HYBRID RAPESEED SEED PRODUCTION IN THE FIELD USING THE *pol* CYTOPLASMIC MALE STERILITY SYSTEM

by

Russel Miles Pinnisch

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ABSTRACT

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Hybrid rapeseed production in the field using the pol cytoplasmic male sterility system.

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

Hybrid rapeseed (*Brassica napus* L.) seed production blocks were established at Glenlea and Portage la Prairie in 1986 and 1987, to examine the effect of distance from a pollen source on the seed yield, yield components and percent hybridity of Marnoo A-line rows. The row ratio employed was a 10:1 ratio of A-line to pollen parent rows. Leafcutter bees (*Megachile rotundata*) were used as the pollen vector to carry pollen from the pollen parent to the A-line flowers,

In 1986 no significant differences in seed yield were found to exist among A-line rows. Although seed yields were excellent on the A-line rows, only a small percentage of the seed produced was hybrid. The percentage of hybrid seed and seed yields were found to decline as distance from the pollen source increased. The high percentage of non hybrid

seed present in the seed lot was due to the heat sensitivity of the _pol_ cytoplasmic male sterility system, which results in a reversion to male fertility.

In 1987 significant differences existed among A-line rows with regards to seed yields. Seed yield and percent hybridity were found to decline as distance from the pollen source increased. Part of this decline in yield appeared to be due to a decrease in thousand seed weight as distance from the pollen source increased. The percentage of hybrid seed produced on the A-line rows was lower in 1987 than in 1986 indicating that a further dilution of the hybrid seed lot with non hybrid seed had taken place.

In conclusion, the 10:1 ratio of A-line to pollen parent rows used in this study gave satisfactory seed yields. However, the heat sensitivity of the _pol_ male sterile cytoplasm resulted in an unacceptably high proportion of non hybrid seed being present in the hybrid seed lot. Improvement in the male sterility of the seed parent is essential if hybrid seed production is to be commercially viable.

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1. INTRODUCTION

Rapeseed (Brassica napus L. and Brassica campestris L.) is a popular crop among western Canadian farmers and has become an integral part of many crop rotations. Rapeseed is second only to wheat as Canada's most valuable crop (Adolphe, 1980). In 1985, 2,783,000 hectares were seeded to rapeseed in western Canada, with a production of 3,422,000 tonnes.[1]

Historically, the development of hybrid cultivars in crops such as corn, sunflowers, sorghum and many horticultural species has led to significant increases in seed yields due to the phenomenon of heterosis or hybrid vigour (Duvick 1984, Furgala et al. 1979, Miller and Kebede, 1984). The success realized with these crops has generated interest in the development of hybrid cultivars in rapeseed. It is hoped that the development of hybrid rapeseed cultivars might lead not only to increased seed yields but also to improvements in quality components such as oil and protein contents.

The development of hybrid cultivars in any crop encompasses three aspects namely: (1) demonstration of heterosis for yield (or some other attribute) in the progeny

1. Canadian Grain Commission , Western Canadian Oilseeds , 1985. Ag. Can. Crop Bulletin No. 167.

of single or double crosses, (2) a form of pollination control to prevent self pollination of the female parent for crops with perfect flowers and (3) methods to produce adequate amounts of hybrid seed for commercial use.

Heterosis in rapeseed has been well documented. Sernyk and Stefansson (1983) found that certain rapeseed (B. napus) cultivars, in crosses with the cultivar Regent, produced progeny that exhibited 38 to 43 percent heterosis for seed yield over Regent. Grant and Beversdorf (1985) found that F_1 B. napus rapeseed hybrids expressed a positive heterosis for seed yield of up to 72 percent over the higher yielding parent in the cross. Shiga (1976) and Buson (1980) noted significant heterosis for seed yield in F_1 B. napus hybrids. Hutcheson et al. (1981) discovered a naturally occurring hybrid from a cross between B. campestris ssp. sarson Prain. cultivar R-500 and B. campestris L. var. oleifera Metzg. It was found that the hybrid yielded 146 percent of the B. campestris var. oleifera cultivar Candle.

Recently, research has been focused on incorporating cytoplasmic male sterility (CMS) into rapeseed, as a means of pollination control in commercial hybrid seed production. Even though other methods of pollination control exist, such as genetic male sterility (Jain 1959) and the use of gametocides (Chopra et al. 1960, Dotlacil and Apltauerova 1978), CMS appears to provide the greatest promise for use in any hybrid seed production program. According to Erickson (1984), CMS is the most economical and practical method available for large scale production of commercial hybrids.

Several male sterility inducing cytoplasms for B. napus have been described. Ogura (1968) found a male sterility inducing cytoplasm in radish (Raphanus sativus L.). Bannerot et al. (1977) transferred the nucleus of B. napus into this radish cytoplasm using intergeneric crossing followed by a series of backcrosses. Shiga and Baba (1973) and Thompson (1972) discovered a male sterility inducing cytoplasm which occurred naturally in B. napus. Shiga (1980) designated these cytoplasms from Raphanus sativus as ogu and from B. napus as nap. Fu (1981) discovered male sterile plants from the seed of the cultivar Polima (B. napus) and this male sterile cytoplasm was designated as pol by Fan and Stefansson (1986).

Shiga et al. (1983) studied the nap CMS types of European B. napus cultivars and their ability to restore male fertility in nap CMS lines. This restoration ability was attributed to the presence of restorer genes whose presence would be essential to restore the male fertility of the hybrid plants. Thompson (1972), Shiga (1976) and Shiga et al. (1983) found that a few cultivars could act as "maintainers" of the nap cytoplasm since they possessed a male fertile cytoplasm but had no male fertility restoration genes

Fan et al. (1986) examined F_1 progenies from crosses involving a number of B. napus strains and male sterile plants containing either the nap or pol cytoplasms. It was found that all the strains examined could act as "maintainers" for the pol cytoplasm. Further work involving F_2 and backcross data with the cultivars Karat and Westar, showed that a single dominant gene was responsible for the restoration of fertility in the case of the nap cytoplasm. The fertility in the pol cytoplasm was found to be restored in the progeny of crosses between pol *B. napus* and the *B. juncea* L. Czern cultivar ZEM.

Thus, all of the necessary components for pollination control for hybrid rapeseed production have been uncovered. The nap or pol CMS cytoplasm could be used to prevent self pollination of the female parent of the cross, and the restorer genes could be incorporated to restore the male fertility of the F_1 hybrid. The "maintainer" line could be used to increase the seed supply of the male sterile line.

The final aspect involved in the development of hybrid rapeseed lies in the production of sufficient seed stocks. Efficient large scale methods of producing the hybrid seed must be developed in order to minimize the costs of the hybrid seed to the farmer. An important aspect of these production methods includes the ratio of male sterile to male fertile rows planted in the field. Row ratios are important because they determine to a significant extent the quantity of hybrid seed that can be produced on a particular production field. If the number of male fertile rows planted is too large then hybrid seed yields per hectare will be reduced since only the seed produced on the male sterile rows is hybrid in nature. If the number of male fertile rows is too small, then pollen dispersal may be so poor over the range of male sterile rows planted that yields of hybrid seed per hectare are reduced.

The objective of this research was to determine the relationship of distance from a pollen source on the yield, yield components and percent hybridity of the seed produced on the male sterile plants. This information is necessary to ensure the efficient and economic production of the components required for hybrid seed production and of the hybrid seed itself.

2. LITERATURE REVIEW

2.1 Pollen Control And Pollen Vectors

2.1.1 Cross versus self pollination

Conventional _B_. _napus_ rapeseed cultivars under field conditions exhibit an average of 22 percent outcrossing (Rakow and Woods, 1987). For male sterile plants, however, it is hoped that outcrossing will approach 100 percent. Unfortunately, because of the heat sensitivity of the CMS systems in rapeseed, it would seem unrealistic to expect a complete absence of selfing. Fan and Stefansson (1986) observed partial restoration of male fertility in both the _nap_ and _pol_ CMS systems in _B_. _napus_ at day/night temperature regimes of 26/20 ^{o}C and 30/24 ^{o}C, respectively. Such temperatures are commonly experienced in western Canada and as a result some pollen production on the male sterile plants can be expected. The presence of this pollen creates the potential for self pollination. If sufficient self pollination occurs the hybrid seed harvested may be so contaminated with selfed seed that the resulting seed lot may be unmarketable. In the United States the Federal Seed Act Regulations stipulates that the term hybrid cannot be used if the seed lot contains less than 75 percent hybrid seed (Childers and Barnes 1972).

2.1.2 Anemophily versus entomophily

Cross pollination in flowering plants can be attributed to either anemophily (wind pollination) or entomophily (insect pollination). In some crops insect pollinators increasd seed yields. In sunflowers, it was found that with a number of cultivars, insect pollination had a significant effect on increasing yield (Freund and Furgala 1982). Bagged sunflower heads and plots produced a lower seed yield than did unbagged heads and plots. For some cultivars, the poor seed set under the bags was attributed to self incompatibility. Thus, sunflower cultivars with low self compatibility exhibit substantial yield increases in the presence of bees. Furgala et al. (1979) found that sunflower cultivars with a self compatibility of 16 percent [where self compatibility equals the yield of self pollinated (bagged) plants divided by yield of open pollinated plants (unbagged) times 100], yielded 1,019 lbs/acre (1143 kg/ha) in cages without insects, 2,850 lbs/acre (3195 kg/ha) in cages with insects, and 2,801 lbs/acre (3140 kg/ha) uncaged.

Krause and Wilson (1981) examined seed set on a number of bagged and unbagged hybrid sunflower heads. It was estimated that approximately 51 percent of the seeds in the bagged heads were unset while only 17 percent of the seeds in the unbagged heads were unset. It was concluded that the presence of an adequate number of insect pollinators would be essential to achieve maximum seed yields in sunflowers. In contrast to these findings, the introduction of honeybees into Australian sunflower fields did nothing to improve seed set.[2]

Free and Spencer-Booth (1963) studied the pollination of white mustard (Sinapsis alba) and brown mustard (B. juncea) by honeybees (Apis mellifera). It was found that the white mustard exhibited higher seed yields in the presence of honeybees but the seed yields of brown mustard were unaffected. In contrast, Ohsawa and Namai (1984) found that using insect pollinators could also improve seed yields in brown mustard.

The flower of B. napus is attractive to bees (Williams 1984), however, good seed yields can be obtained in the absence of insect pollinators (Free and Nuttall 1968). Despite being self fertile, reduced yields were observed on B. napus plants grown in the still air of the greenhouse (Williams 1978). Subsequent shaking of the plants resulted in more pods set and more seeds per pod than unshaken plants. This suggested that wind agitation of plants increased self-pollination. It was concluded that the addition of an insect pollen vector would have minimal effect on seed yields. Work by Williams (1984) indicated that wind pollination or self pollination can sometimes be sufficient to attain maximum seed yields in B. napus.

2. Queensland Dept. of Industries , 1976, Annual Report 1975-76. Australia. 91 pp.

The distance that *B. napus* pollen can be carried by wind has not been determined. Eisikowitch (1981) found that even under high, artificially produced wind velocities, the amount of pollen transport that occurred was relatively low. In the field, *B. napus* pollen has been found 32 m from a pollen source (Mesquida and Renard 1982). Eisikowitch (1981) found that pollen of *B. napus* was sticky and had typical entomophilic characters. It was proposed that insect vectors play a significant role in pollination by direct transfer of the pollen from the anther to stigma and by triggering an unknown pollen release mechanism which creates a cloud of pollen grains above the flower, making them subject to wind dispersal. It was recommended that bee hives be placed in the rapeseed fields to increase seed yields.

Although there is a difference of opinion among researchers as to the importance of insect pollinators in fields of male fertile plants of rapeseed, their inclusion in hybrid sunflower seed production fields appears to be essential. Frank and Farkas (1979) found that only 25 to 30 percent of the pollination in hybrid sunflower production fields may be due to wind borne pollen. Smith (1978) stated that a plot of male sterile sunflower plants in the USSR, situated 1.05 km from a pollen source expressed only 18.7 percent outcrossing.

For hybrid rapeseed production, the inclusion of an insect pollinator has been shown to be essential if maximum yields of hybrid seed are to be attained. Mesquida and Renard (1979) studied the entomophilous pollination of male sterile lines of winter rapeseed (B. napus). It was found that under low wind velocities (9 to 13 km/h) rapeseed pollen was dispersed over a short distance. The density of pollen grains dropped from 178 pollen grains/cm^2 at 1 m distance from the pollen source to 9 pollen grains/cm^2 at 7 m. In another study it was found that 75 percent of the pollen from the male fertile parent was carried less than 6 m (Mesquida and Renard 1982). Wind pollination accounted for 4 to 16 percent of the pod set and 1 to 4 percent of the yield at distances greater than 24 m from the pollen producer. At distances within 6 m, wind pollination contributed 23 to 29 percent of the pod set and 3 to 12 percent of the yield.

The effect of insect pollination on ogu CMS hybrid winter rapeseed (B. napus) yield was determined by including or excluding insect pollinators by means of cages (Mesquida and Renard 1979). In cages without bees, 14.1 percent of the flowers set pods with an average of 3.8 seeds/pod. In cages with bees, 84.8 percent of the flowers set pods with an average of 21.2 seed/pod. It was concluded that wind pollination, although not negligible, was not sufficient by itself to provide maximum seed yields. It was estimated that 70 percent of the yield could be attributed to insect pollinator activity.

2.1.3 Insect pollen vectors

The type of insect pollinator to be used in the hybrid seed production field is determined, to a significant extent, by the instinctive foraging behaviour of the insects (Erickson 1983). Thus certain types of insects will be efficient pollinators of some plant species but be less effective on others. The most frequently used insects in hybrid seed production fields are bees. Insects such as flies are often used as pollinators in cages for research studies and in breeding nurseries.

The most frequently used bee in commercial seed production is the honeybee (Apis mellifera). Honeybees, although less efficient pollinators than other bees, are often used because they can be easily propogated and can be moved from field to field with relative ease. The foraging area of honeybees is very large. Gary (1979) reported that some honeybees will forage as far as 14 km away from the hive. In most pollination situations, the production field represents less than 5 percent of the total potential foraging area of the colonies placed in the field. Thus most of the honeybee population will forage in other areas to satisfy the nutritional requirements of the colony. Such foraging behaviour may serve to bring foreign pollen into the hybrid production field resulting in contamination of the hybrid seed lot.

The leafcutter bee (Megachile rotundata Fabr.) is frequently used to pollinate alfalfa. The foraging range of the leafcutter bee is much smaller than that of the honeybee or bumble bee (Bombus tectorum L.). This is because the leafcutter bee is not a strong long distance flier and does not forage further than necessary to gather food (Richards 1984). Thus yields of alfalfa seed are highest in the rows nearest the shelter and decline as distance from the shelter increases. Bradner et al. (1965) studied the effects of bee species and isolation distance on varietal contamination in alfalfa. It was found that the leafcutter bee did not forage as far as the honeybee did, and thus isolation distance requirements were less for leafcutter bees than honeybees.

Another type of bee that has been used as a pollen vector in commercial seed production fields is the alkali bee (Nomia melanderi)(Erickson 1983). The alkali bee is a ground nesting species that can be maintained in permanent or artificial nest sites near the seed production fields. Permanent sites are only advantageous if the seed production fields are maintained within 1 mile from the nest from year to year. Another bee species, Osmia cornifrons, is utilized for pollination in apple orchards in Japan.

In hybrid seed production, it is important that the insect pollinator collect both pollen and nectar, and maintain a satisfactory level of constancy to the crop. Both of these aspects have been examined for honeybees.

Free and Nuttall (1968) found that all honeybees collected nectar and none pollen only. This is of particular importance in the pollination of male sterile flowers. Male sterile flowers produce little or no pollen, thus insects that primarily collect pollen will exhibit a low visitation frequency on the male sterile plants.

Zahavi et al. (1984) studied flower constancy in honeybees. It was found that honeybees tend to collect pollen only from one source and change only after that source has been exhausted. Pollen-collecting honeybees tend to avoid mixing their pollen loads regardless of the availability of different pollen types. Visual observations of honeybees collecting mixed pollen loads showed that the honeybees had difficulty packing mixed pollen grains. Examination by scanning electron microscope of pure and mixed pollen loads showed that pure loads fit together well while those of mixed loads had clefts and holes. Thus pollen gathering is less efficient if mixed loads are collected. Observations by Waters (1979) also showed that honeybees will not collect pollen from more than one plant species at a time. In this experiment, bees marked while collecting pollen on sweet corn (Zea mays L.) did not visit onion (Allium cepa L.), and bees marked on onions did not visit corn.

2.2 Floral Aspects Affecting Insect Pollination

2.2.1 Nectar production and insect visitation

The efficiency of insect pollination depends to a significant extent on the floral characteristics exhibited by a particular species. Factors such as nectar production, floral morphology, and aromaticity may serve as a positive or negative stimulus to insects to avoid or to forage a particular flower. In the development of hybrid seed parents, floral nectar production and aroma chemistry are frequently altered through plant breeding (Erickson,1984).

Vansell (1934) studied the relation between the nectar concentration in fruit blossoms and honeybee visitation. It was found that the chief factor involved in determining which species or cultivar the honeybee will prefer to forage, is the concentration of the nectar sugar. As well, it was noted that foraging behaviour of the honeybees changed in response to slight differences in nectar sugar concentration.

In cotton (*Gossypium* spp.) it has been shown that floral visitation by honeybees was related to nectar secretion (Moffet *et al*. 1976). Consistent differences were found to exist among different cotton cultivars with respect to floral nectar production. It was found that phenotypes with a high sugar concentration in the floral nectar, usually attracted the most honeybees to their flowers.

Southwick and Schreffler (1983) found that honeybees preferred plants that offered high nectar rewards.

Nectar attractiveness to insects depends not only on the absolute amount the plant produces but also on its sugar composition. Wykes (1952a) investigated the sugars present in the floral nectar of various species. Differences were noted among species with regards to their contents of sucrose, glucose, and fructose. Sihag and Kapil (1984) studied the effect of quality and quantity of nectar on foraging stategies of honeybees. For some plant species, sucrose was the dominant sugar in the nectar while glucose and fructose were more prevalent in others. Different species of *Apis* responded differently to these plant species. *Apis dorsata* preferred sucrose dominated sugars, whereas *Apis florata* were attracted to nectars in which glucose and fructose were dominant. Bachman and Waller (1977) found that honeybees (*Apis mellifera*) preferred sucrose to either glucose or fructose. A mixture of equal parts of sucrose, glucose, and fructose was less attractive than sucrose dominant mixtures. Work by Wykes (1952b) also showed that honeybees preferred sucrose over glucose or fructose solutions.

The rapeseed flower is very attractive to nectar and pollen gathering bees (Free and Nuttall 1968). Szabo (1982) found that a number of cultivars and breeders lines of *B. napus* and *B. campestris* secreted varied quantities of nectar. Sugar concentration was found to vary between 30 and 40 percent (Szabo 1985). Kamler (1984) studied nectar secretion in 21 cultivars of rapeseed (B. napus) and found no difference in the amount of nectar produced between old cultivars and new ones. Four cultivars with high nectar secretion and four cultivars with low nectar secretion were self-fertilized for four generations. The difference between the high and low nectar producing inbreds was 40 percent of the average nectar yield of the parents. It was concluded that breeding cultivars producing large quantities of nectar with a high sugar content would be possible.

2.2.2 Pollen and insect visitation

Honeybees forage pollen to satisfy their requirement for protein, fats, vitamins and minerals. Campana and Moeller (1977) studied the honeybees preference for and nutritive value of pollen from a number of different plant species. It was found that a bee's preference for a certain pollen was of greater importance than the pollen's nutritive value in building colony populations, provided that the diet was not lacking any essential nutrients. A similar study by Boch (1982) examined pollen attractiveness to honeybees of a number of different plant species all producing yellow colored pollen. Although yellow colored pollen is known to be attractive to honeybees, the color pigments did not seem to be associated with the substances that attracted the bees.

The preference of honeybees for the pollen of one plant species over another has been well documented (Campana and Moeller 1977, Boch 1982). In hybrid sunflower seed production fields, it was noted that when honeybees foraged on the male fertile rows, the honeybees carefully removed any sunflower pollen grains that adhered to their bodies (Tepedino and Parker 1982). It was proposed that their pollen requirements were being met elsewhere.

In hybrid cotton seed production fields, it was found that the honeybees would not forage for pollen on the male fertile lines if other more attractive plants were blooming nearby (Eisikowitch and Loper 1984). Honeybees confined in cages in the cotton plots would collect cotton pollen. Loper and Davis (1985) noted that honeybees foraging for nectar would spend as much as 15 to 20 min combing the cotton pollen off their bodies. It was concluded that such behaviour would reduce yields of hybrid cotton seed on the male sterile lines because of this reduction in pollen dispersal.

Waller et al. (1985) studied the amounts of pollen collected by a honeybee colony located in a hybrid cotton production field. It was found that the largest source of pollen was smartweed (Polygonum spp.). Large amounts of sorghum, sunflower, and pearl millet pollen were also found. Later in the growing season ragweed (Ambrosia spp.) became the dominant pollen source. Only four cotton pollen grains were noted among the more than 10,000 pollen grains examined. In the hybrid production field it was found that the bees exhibited an increasing preference for male sterile flowers over male fertile flowers. It was concluded that this avoidance of the male fertile flowers was due to the pollen. It is not known whether this avoidance is due to the large spiny nature of cotton pollen or because of some objectionable chemical(s).

Rapeseed produces large amounts of attractive pollen (Szabo 1985, Boch 1982). Mesquida and Renard (1984) found that honeybees readily forage male fertile B. napus flowers. Thus the problems of avoidance of male fertile cotton plants by honeybees do not exist in hybrid rapeseed seed production fields.

2.2.3 Flower colour, floral morphology and pollination efficiency

Wells et al. (1981) examined flower colour preference in honeybees. It was found that flower colour, either yellow or blue in this case, did not affect bee visitation patterns. Even though the bees did not show a uniform colour preference, it was observed that some bees visited blue flowers exclusively while others visited only yellow. Thus bee performance was not random with respect to colour.

Work by Marden and Waddington (1981) showed that honeybees when presented with equally rewarding yellow or blue artificial flowers exhibited no preference for one colour over the other. It was found that honeybees always foraged the closest flower regardless of its colour. Real (1981) examined the influence of variability in nectar reward per flower on the foraging behaviour of bumblebees (Bombus sandersoni Fkln.). In one experiment yellow and blue artificial flowers contained equal amounts of nectar. Some preference for yellow flowers was noted. In a second experiment, the amount of nectar was kept constant on blue flowers (2 ul in each) and the amount on the yellow flowers was varied from 0 to 6 ul. It was found that bees avoided the variable yellow flowers and preferentially foraged the blue flowers. When the nectar in the blue flowers was made variable and that in the yellow flowers kept constant, the bees showed a strong avoidance for the blue flowers and preferentially foraged the yellow. On the basis of these studies it was hypothesized that choice is based on reward alone, with floral colour used as a cue by the bees to direct them to the desired reward (Waser 1983).

Pollination efficiency also depends on flower structure. In "Delicious" apple (Malus domestica Borkh.) poor fruit set has been associated with blossom morphology (Degrandi-Hoffman et al. 1985). Basal gaps in the androecium allow honeybees to land on the petals and extend their probosces between the stamens without touching the anthers or stigma (Robinson and Fell 1981). This process is known as "sideworking". It has been found that with most apple cultivars, these basal gaps do not exist. This forces "topworking" of the flower by the honeybee. In this case the bee stands with all legs on the stamens and the proboscis is extended down into the nectary, usually resulting in contact with the anthers and the stigma.

Degrandi-Hoffman et al. (1985) examined the effect of "sideworking" behaviour on fruit set in "Delicious" apple. It was found that cross-pollination and fruit set were not significantly limited because of "sideworking".

Free and Ferguson (1983) examined foraging behaviour of honeybees on the winter rapeseed cultivar Primor (B. napus). It was found that approximately 25 percent of the bees "sideworked" the flowers. It was proposed that the effect of flower structure on bee behaviour and pollination efficiency be taken into consideration by plant breeders.

"Sideworking" of male sterile plants in a hybrid seed production field may significantly reduce hybrid seed yields. Ohkawa (1983) reported that the nap male sterile cytoplasm in B. campestris caused plants to produce flowers with small anthers, short filaments and narrow petals. Such flower structure may serve to enhance "sideworking" by bees on some male sterile lines. B. napus cultivars in the nap or pol cytoplasm have similar shaped flowers (Fan and Stefansson 1986).

2.3 Parental Ratios Used In Field Production Of Hybrid Seed

2.3.1 Hybrid sunflower seed production

The hybrid seed of sunflowers is produced on the male sterile plants in alternating groups of rows with the male fertile parent (Smith 1978). Ratios of 2:1 to 7:1 of male sterile to male fertile rows have provided adequate pollination under different environments. This ratio is affected by the pollination requirements of the various male sterile lines, pollen production of the male fertile rows, size of the planter unit and width of the header on the harvester.

Seetharam and Satyanarayana (1983) studied the effect of parental planting ratios and hybrid seed yield per ha and seed set on male sterile sunflowers. It was found that hybrid seed yield per ha increased as the male sterile to male fertile ratio was increased from 1:1 to 4:1 and decreased slightly at 5:1. Seed set and seed yield on the male sterile plants decreased as the row ratio was increased from 1:1 to 5:1.

Robinson (1984) studied the effect of distance from a pollen source on yields of male sterile rows in sunflower. Seed production fields consisted of 25 to 40 male sterile rows on either side of four male fertile rows. Individual male sterile rows were harvested separately and correlations and regression analyses showed that yield per row declined linearly with distance from the pollinator rows. It was found that seed yield per ha on the male sterile rows was greatest when a 3:1 row ratio of male sterile to male fertile was employed.

Drane et al. (1982) found a significant correlation existed between mean bee activity over the male sterile rows and the yield of hybrid seed from the male sterile parent in hybrid sunflower seed production fields. It was also found that satisfactory bee activity and seed yield occurred on the male sterile rows with row ratios of 5:1 of male sterile to male fertile.

Satyanarayana and Seetharam (1983) studied the effect of parental ratios on sunflower seed quality. It was found that the planting ratios employed did not affect the quality of the hybrid seed, percent germination, hypocotyl length, root length, 1000 seed weight, fresh weight, or dry weight.

2.3.2 Hybrid onion, cotton and rapeseed production

The effect of planting ratio on hybrid seed yields in onion were studied by Woyke and Dudek (1984). It was found that honeybee visitation did not differ among the different planting ratios. It was proposed that given good weather for bee activity, ratios of 6 male sterile rows to 1 male fertile row could be used. Waters (1979) stated that the usual practice in hybrid onion seed production is to plant 6 to 12 male sterile rows with 2 rows of male fertile onions.

In hybrid cotton seed production fields a ratio of 6 male sterile to 2 male fertile rows is generally used (Moffet 1983). It has been found that the third male sterile row from the pollen source can yield 95 percent of the male sterile row adjacent to the pollen source.

Renard and Mesquida (1979) studied the effect of planting row ratios on seed yields of hybrid ogu CMS B. napus rapeseed in France. It was recommended that a 14:2 ratio of male sterile to male fertile rows, on a 0.35 m row spacing be utilized for field production of hybrid B. napus rapeseed. It was found that the frequency of bee visitation decreased slightly as distance from the pollen source increased. A significant correlation between bee activity and pod setting was found (r=0.70* to 0.97***)

3. MATERIALS AND METHODS

Rapeseed (*B. napus*) hybrid seed production blocks were established in 1986 and 1987 at the Agriculture Canada Research Station at Glenlea, and at the University of Manitoba's Plant Science Research Station at Portage la Prairie. In both years a pure breeding *pol* CMS system male sterile line (A-line) of the low erucic acid, low glucosinolate rapeseed cultivar Marnoo was used as the female parent. In 1986 a high erucic acid producing line (S82-4362) was used as the pollen parent. In 1987 the pollen parent used was a pure breeding *pol* CMS restorer line of the canola rapeseed cultivar Regent containing a single Mendelian dominant *pol* CMS restorer gene.

3.1 Experimental Design And Procedures

At each location the hybrid seed production field consisted of 2 blocks and 4 replicates. Each block consisted of 2 replicates. Thirty rows of Marnoo A-line were planted on either side of two pairs of three rows each of either pollen parent S82-4362 (1986) or Regent *pol* R-line (1987) (Figure 1). All rows were 12 m in length. A leafcutter bee (*Megachile rotundata*) shelter was placed in

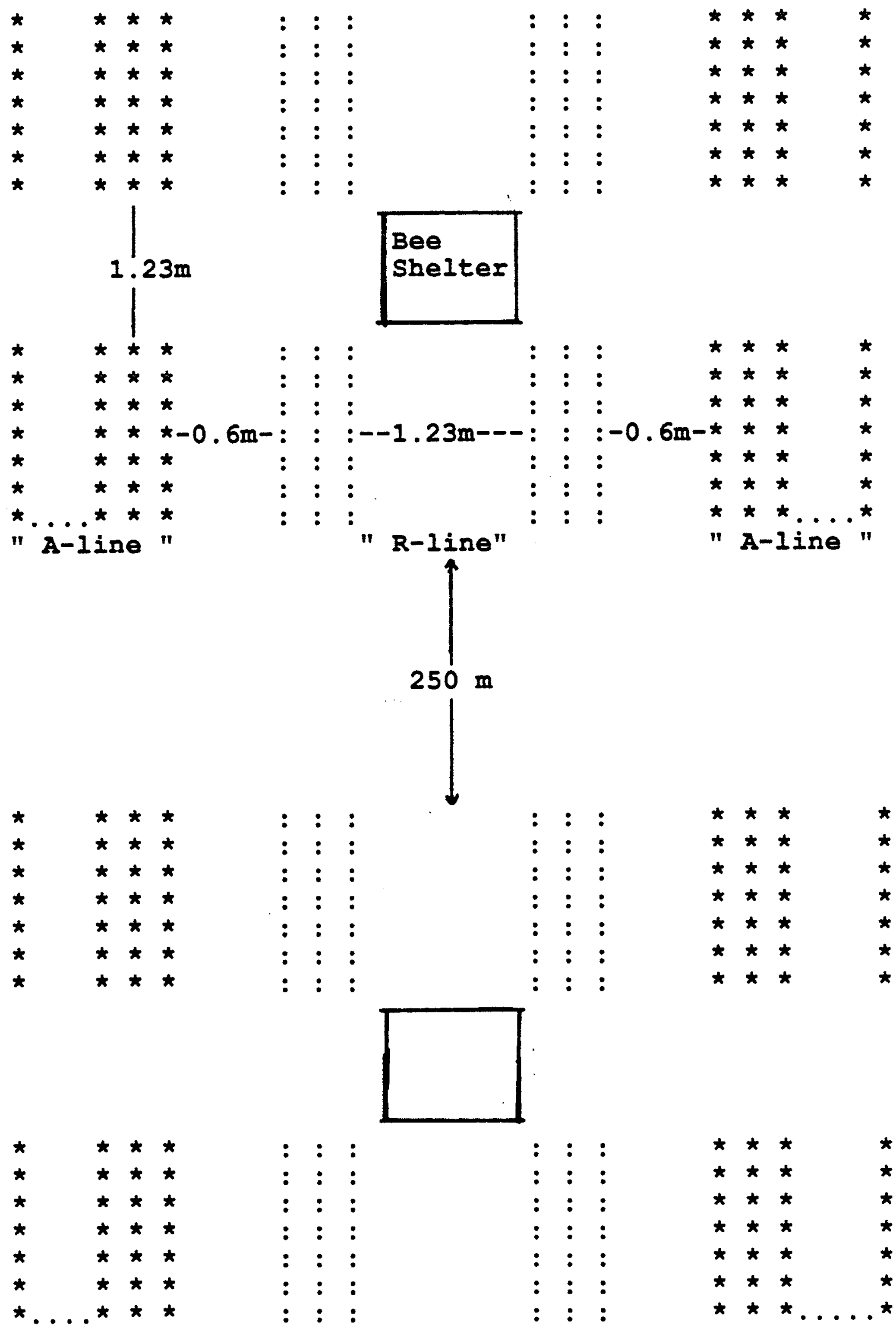


Figure 1. Planting design used at Glenlea and Portage la Prairie in 1986 and 1987.

the middle of the blocks, in the gap between the two pairs of three rows of the pollen parent. An observation pathway ran down the middle of each block. Blocks were separated by a minimum distance of 250 m.

A double disc belt-cone seeder was used for seeding. Carbofuran (10 percent) granules were banded with the seed at a rate of 1.0 kg a.i./ha to control flea beetles (*Phyllotreta cruciferae* Goeze and *P. striolata* F.) and cabbage root maggots (*Delia* spp.). Fertilizer (16-20-0) was incorporated at the rate of 112 kg/ha at seeding.

The Glenlea site was seeded on May 22, 1986 and on May 25, 1987, while the Portage la Prairie site was seeded on May 28 in 1986 and on May 8, 1987. Leafcutter bees were moved into the blocks when both the A-line and pollen parent lines had reached first flower, and were removed when the restorer line had almost completed flowering. Bee populations were maintained through alternate day supplementation of the initial population at 1000 to 1200 bees over each block, which approximates the recommended population of 20,000 bees per 0.4 ha on alfalfa (Richards 1984).

3.2 Seed Yield

When the majority of the seeds in the lower pods on the main raceme were black in colour, the block was considered as having matured. Each A-line row was harvested separately by hand. The above ground plant material was placed in burlap sacks and air dried several weeks before threshing. The contents of each sack were emptied into a stationary thresher and the seed obtained was cleaned and weighed. Thousand seed weights were determined by weighing 1000 seeds from each row.

3.3 Yield Components

Yield components were determined for each A-line row from a random sample of 5 plants. For each plant, the number of pods, the number of aborted flowers, and seeds per pod were determined. Pedicels lacking a pod, pedicel scars and unopened buds were all counted as aborted flowers. Pod setting was calculated by dividing the number of pods by the number of flowers produced, the latter being the sum of the number of pods plus the number of aborted flowers. Seeds per pod was determined by taking the weight of the seed harvested from each of the 5 plants sampled, dividing it by the 1000 seed weight for the row from which the plants were taken and dividing the number obtained by the number of pods on that plant.

3.4 Percent Hybridity

In 1986, the presence of erucic acid in the seed harvested from the A-line rows served as the genetic marker to detect hybridity. Erucic acid was detected via a paper chromatographic technique described by Thies (1971). A 50 seed sample from each row was analyzed. Individual seeds were placed in the cells of a microtiter tray and crushed. Erucic acid was extracted by a solution of potassium hydroxide (KOH), methanol, water, and 2-propanol. The extracted erucic acid from each seed was spotted on chromatography paper coated with a solution of paraffin oil and ether. Ten spots per sheet were made, with 9 spots corresponding to 9 seeds from the male sterile row, and the 10th being a control (a seed from the high erucic acid producing parent). Chromatography sheets were then placed in a 95 percent acetic acid solution and later in a copper salt solution (cupric acetate, sodium acetate, water). For colour development of the spots, the sheets were placed in a 0.03 percent of dithio-oxamide solution in ethanol for 30 seconds. Seed was classified as being hybrid or selfed on the basis of the presence or absence of the erucic acid spot respectively, which occurred near the bottom of the solvent front.

In 1987, the percent hybridity was determined via a greenhouse growout of a 50 seed sample from each A-line row.

Seeds were planted into 2" peat pots containing Metro-mix growing media. At flowering the number of plants bearing sterile and fertile flowers were determined, with 50 plants in total being counted. Plants bearing fertile flowers were considered hybrid (i.e. containing the dominant pol CMS restorer gene) while those plants bearing sterile flowers were believed to have arisen from non hybrid seed.

3.5 Miscellaneous Measurements

The amount of nectar produced by both the pollen parents and the A-line parent was determined in both 1986 and 1987. Hand crosses were made in 1987 between the Regent restorer and Marnoo A-line, to determine the efficiency with which the restorer line could restore male fertility.

Individual plants in 7 rows were bagged with mesh bags in both 1986 and 1987. Mesh bags were used to see how much hybrid seed could be produced solely on the basis of wind pollination.

3.6 Statistical Analyses

Data for this experiment were analyzed using the analysis of variance techniques. The outer A-line rows and the plants at the end of each row were excluded from the analysis to prevent edge effects and to maintain the within row plant competition. Least significant differences were used to detect differences among means. Correlation and regression analyses were also performed. All statistical analyses were performed on the University of Manitoba's AMDAHL 5858 mainframe computer using SAS statistical programs (Joyner 1985).

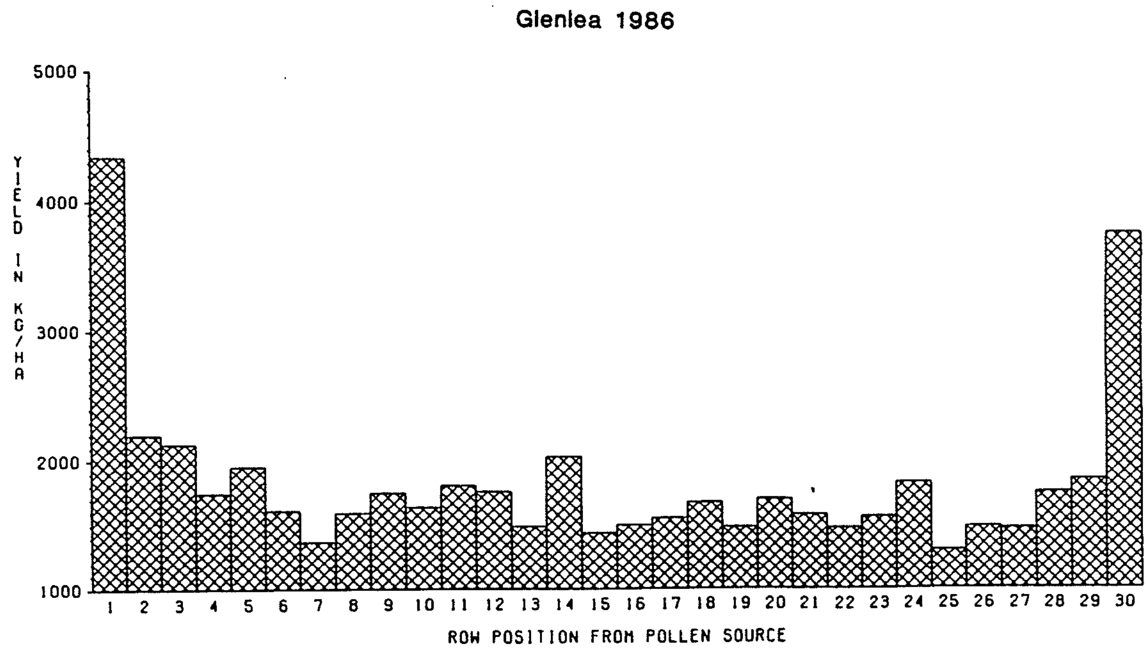
4. RESULTS AND DISCUSSION

4.1 Elimination Of Edge Effects

Because of the size and design of the hybrid seed production block, certain portions of the block produce unusable results because of edge effects. The seed yields of rows 1 and 30 (the first and last rows in the hybrid seed production block) were in some cases double that of the adjacent rows (Figures 2 and 3). The reason for this discrepancy is that rows 1 and 30, being on the outer edge, had a greater resevoir of water and nutrients on which to draw from. As well, it was noted that wild pollinating insects approached the plot from the outside and worked in, while the leafcutter bees tended to work from the inside of the plots to the outer area. Because of the small size of the plots, leafcutter bees also were able to fly around the periphery of the plots.

No edge effects were evident for pod setting (Figures 4 and 5.), seeds per pod (Figures 6 and 7.), thousand seed weight (Figure 8 and 9.), or number of pods per plant (Figures 10 and 11.). Some edge effects were seen for the percentage of hybrid seed produced (Figures 12 and 13).

To eliminate possible edge effects, rows 1 and 30 have been excluded from the statistical analyses of all parameters. As well, at harvest, the outer 60 cm at each



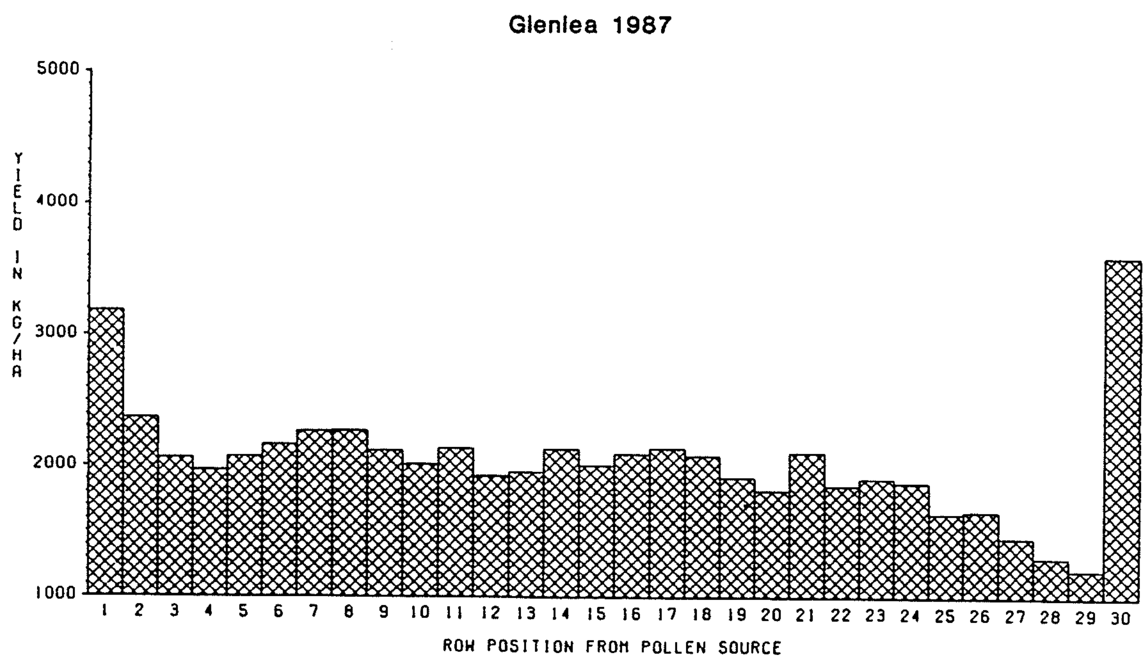
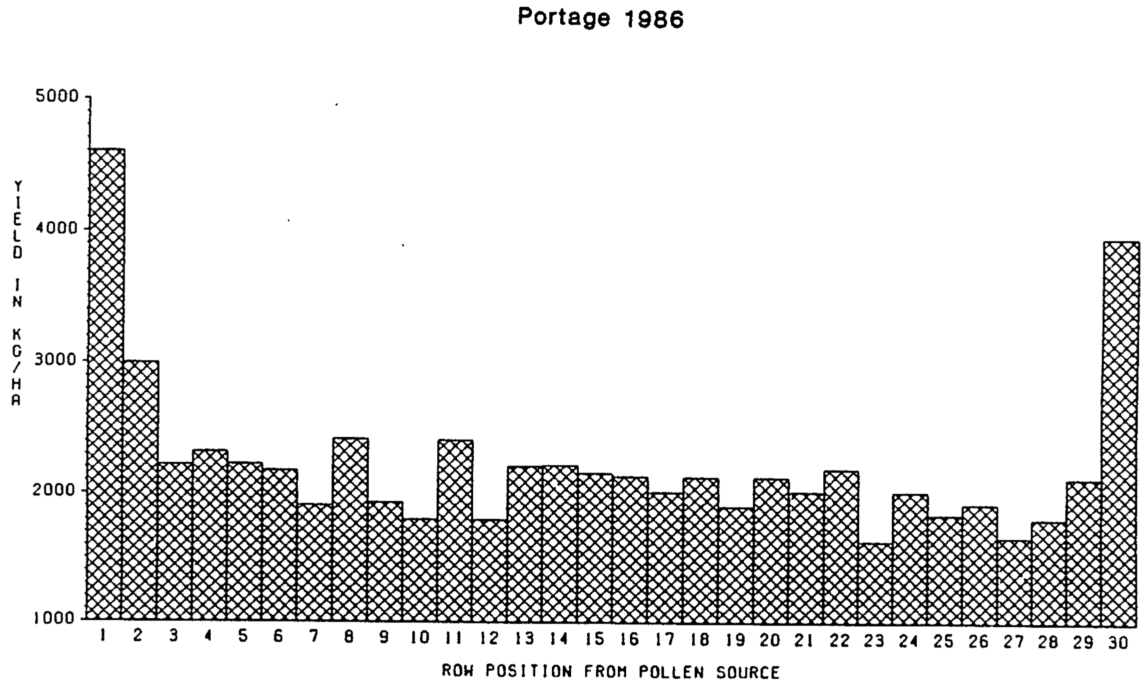


Figure 2. Seed yields for Marnoo A-line rows grown in hybrid seed production blocks at Glenlea in 1986 and 1987.



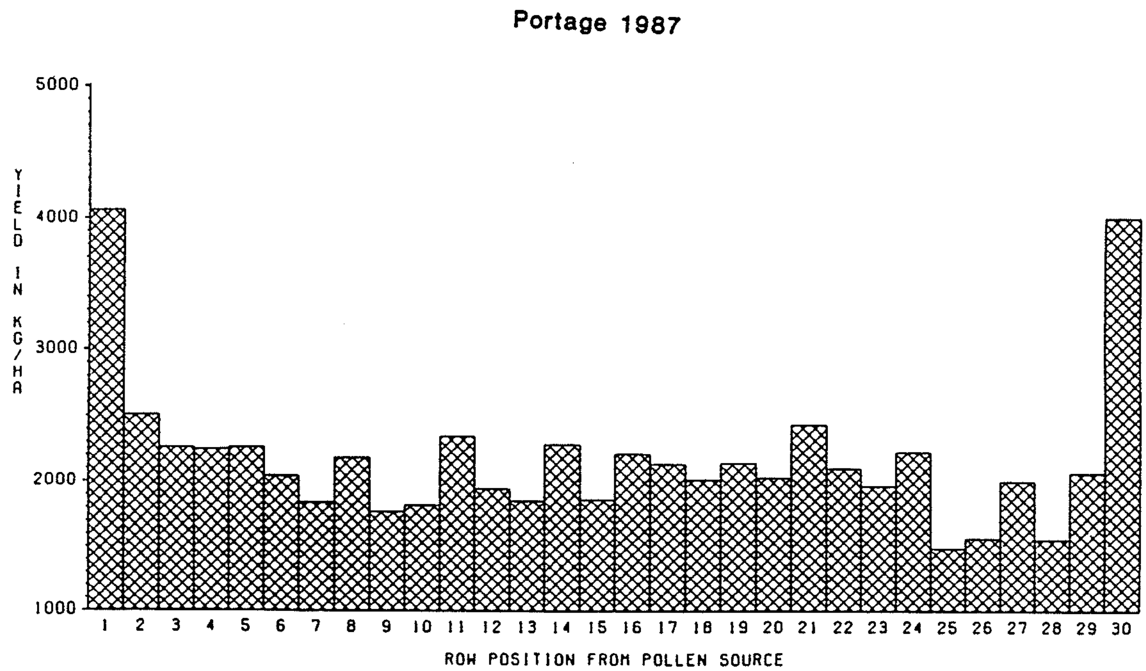


Figure 3. Seed yields for Marnoo A-line rows grown in hybrid seed production blocks at Portage la Prairie in 1986 and 1987.

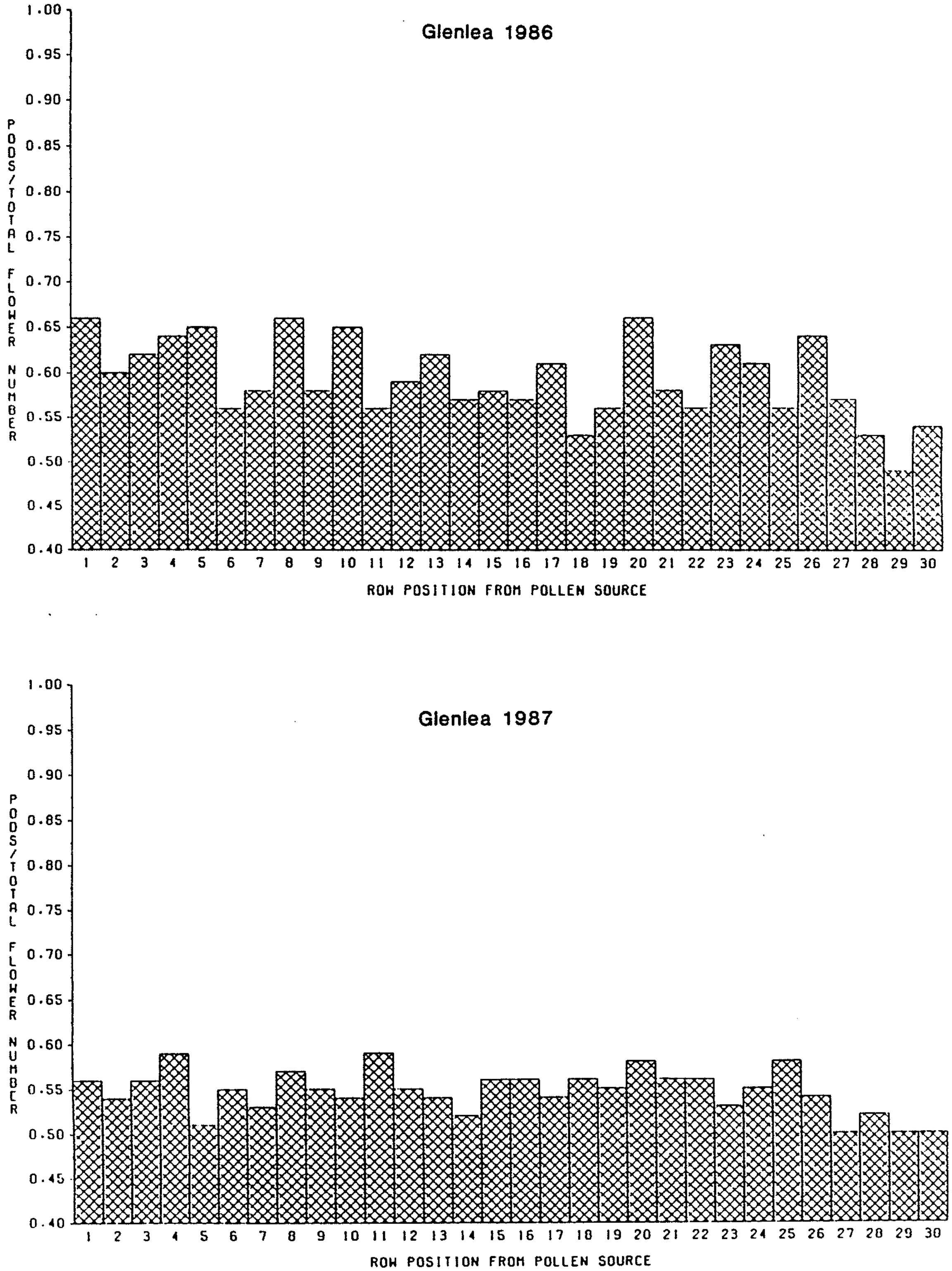
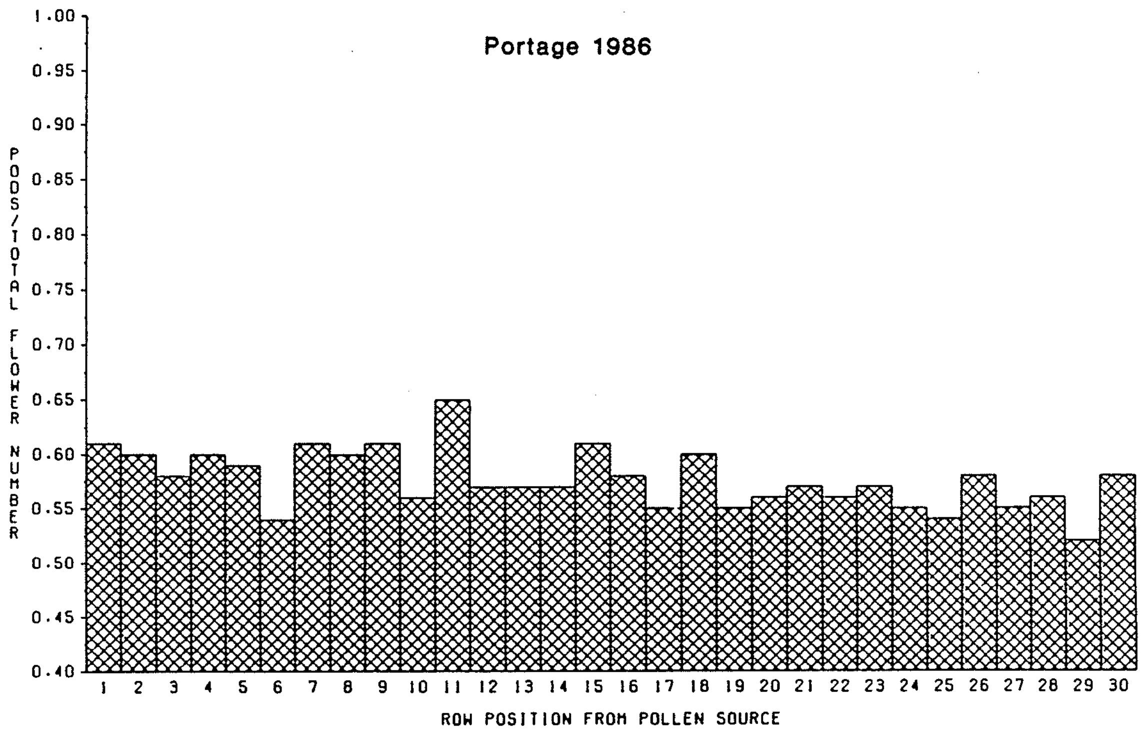


Figure 4. Pod setting on Marnoo A-line rows grown in hybrid seed production blocks at Glenlea in 1986 and 1987.



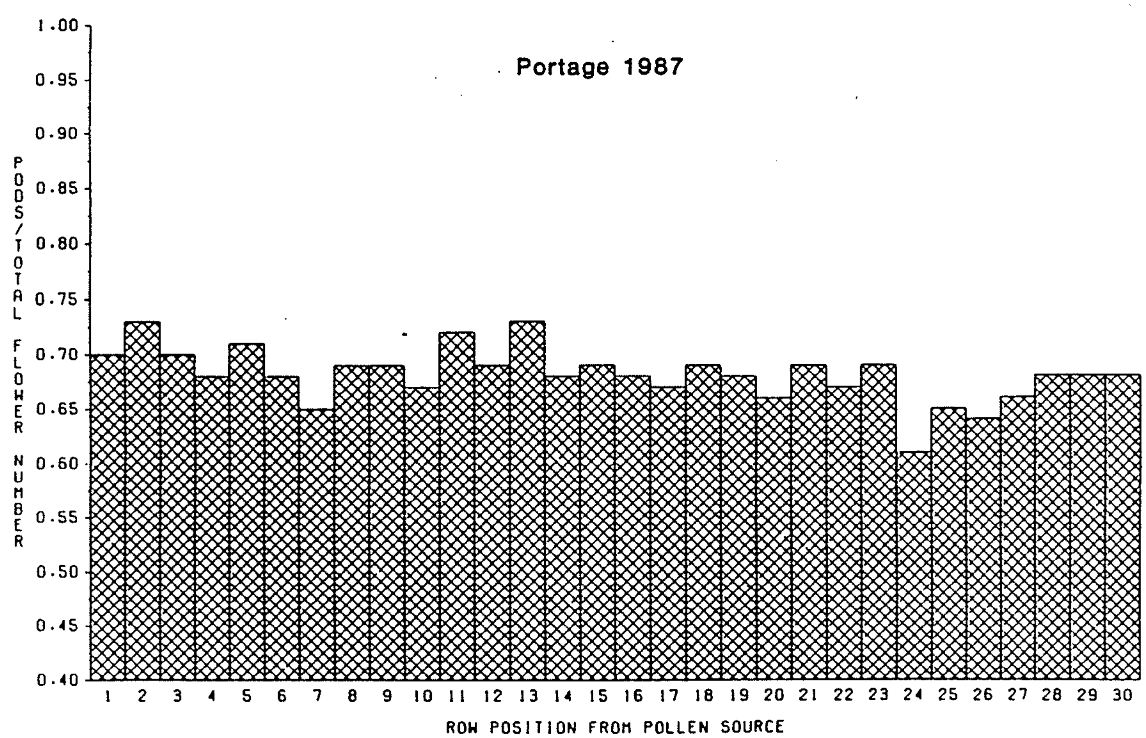
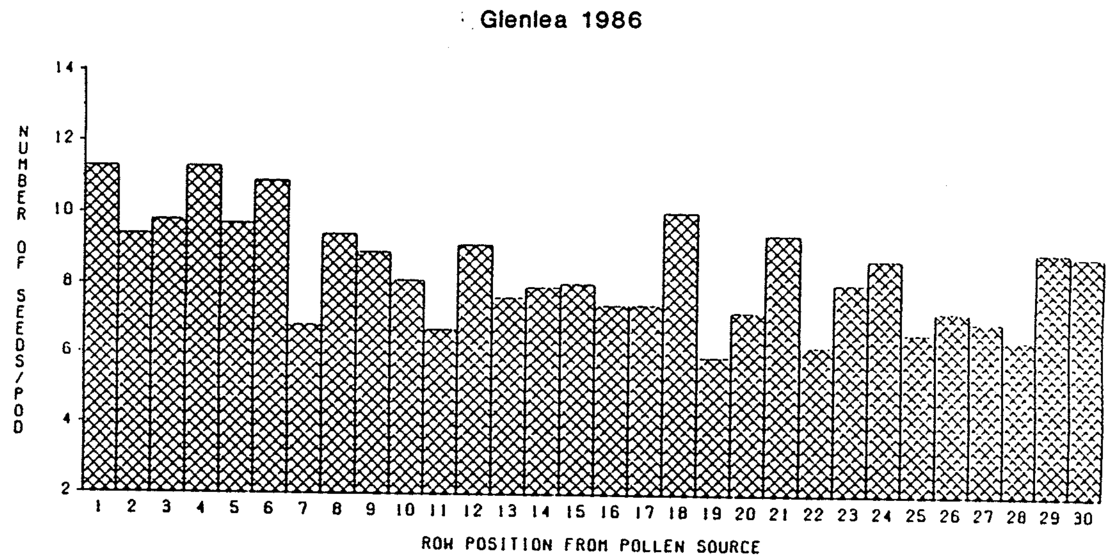


Figure 5. Pod setting on Marnoo A-line rows grown in hybrid seed production blocks at Portage la Prairie in 1986 and 1987.



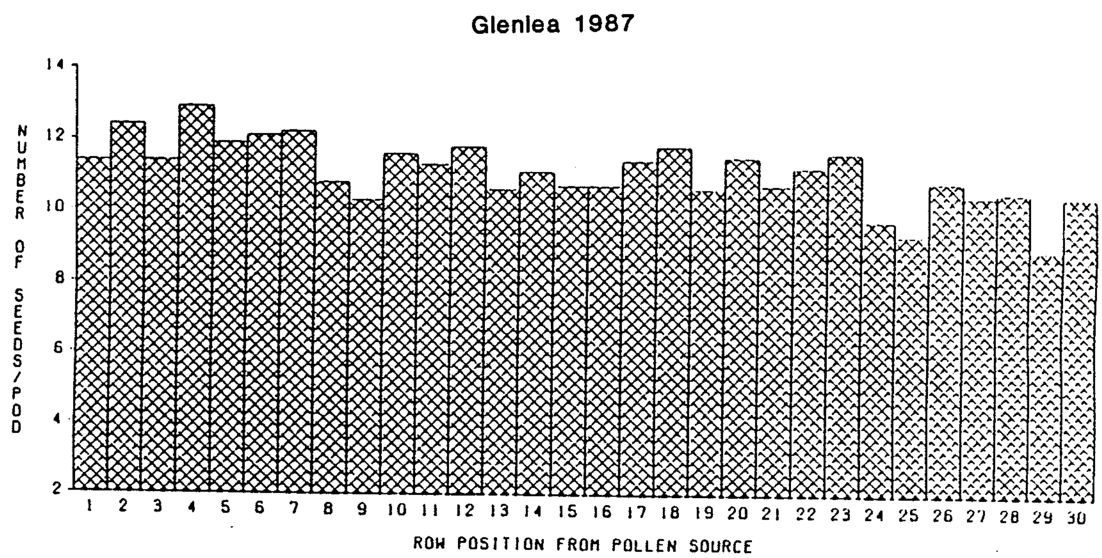
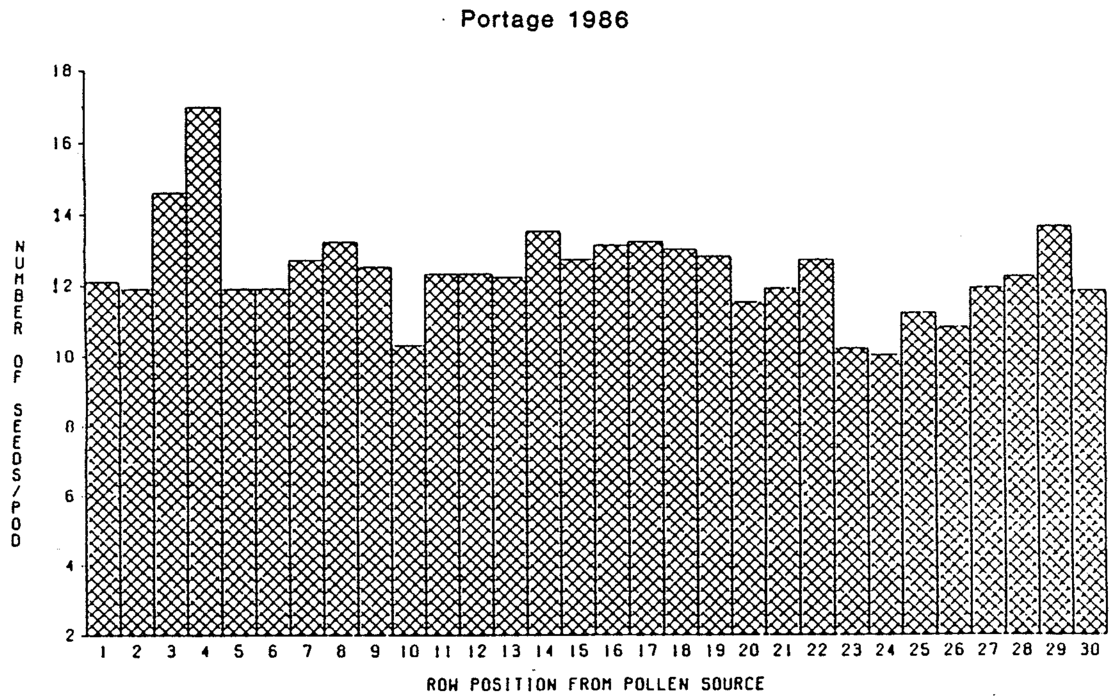


Figure 6. Number of seeds per pod produced on Marnoo A-line rows grown in hybrid seed production blocks at Glenlea in 1986 and 1987.



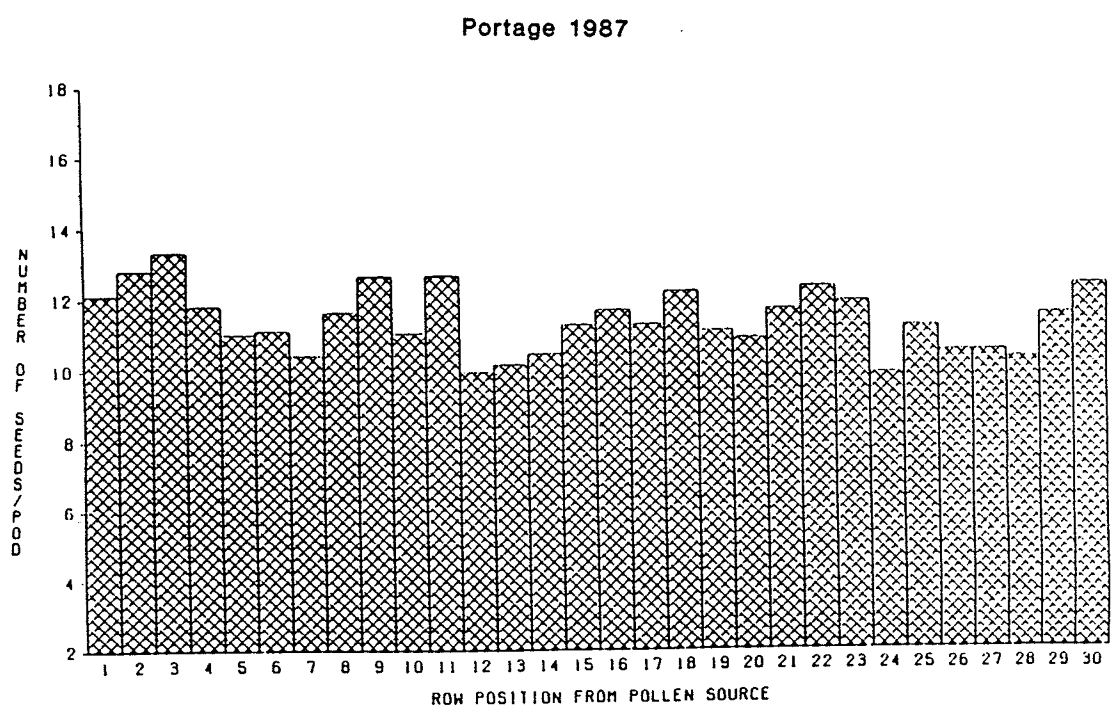
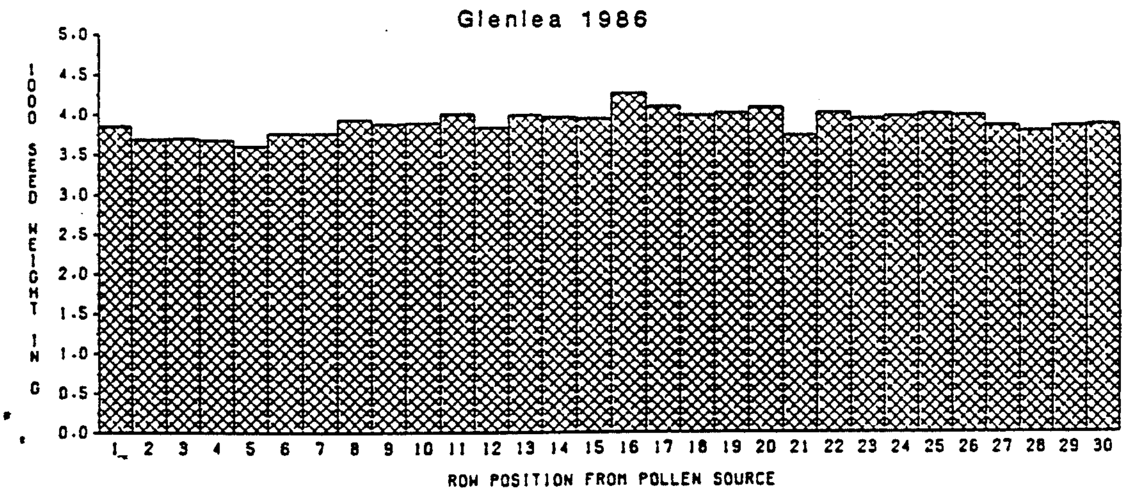


Figure 7. Number of seeds per pod produced on Marnoo A-line rows grown in hybrid seed production blocks at Portage la Prairie in 1986 and 1987.



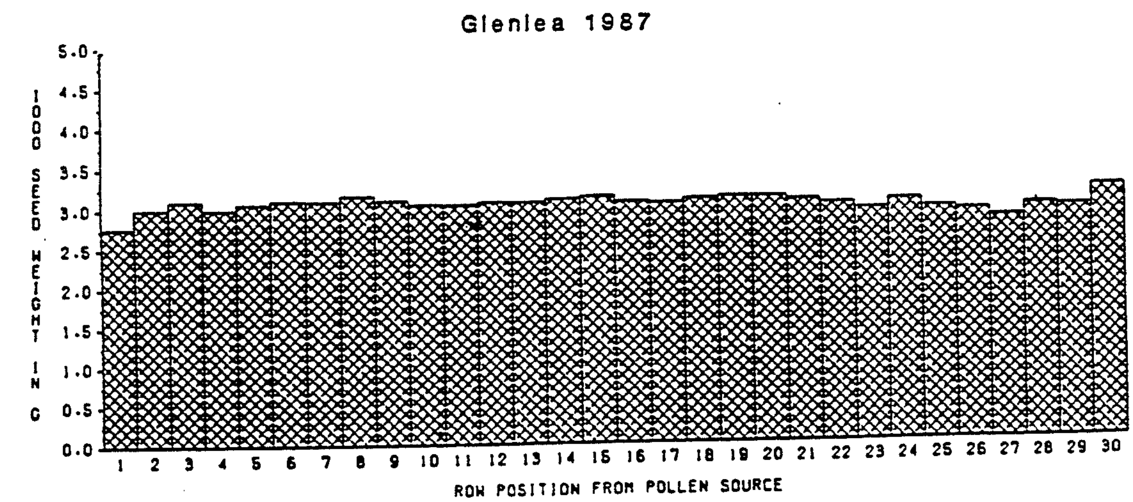


Figure 8. Thousand seed weights for Marnoo A-line rows grown in hybrid seed production blocks at Glenlea in 1986 and 1987.

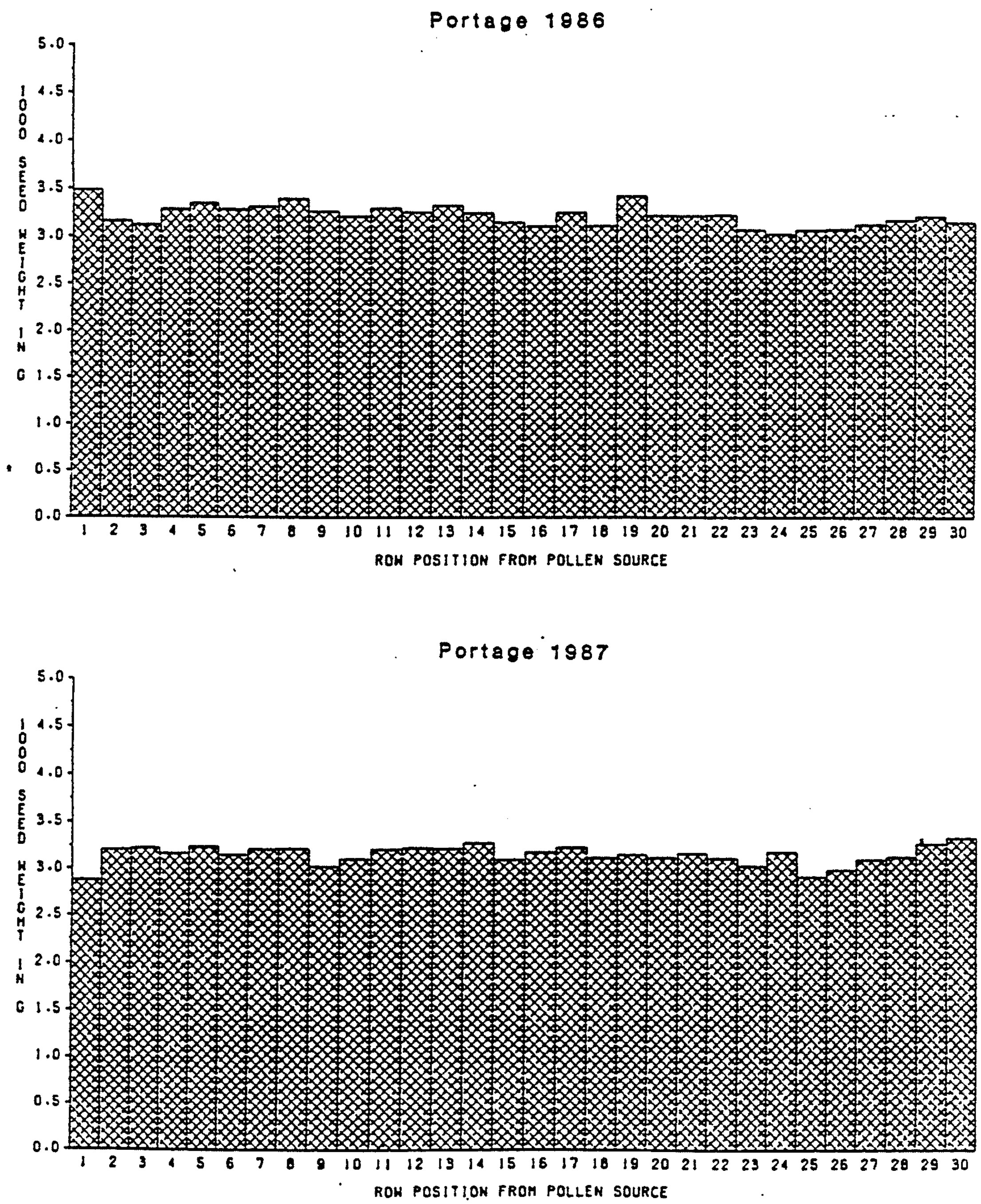


Figure 9. Thousand seed weights for Marnoo A-line rows grown in hybrid seed production blocks at Portage la Prairie in 1986 and 1987.

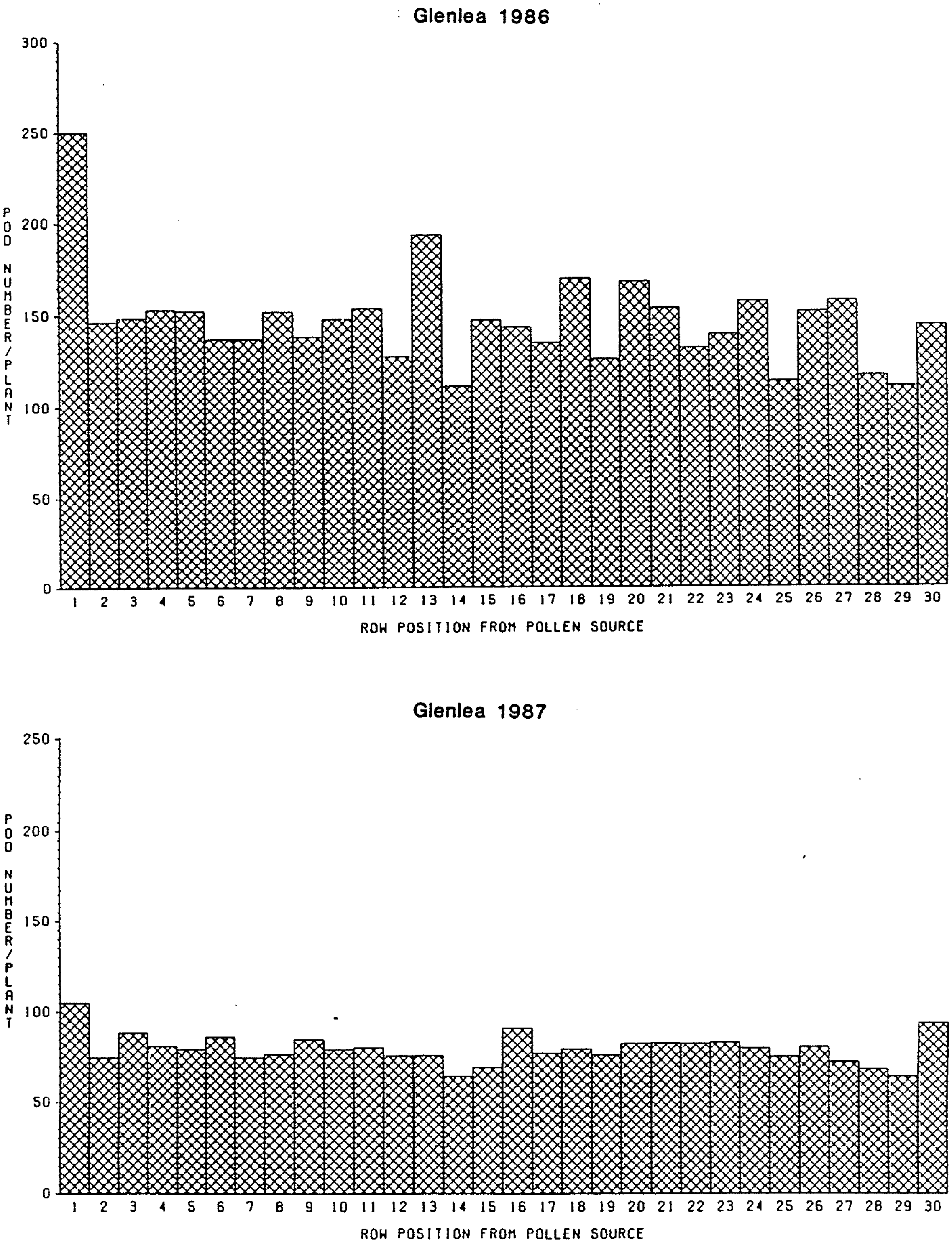


Figure 10. Number of pods produced per plant on Marnoo A-line rows grown in hybrid seed production blocks at Glenlea in 1986 and 1987.

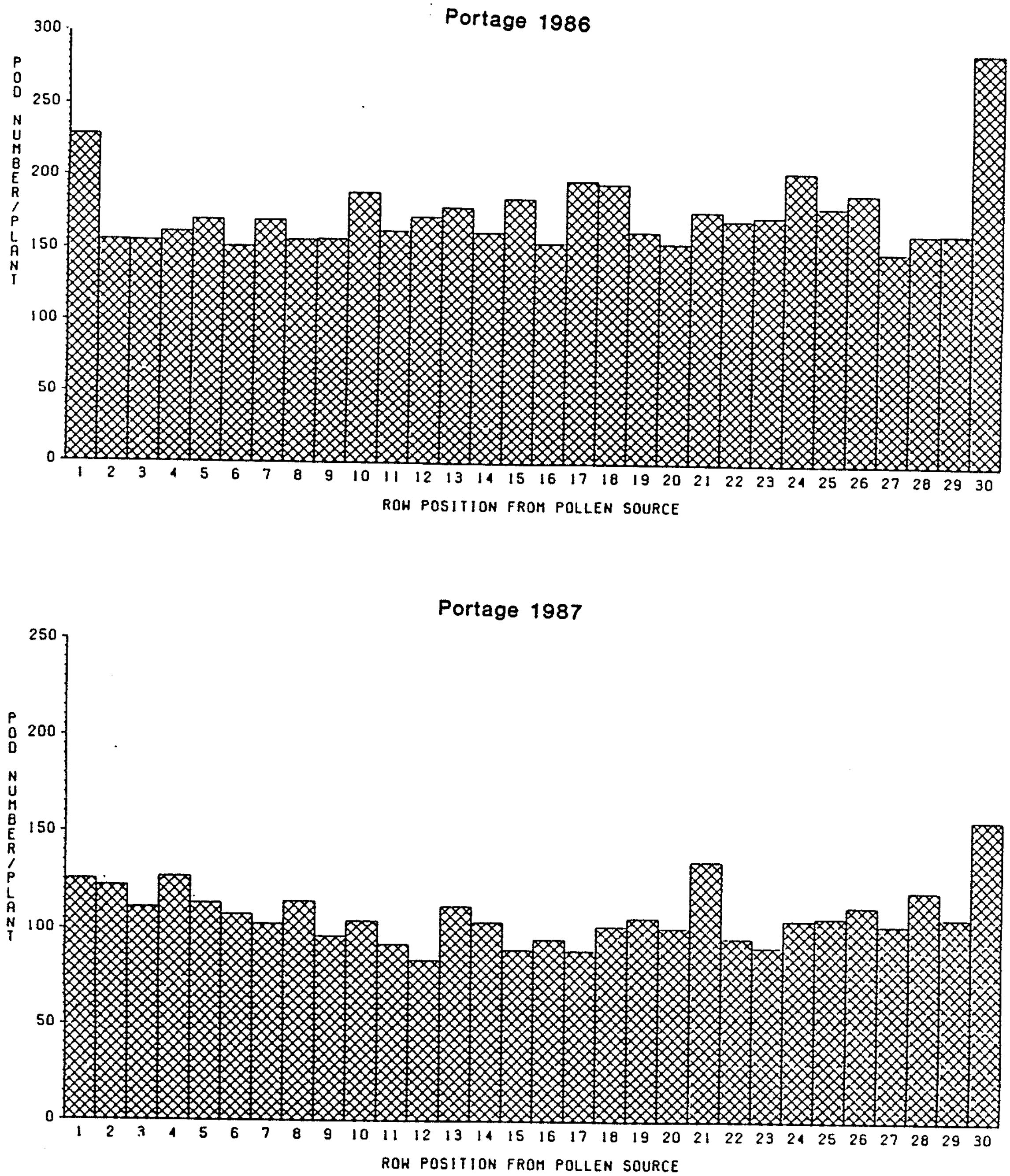


Figure 11. Number of pods produced per plant on Marnoo A-line rows grown in hybrid seed production blocks at Portage la Prairie in 1986 and 1987.

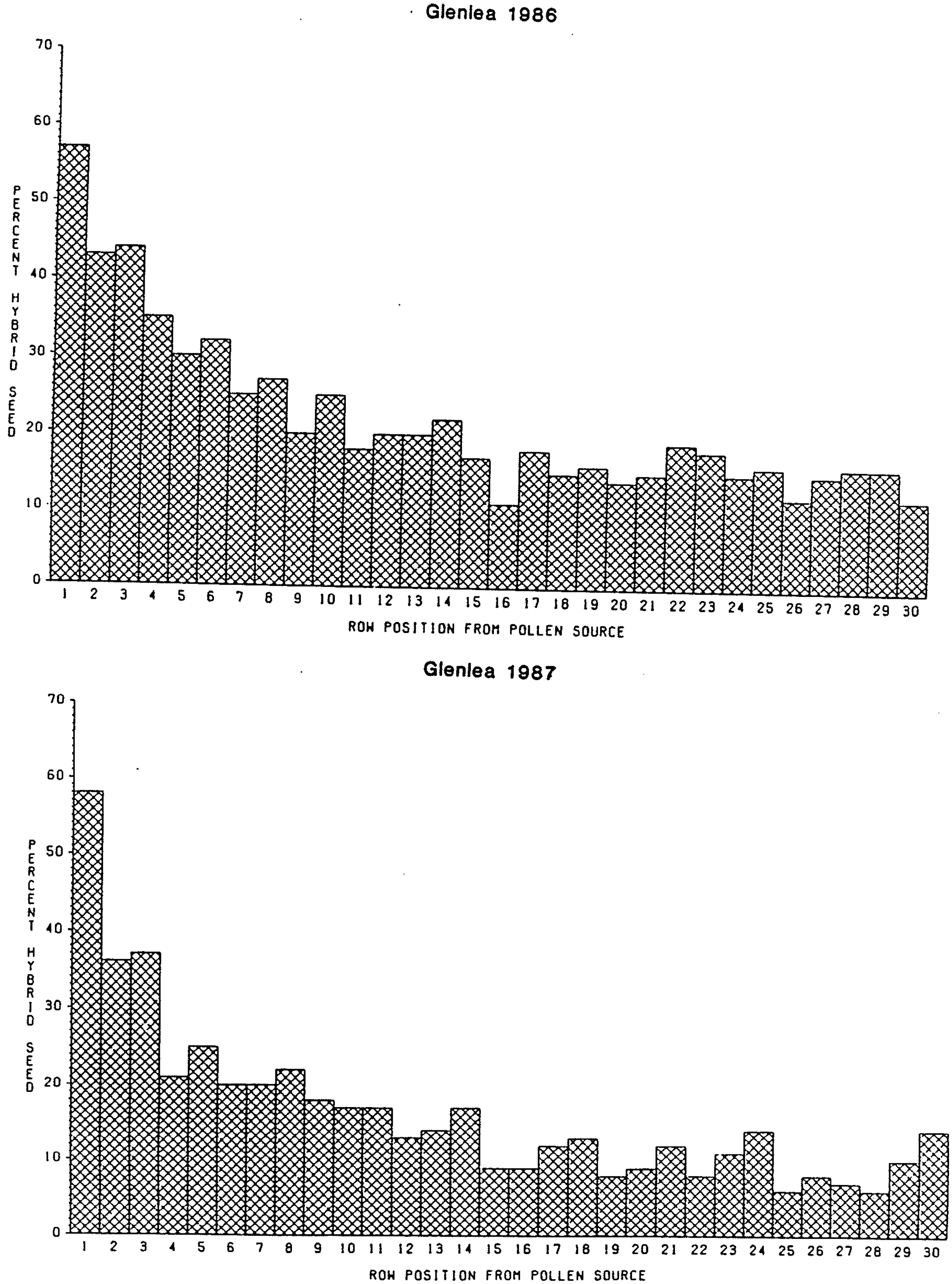


Figure 12. The percentage of hybrid seed produced on Marnoo A-line rows grown in hybrid seed production blocks at Glenlea in 1986 and 1987.

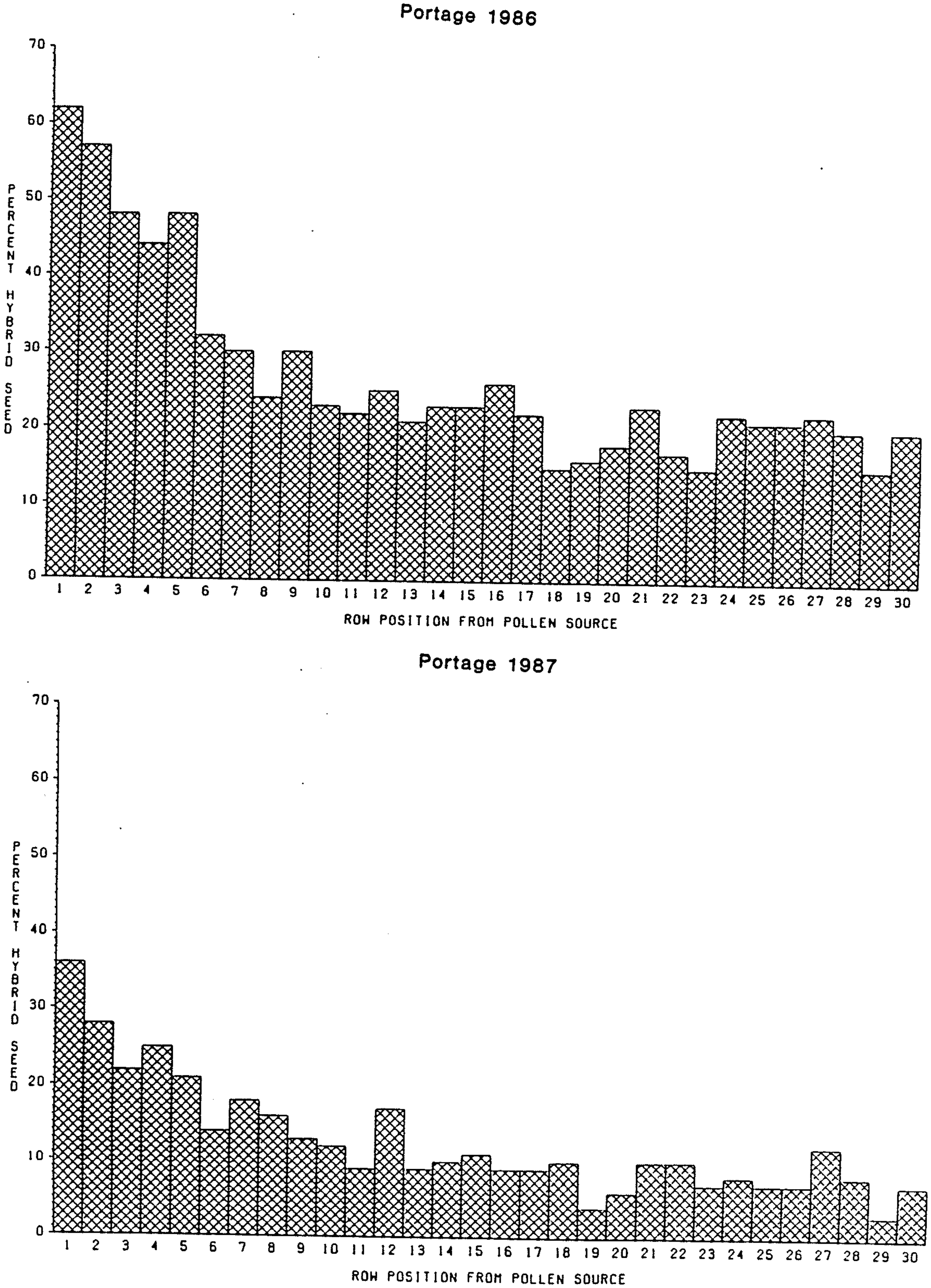


Figure 13. The percentage of hybrid seed produced on Marnoo A-line rows grown in hybrid seed production blocks at Portage la Prairie in 1986 and 1987.

end of the row were removed to partially compensate for the wild pollinator activity and to maintain uniform inter-plant competition.

4.2 Seed Yield

No significant differences in seed yield were found to exist among rows at either Glenlea or Portage la Prairie in 1986, while in 1987, significant differences in seed yield were found to exist among rows at both locations (Table 1.). R-square values (Appendix Tables 1 and 3) indicated that only a small percentage of the variation in seed yield could be attributed to distance from the pollen source.

Despite the low r-square values, regression coefficients of yield on distance from a pollen source for each trial (Table 2.) indicated that seed yield declined as distance from the pollen source increased. These results are in agreement with those reported for ogu CMS hybrid rapeseed (B. napus) seed production (Renard and Mesquida 1979). In this study seed set on the female parents decreased as distance from the pollen source increased. Robinson (1984) found that yield decreased linearly as distance from the pollen source increased in hybrid sunflower seed production. The reason for this decline can be explained by bee foraging behaviour. Bees tend to forage mainly on the male fertile rows and most bees forage along a row without crossing rows. As a consequence, pollen dispersal is greater on the male

Table 1. Effect of number of rows from the pollen source on the mean seed yield of Marnoo A-line rows grown in hybrid seed production blocks.

Row Number	Seed Yield (kg/ha.) Glenlea 1986	1987	Portage 1986	1987
2	2197	2369	2997	2508
3	2127	2066	2219	2261
4	1741	1972	2316	2244
5	1952	2075	2225	2261
6	1611	2166	2172	2041
7	1369	2272	1905	1838
8	1591	2275	2416	2180
9	1750	2122	1930	1769
10	1641	2019	1797	1819
11	1811	2141	2408	2347
12	1763	1930	1794	1944
13	1491	1958	2208	1855
14	2027	2133	2216	2283
15	1438	2008	2155	1863
16	1500	2100	2130	2211
17	1550	2141	2013	2136
18	1672	2086	2125	2019
19	1483	1916	1900	2141
20	1700	1819	2125	2030
21	1577	2111	2019	2433
22	1477	1855	2191	2100
23	1563	1908	1630	1963
24	1827	1877	2016	2227
25	1300	1638	1836	1488
26	1483	1655	1919	1566
27	1466	1455	1658	2002
28	1744	1308	1805	1555
29	1841	1213	2116	2066
LSD (0.05)	NS	95 ***	NS	181 **
C.V. (%)	24.85	9.67	25.09	17.50

NS non significant
** *** significant at p=0.01 and 0.001 respectively

Table 2. Regression coefficients for yield versus number of rows from the pollen source for Marnoo A-line rows grown in hybrid seed production blocks.

Location	Year	Regression Coefficient
Glenlea	1986	-11.37 *
	1987	-26.78 ***
Portage	1986	-18.90 **
	1987	-12.74 *
Glenlea/Portage	86/87	-17.72 ***

* ** *** significant at p=0.05 , .01 and .001 respectively

sterile rows located near the pollen source than those located further away.

Leafcutter bee populations were difficult to maintain in the hybrid seed production blocks at both locations in 1986, and rainy cool weather during most of the flowering period reduced the amount of insect activity that occurred. Despite these problems, seed yields were high in 1986 suggesting that wild species of insect pollinators contributed significantly to the production of these high seed yields.

In 1987 the flowering stages for the two parents were nearly identical. The warm and dry weather experienced for the majority of the flowering period was conducive to bee activity. Significant seed yield differences at both locations in this year may have been due to differences in numbers of bees foraging different parts of the block, differences in the pollen loads carried by the bees, or in the viability of the pollen of the pollen parent. It was noted that little discrimination appeared to be made by the bees between the pollen parents used and the A-line rows. Bees readily foraged either parent in the production block in both 1986 and 1987.

The majority of the seed yield obtained in both years and at both locations, was the result of entomophilic pollination. Plants covered by mesh bags to exclude insects but not wind borne pollen, were found to set few pods and few seeds per pod. As well, a plot of <u>pol</u> CMS Marnoo A-line material planted several hundred metres from a pollen source, in the absence of leafcutter bees set very few pods and very few seeds per pod. These results are in agreement with those of Mesquida and Renard (1979) who found that on *ogu* male sterile *B. napus* wind pollination could contribute, at most, 12 percent of the seed yield within 6 m from the pollen source.

Regression coefficients and intercept values for each trial were significantly different from all other trials. This suggests that the relationship between distance from a pollen source and seed yield is affected by a variety of environmental factors.

4.3 Yield Components

No significant differences were found to exist among rows for pod setting or for pod number per plant in any trial (Table 3 and 4.). Non significant differences indicated that insect pollinator activity was uniform over the hybrid seed production block, if it is assumed that a single bee visit results in the formation of a pod. Non significant regression coefficients for pod setting and pod number per plant (Table 5.) for most trials also support this conclusion. Even though bee activity was uniform across the plot, yields declined with increasing distance from the pollen source. This is because of the difference in pollen loads that bees foraging near the pollen source would carry as opposed to the pollen loads of bees foraging at the end of the block. In other studies, significant differences among rows existed with regards to pollinator activity. In hybrid sunflowers, (Drane et al. 1982), it was found that bee activity over the male sterile rows increased slightly as distance from the pollen source increased. In contrast to these findings, Renard and Mesquida (1979) found that in rapeseed (ogu CMS) the frequency of insect pollinator activity decreased as the distance from the pollen source increased.

It was also found that seeds per pod did not differ significantly among rows except for Glenlea in 1986 (Table

Table 3. Effect of number of rows from the pollen source on pod settig on Marnoo A-line rows grown in hybrid seed production blocks.

Row Number	Pod Setting			
	Glenlea		Portage	
	1986	1987	1986	1987
2	0.60	0.54	0.60	0.73
3	0.62	0.56	0.58	0.70
4	0.64	0.59	0.60	0.68
5	0.65	0.51	0.59	0.71
6	0.56	0.55	0.54	0.68
7	0.58	0.53	0.61	0.65
8	0.66	0.57	0.60	0.69
9	0.58	0.55	0.61	0.69
10	0.65	0.54	0.56	0.67
11	0.56	0.59	0.65	0.72
12	0.59	0.55	0.57	0.69
13	0.62	0.54	0.57	0.73
14	0.57	0.52	0.57	0.68
15	0.58	0.56	0.61	0.69
16	0.57	0.56	0.58	0.68
17	0.61	0.54	0.55	0.67
18	0.53	0.56	0.58	0.68
19	0.56	0.55	0.55	0.68
20	0.66	0.58	0.56	0.66
21	0.58	0.56	0.57	0.69
22	0.56	0.56	0.56	0.67
23	0.63	0.53	0.57	0.69
24	0.61	0.55	0.55	0.61
25	0.56	0.58	0.54	0.65
26	0.64	0.54	0.58	0.64
27	0.57	0.50	0.55	0.66
28	0.53	0.52	0.56	0.68
29	0.49	0.50	0.52	0.68
LSD (0.05)	NS	NS	NS	NS
C.V. (%)	13.82	8.09	9.38	6.81

Table 4. Effect of number of rows from the pollen source on the number of pods produced per plant on Marnoo A-line rows grown in hybrid seed production blocks.

Row Number	Pod Number/Plant Glenlea 1986	Glenlea 1987	Portage 1986	Portage 1987
2	146.2	74.9	155.5	122.3
3	148.3	88.6	155.1	111.1
4	152.9	81.2	161.4	126.7
5	152.0	79.4	169.8	113.2
6	136.8	86.5	151.4	107.6
7	136.6	74.9	169.6	102.7
8	151.7	76.7	156.0	114.1
9	138.3	85.0	156.6	96.1
10	147.7	79.4	188.7	104.1
11	153.5	80.4	162.4	92.1
12	127.2	75.9	172.3	83.7
13	192.7	76.2	178.8	111.6
14	111.2	64.4	162.1	103.6
15	147.0	69.3	185.6	89.1
16	143.1	91.0	154.8	94.7
17	134.5	77.1	197.5	88.8
18	169.2	79.4	195.3	101.5
19	125.7	76.2	162.8	105.9
20	167.5	82.3	154.8	100.7
21	153.5	82.7	176.9	134.8
22	131.9	82.4	170.7	95.4
23	139.1	83.0	173.2	90.8
24	156.9	79.8	203.8	104.9
25	113.8	75.2	179.8	106.2
26	151.2	80.4	189.1	111.8
27	156.8	72.4	148.8	102.3
28	116.8	68.1	161.8	119.8
29	110.8	64.1	162.5	106.1
LSD (0.05)	NS	NS	NS	NS
C.V. (%)	21.59	17.66	19.53	21.47

Table 5. Regression coefficients for various yield components versus the number of rows from the pollen source for Marnoo A-line rows grown in hybrid seed production blocks.

Location	Year	Pods/Total Flowers	Pods/ Plant	Seeds/ Pod	1000 Seed Weight
Glenlea	1986	-0.001	0.11	-0.10**	0.02***
	1987	-0.001	-0.25	-0.08***	-0.05***
Portage	1986	-0.002*	0.53	-0.07*	-0.006***
	1987	-0.001	-0.18	-0.04	-0.004***
Glenlea/ Portage	86/87	-0.002***	-0.11	-0.06***	-0.002

* ** *** significant at p=0.05 , 0.01 and 0.001 respectively

6.). In this particular trial, seeds per pod declined as distance from the pollen source increased. Regression coefficients for seeds per pod on number of rows from the pollen source (Table 5.) also indicated that in most cases a significant negative relationship existed.

These results are in agreement with work done by Waller et al. (1985) on hybrid cotton seed production, where the number of seeds per boll declined as distance from the pollinator rows increased. Renard and Mesquida (1979) also found that seed set on ogu B. napus A-lines declined as distance from the pollen source increased.

Thousand seed weights differed significantly among rows for all trials except for Portage la Prairie in 1986 (Table 7). It was found that thousand seed weights tended to decline slightly as distance from the pollen source increased, except for Glenlea in 1986 where they tended to increase with increasing distance. Mesquida and Renard (1982) found that the influence of honeybee pollination served to decrease the weight of the seed produced by the ogu B. napus A-lines. These results are in contrast to those found by Drane et al. (1982) on male sterile sunflowers. In this case no significant differences between mean bee activity per sunflower head and 100 seed weight were found.

Table 6. Effect of number of rows from the pollen source on seed number per pod on Marnoo A-line rows grown in hybrid seed production blocks.

	Number of Seeds/Pod			
	Glenlea		Portage	
Row Number	1986	1987	1986	1987
2	9.4	12.3	11.9	12.8
3	9.8	11.4	14.6	13.3
4	11.3	12.9	17.0	11.8
5	9.7	11.9	11.9	11.0
6	10.9	12.1	11.9	11.1
7	6.8	12.2	12.7	10.4
8	9.4	10.8	13.2	11.6
9	8.9	10.3	12.5	12.6
10	8.1	11.6	10.3	11.0
11	6.7	11.3	12.3	12.6
12	9.1	11.8	12.3	9.9
13	7.6	10.6	12.2	10.1
14	7.9	11.1	13.5	10.4
15	8.0	10.7	12.7	11.2
16	7.4	10.7	13.1	11.6
17	7.4	11.4	13.2	11.2
18	10.0	11.8	13.0	12.1
19	5.9	10.6	12.8	11.0
20	7.2	11.5	11.5	10.8
21	9.4	10.7	11.9	11.6
22	6.2	11.2	12.7	12.2
23	8.0	11.6	10.2	11.8
24	8.7	9.7	10.0	9.8
25	6.6	9.3	11.2	11.1
26	7.2	10.8	10.8	10.4
27	6.9	10.4	11.9	10.4
28	6.4	10.5	12.2	10.2
29	8.9	8.9	13.6	11.4
LSD (0.05)	2.3 **	NS	NS	NS
C.V. (%)	13.66	14.64	20.98	17.49

** significant at p=0.01

Table 7. Effect of number of rows from the pollen source on thousand seed weight of Marnoo A-line rows grown in hybrid seed production blocks.

Row Number	1000 Seed Wt. (g) Glenlea 1986	Glenlea 1987	Portage 1986	Portage 1987
2	3.69	3.00	3.16	3.20
3	3.70	3.10	3.12	3.22
4	3.68	2.99	3.28	3.16
5	3.60	3.06	3.34	3.23
6	3.76	3.10	3.28	3.14
7	3.76	3.09	3.31	3.20
8	3.93	3.16	3.39	3.21
9	3.88	3.10	3.26	3.02
10	3.89	3.05	3.21	3.10
11	4.00	3.04	3.29	3.20
12	3.83	3.07	3.25	3.22
13	3.99	3.06	3.32	3.21
14	3.96	3.09	3.24	3.27
15	3.94	3.12	3.14	3.09
16	4.25	3.05	3.10	3.17
17	4.09	3.03	3.24	3.22
18	3.98	3.08	3.10	3.11
19	4.01	3.10	3.41	3.14
20	4.08	3.10	3.21	3.11
21	3.73	3.06	3.20	3.15
22	4.01	3.01	3.21	3.10
23	3.94	2.94	3.05	3.02
24	3.97	3.05	3.01	3.17
25	4.00	2.95	3.05	2.91
26	3.98	2.91	3.06	2.98
27	3.85	2.82	3.11	3.09
28	3.75	2.97	3.15	3.12
29	3.84	2.94	3.19	3.26
LSD (0.05)	0.27 **	0.13 ***	NS	0.18 *
C.V. (%)	5.03	2.96	5.75	3.99

* ** *** significant at p=0.05 , 0.01 and 0.001 respectively

4.4 Percent Hybridity

Highly significant differences were found to exist among A-line rows with regards to the percentage of hybrid seed harvested from them for all trials (Table 8.). It was found that the majority of seed produced on the A-line rows was unrestored male sterile seed, not hybrid seed. To calculate the theoretical hybrid seed yield per row (Table 9.), the total seed yield per row values for each row (Table 1.) were multiplied by the percentage of hybrid seed (Table 8.). From Table 9. it can be seen that the highest hybrid seed yields were obtained from those rows nearest the pollen source and declined as distance from the pollen source increased.

It was also found that the percentage of hybrid seed produced per row was significantly higher (p=0.01) in 1986 than in 1987 at both Glenlea and Portage la Prairie. It is possible that the change observed in percent hybridity from 1986 to 1987 was due to differences in the pollen sources. Both pollen sources were found to produce a nectar sugar concentration, approximately 7.5% lower than that of the A-line. At Portage la Prairie it was found that the theoretical hybrid seed yield did not differ between years even though the percentage of the hybrid seed in the seed lot in 1987 declined from the 1986 level. This suggests that the hybrid seed was diluted with unrestored, male

Table 8. Effect of number of rows from the pollen source on the percentage of hybrid seed produced on Marnoo A-line rows grown in hybrid seed production blocks.

	Percent Hybrid Seed			
	Glenlea		Portage	
Row number	1986	1987	1986	1987
2	43	36	57	28
3	44	37	48	22
4	35	21	44	25
5	30	25	48	21
6	32	20	32	14
7	25	20	36	18
8	27	22	24	16
9	20	18	30	13
10	25	17	23	12
11	18	17	22	9
12	20	13	25	17
13	20	14	21	9
14	22	17	23	10
15	17	9	23	11
16	11	9	26	9
17	18	12	22	9
18	15	13	15	10
19	16	8	16	4
20	14	9	18	6
21	15	12	23	10
22	19	8	17	10
23	18	11	15	7
24	15	14	22	12
25	16	6	21	7
26	12	8	21	7
27	15	7	22	12
28	16	6	20	8
29	16	10	15	3
LSD (0.05)	10 ***	6 ***	10 ***	7 ***
C.V. (%)	23.35	16.55	19.48	23.06

*** significant at p=0.001

Table 9. Effect of number of rows from the pollen source on theoretical hybrid seed yield on Marnoo A-line rows grown in hybrid seed production blocks.

	Hybrid Seed Yield (kg/ha)			
	Glenlea		Portage	
	1986	1987	1986	1987
2	961	614	539	655
3	822	414	961	489
4	597	411	783	508
5	586	530	611	483
6	552	453	675	302
7	347	378	544	336
8	438	519	575	386
9	369	400	577	228
10	452	342	438	228
11	352	369	536	208
12	347	253	438	328
13	300	269	563	192
14	458	453	522	255
15	258	178	494	192
16	161	189	569	189
17	280	250	441	178
18	250	280	344	186
19	244	208	355	147
20	258	164	405	130
21	255	244	480	228
22	283	180	383	225
23	297	217	244	150
24	308	267	447	183
25	217	128	402	114
26	183	161	411	136
27	211	111	355	244
28	280	89	347	119
29	286	122	297	142
LSD (0.05)	93 ***	50 ***	79 ***	59 ***
C.V. (%)	33.80	49.52	31.79	45.28

*** significant at p=0.001

sterile seed. This can be most easily explained in terms of male fertility reversion of the previously male sterile pol CMS, which was first described by Fan and Stefansson (1986). They found that under high temperatures, anthers of pol male sterile flowers would become partially male fertile.

It was also found that the percentage of hybrid seed produced declined as the number of rows from the pollen source increased (Table 8.). This was again reflected by the regression of theoretical hybrid seed yield (Table 10.) on row number from the pollen source. A very highly significant and negative relationship was found to exist. Thus flowers of A-line rows nearest the pollen source are more likely to be foraged by bees carrying pollen from the pollen parent than are flowers in A-line rows farther away. It is possible that the viability of the pollen from the pollen parent may not deteriorate as much if it is carried to a nearby A-line flower than it would if carried to the end of the block.

In this study, the morphology of the A-line flower did not appear to be conducive to self pollination. Marnoo A-line flowers had short filaments and reduced anthers, characteristics similar to those described by Fan and Stefansson (1986) for B. napus cultivars with the pol cytoplasm. The distance between the stigma surface and any pollen produced on these anthers seemed to preclude any self pollination even in the presence of strong winds. Poor pod and seed set under the mesh bags in this study supports this

Table 10. Regression coefficients for percent hybrid seed and theoretical hybrid seed yield on number of rows from the pollen source for Marnoo A-line rows grown in hybrid seed production blocks.

Location	Year	Percent Hybridity	Theoretical Hybrid Seed Yield
Glenlea	1986	-1.0 ***	-20.85***
	1987	-0.6 ***	-15.11***
Portage	1986	-0.5 ***	-14.47***
	1987	-0.5 ***	-13.21***
Glenlea/ Portage	86/87	-0.6 ***	-15.20***

*** significant at p=0.001

conclusion. However, the presence of an insect pollen vector causes an increase in pollen dispersal since the bees brush against the reduced anthers while foraging for nectar. Any pollen grains that adhere to the bodies of the bee may be deposited on the stigma of the same flower or on the stigma of other A-line flowers. Thus pollination could have occurred on the A-line flowers without the bees having ever visited a pollen parent or R-line flower. This explains the large proportion of the unrestored seed in the seed harvested from the A-line rows.

It was also found that the seed produced under the mesh bags had a percent hybridity lower than that for the row from which it was taken. This again shows the ineffectiveness of wind in moving R-line pollen. Most hybrid seed produced on the A-line rows must have been the result of entomophilic deposition of pollen from the pollen parent.

4.5 Correlation Between Number Of Rows From The Pollen Source, Yield, Yield Components And Percent Hybridity.

To determine how the number of rows from the pollen source, seed yield and yield components are interrelated, correlation coefficients between these parameters were calculated (Tables 11 and 12.). It was found that yields on the A-line rows were only weakly negatively correlated with distance from the pollen source for most trials (r= -0.22* to -0.24*) (Tables 11 and 12.), except for Glenlea in 1987 (r= -0.65***) (Table 11.). It would be expected that yield would decline as distance from the pollen source increased, however the pollen production of the A-line plants served to weaken this correlation. As well, a strong negative correlation was found to exist between theoretical hybrid seed yield and distance from a pollen source. Strong negative correlations between yield of hybrid seed and distance from a pollen source were found in hybrid sunflower seed production (Robinson 1984, Drane et al. 1982).

Significant correlations between number of rows from the pollen source and pod number, or pod setting were largely non existent. This suggests that bee foraging activity was more or less uniform across rows. Results from studies by Renard and Mesquida (1979) in ogu CMS B. napus showed that correlations between bee activity and pod setting were significant (r= 0.70* to 0.97**). The

Table 11. Correlation coefficients between the number of rows from the pollen source, seed yield, yield components and percent hybridity for Marnoo A-line grown in hybrid seed production blocks at Glenlea in 1986 (top line) and 1987 (lower line).

Character	Seed Yield	Pods/ Total Flowers	Seeds/ Pod	1000 Seed Weight	Pods/ Plant	Percent Hybridity	Theoretical Hybrid Seed Yield
Row Number	-0.22* -0.65***	-0.24** -0.12	-0.48*** -0.36***	0.24 0.34***	-0.12 -0.14	-0.62*** -0.70***	-0.58*** -0.75***
Seed Yield		0.00 0.23*	0.21 0.25**	-0.08 0.47***	0.10 0.21*	0.34*** 0.48***	0.63*** 0.70***
Pods/Total Flowers			-0.09 0.14	0.06 0.20*	0.29** 0.40***	0.19* 0.10	0.15 0.12
Seeds/Pod				-0.55*** 0.05	0.01 0.18	0.49*** 0.36***	0.47*** 0.35***
1000 Seed Weight					0.08 0.12	-0.33*** 0.20*	-0.30*** 0.27**
Pods/Plant						0.07 0.04	0.07 0.07
Percent Hybridity							0.90*** 0.97***

* ** *** significant at p = 0.05, 0.01 and 0.001 respectively

Table 12. Correlation coefficients between the number of rows from the pollen source, seed yield, yield components and percent hybridity for Marnoo A-line grown in hybrid seed production blocks at Portage la Prairie in 1986 (top line) and 1987 (lower line).

Character	Seed Yield	Pods/ Total Flowers	Seeds/ Pod	1000 Seed Weight	Pods/ Plant	Percent Hybridity	Theoretical Hybrid Seed Yield
Row Number	-0.24**	-0.22*	-0.21*	-0.23*	0.12	-0.50***	-0.55***
	-0.22*	-0.17	-0.17	-0.20*	-0.05	-0.62***	-0.63***
Seed Yield		-0.20*	0.09	0.33***	0.01	-0.06	0.60***
		-0.04	-0.11	0.59***	0.05	0.17	0.56***
Pods/Total Flowers			0.10	-0.11	-0.10	0.15	0.01
			0.38***	-0.28***	0.48***	0.11	0.08
Seeds/Pod				-0.01	-0.16	0.28**	0.30*
				-0.22*	0.25**	0.10	0.05
1000 Seed Weight					0.05	0.05	0.23*
					-0.16	0.16	0.33***
Pods/Plant						-0.10	0.06
						0.22*	0.21**
Percent Hybridity							0.77***
							0.93***

* ** *** significant at p = 0.05, 0.01 and 0.001 respectively

highly significant and positively correlated to thousand seed weight at Portage la Prairie for both 1986 and 1987 (r= 0.33*** to 0.59***) (Table 12).

Percent hybridity was found to be negatively correlated to the number of rows from the pollen source (r= -0.50*** to -0.70***) (Tables 11 and 12.). As well the theoretical hybrid seed yield was found to be negatively correlated (r=-0.55*** to -0.75***) (Tables 11 and 12) to number of rows from the pollen source.

4.6 Row Ratio Recommendations

Because of the heat sensitivity of the pol CMS system and the subsequent pollen production on the A-line rows, distinct recommendations on the appropriate ratio of A-line to pollen source rows (whether restorer or maintainer) are difficult to make. Total seed yields (Table 1) are satisfactory over the range of the experimental field, suggesting that the 30:3 (or 10:1) ratio of A-line rows to pollen source rows used in this study would be appropriate. However, the theoretical hybrid seed yield (Table 9.) suggests that a somewhat smaller ratio may be more practical. Hybrid seed yields appeared to drop off after the first 10 rows, with little change noted among the following rows. This suggests that a 3:1 ratio of A-line to pollen source rows may give optimum yield of hybrid seed per hectare.

Renard and Mesquida (1979) found that a 14:2 (or 7:1) ratio of A-line to pollen source rows gave the highest hybrid seed yields per hectare with the ogu CMS hybrid rapeseed (B. napus) production system. Robinson (1984) found that yields of hybrid sunflower seed per hectare were greatest when a 4:2 (or 2:1) to 16:2 (or 8:1) ratio of A-line to pollen source was used.

These results are similar to the 10:1 ratio being proposed in this study. However, this row ratio may have to be altered if upon improving the sterility of the A-line material, the bees fail to provide sufficient pollen dispersal. Improvements in male sterility stability to heat stress would eliminate any pollen production on the A-line rows. This would force all bees in the field to forage the R-line to meet their nutritive requirements for pollen. However, improvements in male sterility stability might have some effect on nectar production of the A-line flowers. This may cause the bees to prefer or reject the A-line flowers thus affecting pollen dispersal. It is important that both parents present in the block have similar nectar contents and sugar concentrations.

Efficient production of hybrid rapeseed should endeavour to minimize the number of pollen source rows required. Seed from pollen source rows is of little commercial value and by having a large number of pollen source rows in the field, the possibility of contaminating the hybrid seed lot with pollen source seed is greater.

5. CONCLUSION

The purpose of this study was to ascertain the relationship between number of rows from the pollen source, and seed yield, yield components and percent hybridity on the *pol* CMS A-line rows of hybrid seed production blocks. It was found that the seed yield on the Marnoo A-line rows declined linearly as the number of rows from the pollen source increased. The yield components that seemed to be the most responsible for this yield decline were seeds per pod and to a lesser extent, thousand seed weight. Percent hybridity was the parameter most influenced by the number of rows from the pollen source. Thus the amount of hybrid seed produced on the outer rows of the experimental field was less than that produced by those rows adjacent to the pollen source.

The heat sensitivity of the *pol* CMS system resulted in some A-line pollen being produced. This, in turn, led to the presence of unrestored seed in the hybrid seed lot. None of the seed produced on any of the rows could be considered to be of commercial quality because of the contamination with A-line seed.

It has been shown that insect pollen vectors will transport pollen from the pollen source over the A-line rows, which is important since wind pollination has been shown to be negligible.

In this study a row ratio of 10:1 of A-line to pollen source appeared to give satisfactory yields. However improvement of the male sterility of the A-line material may require a reduction in this ratio to 7:1 or lower. As well, the market price for hybrid rapeseed seed will determine to some extent what row ratio is economically feasible.

Although considerable morphological differences exist between A-line and pollen source plants, especially with respect to floral characters, little discrimination appears to be made by the insect pollen vector between the two lines. Thus many of the problems experienced in hybrid cotton seed production are unlikely to be experienced in hybrid rapeseed seed production.

It is essential for further studies on hybrid rapeseed seed production, that the male sterility of the A-line parent be complete. Even small amounts of pollen produced by A-line plants can be readily transported by insects to other A-line plants. Breeding A-lines resistant to heat stress reversion to male fertility as described by Fan and Stefansson (1986) is essential to prevent contamination of the hybrid seed lot with non hybrid seed.

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Appendix Table 1. Analysis of variance for characters studied on Marnoo A-line rows grown in hybrid seed production blocks at Glenlea in 1986.

		Mean Squares					
Source	df	Seed Yield	Pods/ Total Flowers	Pods/Plant	Seeds/Pod	1000 Seed Weight	Percent Hybridity
Rows	27	25485	0.007	1363	4.14**	0.08**	0.03***
Error	81	22281	0.007	958	1.26[a]	0.04	0.01
R-Square		0.28	0.32	0.45	0.77	0.60	0.62

** *** significant at $p = 0.01$ and 0.001 respectively

[a] based on 27 df

Appendix Table 2. Analysis of variance for characters studied on Marnoo A-line rows grown in hybrid seed production blocks at Glenlea in 1987.

		Mean Squares					
Source	df	Seed Yield	Pods/ Total Flowers	Pods/Plant	Seeds/Pod	1000 Seed Weight	Percent Hybridity
Rows	27	40104***	0.002	169	3.28	0.02***	0.01***
Error	81	4610	0.002	190	2.63	0.01	0.00
R-Square		0.76	0.43	0.29	0.36	0.61	0.69

*** significant at p = 0.001

Appendix Table 3. Analysis of variance for characters studied on Marnoo A-line rows grown in hybrid seed production blocks at Portage la Prairie in 1986.

		Mean Squares					
Source	df	Seed Yield	Pods/ Total Flowers	Pods/Plant	Seeds/Pod	1000 Seed Weight	Percent Hybridity
Rows	27	39005	0.003	914	7.29	0.04	0.02***
Error	81	35315	0.003	1101	6.80	0.03	0.00
R-Square		0.49	0.50	0.37	0.28	0.54	0.55

*** significant at p = 0.001

Appendix Table 4. Analysis of variance for characters studied on Marnoo A-line rows grown in hybrid seed production blocks at Portage la Prairie in 1987.

		Mean Squares					
Source	df	Seed Yield	Pods/ Total Flowers	Pods/Plant	Seeds/Pod	1000 Seed Weight	Percent Hybridity
Rows	27	3426**	0.003	566	3.35	0.03*	0.01***
Error	81	16541	0.002	509	3.89	0.02	0.00
R-Square		0.56	0.73	0.50	0.34	0.61	0.63

* ** *** significant at p = 0.05, 0.01 and 0.001 respectively