 (a)	17.3 (cd)
Average		**46**	**1.7**	**25.4**	**-7.4**		**17.7**
F_1 Carman Synthetic	No	45 (cdefg)	-0.4	26.4 (abc)	-3.6	0 (a)	17.1 (cd)
F_1 Winnipeg Synthetic	No	44.9 (defg)	-0.7	26.0 (bcde)	-5.3	0 (a)	15.6 (cd)
F_1 Glenlea Synthetic	No	45.4 (bcdef)	0.4	26.0 (bcde)	-5.2	0 (a)	14.9 (cd)
Average		**45.1**	**-0.2**	**26.1**	**-4.7**		**15.9**
Mavrick	No	44.6 (efgh)		26.4 (abc)		0 (a)	27.1 (b)
Parkland	No	45.0 (defg)		25.7 (bcde)		0 (a)	26.2 (b)
Reward	No	45.6 (abcde)		25.1 (e)		0 (a)	37.8 (a)
MBRR195	No	45.3 (bcdef)		25.9 (bcde)		0 (a)	18.7 (c)
MBRR195	Yes	45.1 (cdefg)		25.7 (bcde)		0 (a)	16.6 (cd)
SW03375	No	43.8 (h)		27.4 (a)		0 (a)	14.3 (d)
Average		**44.9**		**26**			**23.45**
CV		1.35		2.56		0	8.81
MSE		0.375		0.43		0	2.86
R^2		0.68		0.59		0	0.95
Alpha		0.05		0.05		0.05	0.05

5.0 Conclusions

The purpose of this study was to develop bromoxynil resistant *B. rapa* hybrid and synthetic seed production protocols using two different self-incompatible *B. rapa* populations, one with bromoxynil resistance and one without. After this, the hybrid and synthetic seed produced in these trials was evaluated in a preliminary yield trial.

The findings in this study suggest that row ratio in the hybrid seed production trials affected degree hybridity. The 1:1 row ratio treatment produced the highest hybridity averaged over all environments.

The synthetic seed lots did not express as high a proportion of resistant material as expected. The low proportion of resistant material (both pure breeding male parental material and hybrid material) in the synthetic seed lots was primarily due to the decrease in the frequency of the resistant parental genotype during the growing season in the synthetic seed production plots.

The preliminary yield trial produced some very promising results. Commercial heterosis of over 20% for yield was observed for the enriched (sprayed) hybrids. This was combined with enhanced oil content, non-significant changes in protein contents and fully acceptable erucic and glucosinolate levels. Synthetics also displayed increases in yield combined with fully acceptable seed quality.

The production of herbicide resistant enriched (sprayed) hybrids or synthetics in *B. rapa* may be the next logical wave of cultivar types in this species.

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Appendix Table 1. Split plot analysis for vigor between the different hybrid planting ratios over all environments.

Source of Variation	DF	Sums of Squares	Mean Square	F Value	Pr>F
Replicate	1	0.02	0.02	0.04	0.856ns
Environment	5	34.07	6.81	15.22	0.005***
Error a (Env*Rep)	5	2.23	0.44	1.9	0.17ns
Main Plot Total	11	36.32			
Ratio	2	2.48	1.244	5.28	0.022*
Ratio*Env	10	6.75	0.67	2.86	0.04*
Error b	12	2.82	0.23		
Total	35	48.37			

Appendix Table 2. Split plot analysis for days to flower between the different hybrid planting ratios over all environments.

Source of Variation	DF	Sums of Squares	Mean Square	F Value	Pr>F
Replicate	1	0.06	0.06	0.23	0.65 ns
Environment	5	201	40.2	135.73	0.0001 ***
Error a (Env*Rep)	5	1.48	0.29	4.34	0.0173**
Main Plot Total	11	202.54			
Ratio	2	0.02	0.012	0.18	0.8358 ns
Ratio*Env	10	0.63	0.06	0.93	0.53 ns
Error b	12	0.819	0.06		
Total	35	204.009			

Appendix Table 3. Split plot analysis for lodging between the different hybrid planting ratios over all environments.

Source of Variation	DF	Sums of Squares	Mean Square	F Value	Pr>F
Replicate	1	0.02	0.02	0.11	0.76 ns
Environment	5	38.5	7.7	39.21	0.0005 ***
Error a (Env*Rep)	5	0.98	0.19	3.94	0.024**
Main Plot Total	11	39.5			
Ratio	2	0.208	0.104	2.08	0.1672 ns
Ratio*Env	10	0.59	0.059	1.19	0.38 ns
Error b	12	0.599	0.05		
Total	35	40.897			

Appendix Table 4. Split plot analysis for height between the different hybrid planting ratios over all environments.

Source of Variation	DF	Sums of Squares	Mean Square	F Value	Pr>F
Replicate	1	208.59	208.59	1.09	0.34 ns
Environment	5	6569.8	1319.36	6.92	0.02 *
Error a (Env*Rep)	5	953.51	190.71	2.75	0.07 ns
Main Plot Total	11	7731.9			
Ratio	2	111.03	55.51	0.8	0.47 ns
Ratio*Env	10	678.31	67.83	0.98	0.51 ns
Error b	12	831.79	69.31		
Total	35	9353.03			

Appendix Table 5. Split plot analysis for total hybrid seeds within hybrid ratios between the different hybrid planting ratios over all environments.

Source of Variation	DF	Sums of Squares	Mean Square	F Value	Pr>F
Replicate	1	1273.04	1273.04	0.06	0.821 ns
Environment	5	334762.09	6652.41	3.01	0.126 ns
Error a (Env*Rep)	5	111287.55	22257.51	3.97	0.0234 *
Main Plot Total	11	447322.68			
Ratio	2	145671.7	72835.8	13	0.001***
Ratio*Env	10	58909.74	5890.97	1.05	0.46ns
Error b	12	67232073	5602.72		
Total	35	67883977.12			

Appendix Table 6. Split plot analysis for total yield (kg/ha) between the different hybrid planting ratios over all environments.

Source of Variation	DF	Sums of Squares	Mean Square	F Value	Pr>F
Replicate	1	1944.58	1944.58	0.01	0.944 ns
Environment	5	9523064.72	1904612.95	5.44	0.0434 *
Error a (Env*Rep)	5	1752010.31	350402.06	15.06	0.0001***
Main Plot Total	11	11277019.61			
Ratio	2	166342.95	83171.48	3.57	0.06 ns
Ratio*Env	10	398816.34	39881.64	1.71	0.187 ns
Error b	12	279202.98	23266.91		
Total	35	12121381.88			

Appendix Table 7. Split plot analysis for Degree of Hybridity between the different hybrid planting ratios over all environments.

Source of Variation	DF	Sums of Squares	Mean Square	F Value	Pr>F
Replicate	1	1.24	1.242	0.17	0.69 ns
Environment	5	73.51	14.7	1.98	0.23 ns
Error a (Env*Rep)	5	37.188	7.437	0.82	0.56 ns
Main Plot Total	11	111.938			
Ratio	2	864.58	432.29	47.67	0.0001 ***
Ratio*Env	10	68.38	6.84	0.75	0.67 ns
Error b	12	108.83	9.069		
Total	35	1153.728			

Appendix Table 8. GLM analysis for 1998 Carman synthetic inter-genotype competition trial determining the different proportions of male material over the growing season.

Source	df	SS	MS	F Value	Pr>F
Model	7	1793.94	256.28	359.04 ***	0.0001***
Error	6	4.28	0.714		
Total	13	1798.22			
Replicate	1	0.47	0.47	0.65 ns	0.45 ns
Treatment	6	1793.48	298.91	418.77 ***	0.0001 ***