

**Insect-mediated cross-pollination in male-sterile, female-fertile mutant
soybean [*Glycine max* (L.) Merrill] lines**

by

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For the Major Program

DEDICATION

This work is dedicated to my parents,
Esteban Ortiz Carbajal, Teodomira Perez De Ortiz, and my grandmother Alejandra
Carvajal Santa Maria.

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GENERAL INTRODUCTION

DISSERTATION ORGANIZATION

The dissertation includes a general introduction followed by three manuscripts. The first manuscript describes the evaluation of seed-set among thirty-four pairs of near-isogenic soybean lines segregating for the *ms6* male-sterile allele, the *ms6* donor parent, the *ms6* donor parent isogenic purple flower *W1W1*, and *W1w1* in a three-year study. The second manuscript describes the heterosis evaluation of F_1 hybrid soybean plants from single-crosses, three-way crosses, and backcrosses (BC_1F_1) produced by male-sterile lines as female parents and a selected group of male parents. Several bee species were used as the pollinator vector. The third manuscript describes the evaluation of the response to phenotypic recurrent selection for increased seed set on male-sterile, female-fertile soybean lines segregating for male-sterile alleles *ms2* (low seed set), *ms3*, *ms6*, *ms8*, *ms9*, and *ms2* (high seed set) by using a selected group of male lines. Bees also were the pollinator vectors. The manuscripts are followed by a general conclusions section.

BACKGROUND

Soybean, *Glycine max* (L.) Merr., combines in one crop both the primary world supply of edible vegetable oil and the main supply of high-protein feed supplements for livestock. Other fractions and derivatives of soybean seeds have

substantial economic importance in a wide range of industrial, food, pharmaceutical, and agricultural products (Smith and Huyser, 1987). In the US, soybean was planted on 75.2 million acres (30.4 million hectares) in 2004, producing a record 3.141 billion bushels (85.49 million metric tons), with the total crop value exceeding \$17.7 billion. The United States exported a record 1.1 billion bushels (29.94 million metric tons) of soybean, which accounted for 48 percent of the world's soybean trade (Soy stats, 2005).

Since soybean is typically a self-pollinated crop, efforts to increase grain yield and other agronomic traits have been conducted throughout pure-line development programs. Although hybridization has been utilized to produce pure line cultivars, the use of hybrids *per se* has been impractical. One reason is that manual cross-pollination to produce large quantities of hybrid soybean seed is difficult and time-consuming. A low success rate in cross-pollination and the few seeds obtained per pod have contributed to the difficulty of producing large quantities of hybrid seed, necessary if they were used in commercial plantings.

When nuclear male sterility was discovered in soybean, it was viewed as a useful tool for intermating large numbers of plants. Mutations that selectively eliminate male reproductive function and leave female function unimpaired have potential application in hybrid seed production (Horner and Palmer, 1995). The use of nuclear male-sterile and cytoplasmic-genetic male-sterile soybean has raised the real possibility that F_1 hybrid soybean could be put into commercial production. In

the final analysis the success of F_1 hybrid soybean will require the efficient transfer of pollen from the male parent to the female parent.

Because of flower morphology, insect pollen vectors may be the preferred means by which pollen can be transferred. Compared to honey bees, some wild bees pollinate certain crops more efficiently because of unique and desirable behaviors. For example, Southeastern blueberry bees *Hapropoda laboriosa* (F.), *buzz-pollinate* blossoms by shaking pollen from the flower with high frequency muscle vibrations; for blueberry, this greatly improves pollination efficiency (Pichersky and Gershenzon, 2002). Another insect species used for commercial pollination of crops is the alfalfa leaf cutter bee *Megachile rotundata* F. It is extensively employed for the production of alfalfa seed. Introducing alfalfa leaf cutter bees into both open and caged plots of soybeans, Bradner (1977) observed that these pollinating insects also are possible agents of soybean cross-pollination.

In soybean, however, little attention has been paid to the prevalence of pollinating insects. Blickenstaff and Huggans (1962) recorded only one honey bee, three leafcutter bees, and nine bees from the Halictidae family among 25,346 individual insects collected on soybeans with a sweepnet. Culter (1934) and Gordienko (1977) showed that honey bee populations can be established on soybeans. Beard and Knowles (1971) showed that crossing at different locations in California varied: 10 percent at Five Points, 1 to 5 percent at Davis, and 0 to 7 percent at Shafter. No studies have been done on the value of using gregarious types such as wild bees on soybeans,

although such insects might prove to be more efficient pollinators than are honey bees.

Caviness (1970) showed that thrips are ineffective as pollinators of soybean, and that honey bees were responsible for 7.7 percent outcrossing. According to Erickson (1975), increases in soybean yields due to bee pollination are possible in some but not all cultivars. Robacker *et al.* (1983) stated that environmental conditions which promote greater flower production, larger flower size, more intensely colored flowers and higher nectar secretion are the conditions which promote greater honey bee attractiveness. Thus, environmental factors might change plant attractiveness to honey bees through effects on flower characteristics.

The foraging activity of pollinators is also affected by plant density (Handel, 1983). It has been reported that seed set in *Cassia biflora* was higher in dense populations, and pollinator activity was possibly correlated with high plant density (Silander, 1978). According to Chiang and Kiang (1986) is possible to increase the rate of outcrossing and seed yield in soybean by attracting bee visitation using a high density stand with each plant surrounded by a different genotype.

Bradner (1969) stated that all of the breeding components have been described for the production of hybrid soybean seeds, except that a suitable pollen vector has not been found, although the honey bee "looks" encouraging. Parental

lines might be selected with aroma or attractiveness that when incorporated in a hybrid seed program could attract pollinating insects.

Understanding of the different pollination needs of both the male and female parents is of paramount importance if the ultimate goal is hybrid seed production. The processes by which parental and maintainer lines are developed may lead to diminished or altered pollinator foraging cues and rewards. Frequently flowers on male-sterile plants have less nectar than those on male-fertile plants, thus suggesting a pleiotropic effect resulting from selection for male sterility (Palmer *et al.* 2001). Since floral volatiles are usually associated with nectar production, aromatics may be changed along with nectar quality or quantity (Erickson, 1983). Soybean programs that rely on insect pollination for hybrid seed production should monitor parent plants for insect preference simultaneously with selection for agronomic traits (Palmer *et al.*, 2001).

Palmer and Lewers (1998) developed 34 pairs of soybean lines by backcrossing the *ms6* and *w1* alleles to 34 recurrent parents. The crosses produced near-isogenic lines cosegregating for the *ms6* allele (male-sterility) and *w1* (white flower) alleles in coupling phase or white-flowered lines segregating for the *ms6* allele. Neither seed set, nor the attractiveness to pollinator insects has been evaluated on these near-isogenic lines. Previous studies indicated that seed set on male-sterile, female-fertile plants is a good indicator of insect attraction (Lewers *et al.*, 1996, 1998; Lewers and Palmer, 1997; Ortiz-Perez *et al.*, 2004). Considering this background information, the objective of the research reported in the first study was

to evaluate seed set among male-sterile, female-fertile lines segregating for the *ms6* allele by using *Megachile rotundata* as pollinator vector.

Heterosis or hybrid vigor has been studied extensively in cross-pollinated crops such as maize, sorghum, pearl millet, rapeseed, onion, sunflower, cotton, and tomato (Palmer et al., 2001). Traditionally, heterosis has been attributed to dominance interactions and masking of deleterious recessive alleles, although other possible causes of heterosis are considered. Schnell and Cockerman (1992) reported that additive X additive epistasis from multiplicative gene action can cause heterosis.

Earlier maturity, higher yield, and uniformity of the hybrids have resulted in higher prices for the farmers. The stacking of several useful traits such as disease and herbicide resistance is common in hybrid production systems (Palmer *et al.*, 2003) where hybrids offer additional advantages in crops where important traits are controlled by dominant genes. Use of hybrids in self-pollinated crops such as rice, wheat, tobacco and soybean, has been considered impractical because of the strict self-pollination mechanisms that discourage cross-pollination (Palmer *et al.* 2001). In soybean, manual cross-pollination to produce large quantities of hybrid seed for yield trials is not practical, and it is not cost effective. Thus parental combinations that produce heterosis levels superior to the best pure-line cultivars, (i.e., identification of heterotic patterns) have not been identified in soybean. Use of nuclear male sterility in soybean has provided some information on levels of out-crossing and heterosis (Graybosch and Palmer, 1988). Male-sterile, female-fertile

lines have been used to produce F_1 seed to test for heterosis. Average mid-parent yield heterosis for 2, 8, 27, 18, and 18 hybrid combinations were 28%, 13%, 8%, 2%, and 8%, respectively (Brim and Cockerham, 1961; Hillsman and Carter, 1981; Nelson and Bernard, 1984; Lewers *et al.*, 1996).

The first requirement to establish a successful hybrid soybean program is the use of a stable male-sterile, female-fertile system; however, the limiting factor is the efficient transfer of pollen from the male to the female parent (Palmer *et al.*, 2001). Insect-mediated cross-pollination has been shown to produce large quantities of hybrid soybean seed (Lewers and Palmer, 1997; Lewers *et al.*, 1996, 1998; Ortiz-Perez *et al.*, 2004). This would facilitate seed production and the further identification of heterotic patterns in soybean. The objective of the research reported in the second study was to compare F_1 hybrid soybean yield and agronomic traits from single-crosses, three-way crosses, and backcrosses (BC_1F_1) produced by male-sterile lines as female parents and a selected group of male parents.

The objective of the research reported in the third manuscript was to evaluate the response to phenotypic recurrent selection for increased seed set on male-sterile, female-fertile soybean lines segregating for male-sterile alleles *ms2*, *ms3*, *ms6*, *ms8*, *ms9*, and one uncharacterized male-sterile allele using bees as pollinator vector. Recurrent selection is a method of population improvement, designed to improve population performance, it involves the systematic selection of desirable individuals from a population followed by recombination of the selected individuals to

form a new population (Fehr, 1991). In soybean, RS schemes have been used to improve traits such as grain yield (Kenworthy and Brim, 1979; Piper and Fehr, 1987; Holbrook *et al.*, 1989; Burton *et al.*, 1990; and Werner and Wilcox, 1990); seed-protein content (Brim and Burton, 1979; Miller and Fehr, 1979; Holbrook *et al.*, 1989; and Xu and Wilcox, 1992); seed-oil content (Burton and Brim, 1981) iron-deficiency chlorosis (Prohaska and Fehr, 1981), and seed size in male-sterile plants (Tinius *et al.*, 1993).

Nuclear male-sterility with insect-mediated cross-pollination has been successfully used in recurrent selection schemes in soybean. Wilson *et al.* (1981) implemented three cycles of mass recurrent selection in an attempt to increase the percentage of seed oleic acid in a population derived from an $F_{3.4}$ progeny of cross-pollination between two exotic lines segregating for the *ms1* male-sterile allele. Burton and Brim (1981) reported an increase in seed-oil content using high oil content lines as male parents to insect-mediated pollination of male-sterile plants segregating for the *ms1* allele.

Recurrent selection strategies in soybean have been mainly used to increase traits directly related to agronomic performance. However, selection to increase seed set on male-sterile plants *per se* has received minimal attention. Graybosch and Palmer (1988) used *ms1ms1*, *ms2ms2*, and *ms3ms3* male-sterile plants to evaluate seed-set using alfalfa leaf cutter bees and honey bees as pollinators with two planting dates. Seed set on male-sterile plants as a percentage of male-fertile

plants ranged from 1.6% to 32.6%. Roumet and Magnier (1993) evaluated seed set on male-sterile plants segregating for the *ms2* allele in caged plots with alfalfa leaf cutter bees as pollinator. The seed set observed represented 60% of their fertile counterpart.

Although insect-mediated cross-pollination has been used to produce seed on male-sterile plants, traits influencing preferential pollination on male-sterile, female-fertile soybean lines have not been clearly elucidated. Preferential pollination observed through seed-set suggested that selection for high seed-set on male-sterile plants can be attained. Thus selected male-sterile, female-fertile lines could be suitable to produce larger amounts of hybrid soybean seed.

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EVALUATION OF INSECT-MEDIATED SEED SET AMONG SOYBEAN LINES SEGREGATING FOR MALE STERILITY AT THE *ms6* LOCUS

A paper to be published in Field Crops Research

E. Ortiz-Perez, H.T. Horner, S.J. Hanlin, and R.G. Palmer

ABSTRACT

Currently, there is no economical way to produce large quantities of F₁ hybrid soybean seed in the USA. One of the fundamental requirements for hybrid seed production is the availability of a stable male-sterile, female-fertile system. However, the more challenging barrier is the efficient transfer of pollen from the male parent to the female parent. This could potentially be achieved through pollinator insects. Our observations suggested that seed set on male-sterile, female-fertile plants is a good indicator of insect attraction. The objective of this study was to evaluate seed set among male-sterile, female-fertile lines segregating for male-sterile, female-fertile *ms6* allele by using *Megachile rotundata* as pollinator vector. Thirty-four pairs of near-isogenic lines, the *ms6 w1w1* donor parent, and its two isogenic lines *W1w1* and *w1w1* segregating for male-sterile (*ms6*) allele were used. The *W1* locus controls flower color and hypocotyle pigmentation. Seed-set was evaluated on field-grown plants in 2001, 2002, and 2003 near Ames, IA. Although the observed seed set was not commercially acceptable, our results indicated significant differences in seed set among lines. This suggests that preferential attraction of pollinators occurred, and selection among male-sterile, female-fertile lines could be used to

obtain female parents suitable to produce larger amounts of hybrid soybean seed. In addition, the effect of flower color on seed set was statistically significant. White-flowered lines (*w1w1*) produced more seed set compared to purple-flowered lines (*W1W1*). Lastly, the important effect of year suggested that the effect of environmental conditions on seed set among lines segregating for male sterility was of paramount importance to plant-pollinator interactions. This needs to be assessed in order to establish an efficient hybrid soybean program.

INTRODUCTION

Since soybean [*Glycine max* (L.) Merrill] is a self-pollinated species, hybrids for commercial production have received little attention. One reason is that manual cross-pollination to produce large quantities of hybrid soybean seed is difficult and time-consuming. The low success rate, and the few seeds obtained per pod have contributed to the difficulty of producing large quantities of hybrid seed. Commercial success of F₁ hybrid soybean will require the efficient transfer of pollen from the male parent to the female parent (Palmer et al., 2001). The use of nuclear male sterility and cytoplasmic-genetic male sterility in soybean has raised the real possibility that F₁ hybrid soybean could be produced commercially. Mutations that selectively eliminate male reproductive function and leave female function unimpaired have potential application in hybrid seed production (Horner and Palmer, 1995).

Insect pollen vectors may be the preferred means by which pollen can be transferred between parents. There are over 3000 species of bees in North America. Most are solitary bees, but a well-known minority of them is social, meaning that they live together in colonies and cooperate in colony tasks. Both solitary and social species are important in crop pollination, but the social species are more easily managed. The honey bee, *Apis mellifera* (L.) represents the best example of social organization. In this species, the colony is the basic reproductive unit, which contains a single long-lived reproductive queen, a large number of non-reproductive workers, and a few males (drones). The workers tend the brood from the time the egg is laid until it is a mature larva, feeding it small quantities of food at intervals during each day. Even more significant in terms of social development is the complex division of labor made possible through the development of communication and the constant food inter-exchange and the stimulation they provide through body contact. In contrast, solitary bees occur in groups or colonies, in which each female builds and provisions her own nest without assistance from other bees (Stephen et al., 1969). One solitary insect species used for commercial pollination of crops is the alfalfa leaf cutter bee *Megachile rotundata* (F.), which is extensively employed for the production of alfalfa seed (Pedersen et al., 1972). Although soybean is an autogamous species, it possesses most characteristics of an entomophilus plant species (Juliano, 1976; Erickson and Garment, 1979; Arroyo, 1981; Erickson, 1983; Delaplane and Mayer, 2000; Horner et al., 2003).

Pollinator activity has been reported in soybean (Bradner, 1969); however, traits influencing preferential pollination on male-sterile, female-fertile lines that could be used as female parents in a hybrid system have not been clearly elucidated. Palmer and Lewers (1998) developed 34 pairs of soybean lines by backcrossing the *ms6* and *w1* alleles to 34 recurrent parents. The crosses produced near-isogenic lines cosegregating for the *ms6* allele (male sterility) and *w1* (white flower) alleles in coupling phase or white-flowered lines segregating for the *ms6* allele. Neither seed set, nor the attractiveness to pollinator insects has been evaluated on these near-isogenic lines. Previous studies indicated that seed-set on male-sterile, female-fertile plants is a good indicator of insect pollinator attraction (Lewers and Palmer, 1997; Lewers et al., 1996, 1998; Ortiz-Perez et al., 2004). The objective of this study was to evaluate seed set among male-sterile, female-fertile lines segregating for the *ms6* allele using *Megachile rotundata* as pollinator during three growing seasons near Ames, Iowa.

MATERIALS AND METHODS

Plant material

Thirty-four pairs of soybean lines were developed by backcrossing the *ms6* and *w1* alleles to 34 recurrent parents (Palmer and Lewers, 1998). The lines were developed with the *ms6* donor cytoplasm and the recurrent parent cytoplasm. Each pair of near-isogenic lines consisted of one line developed with the recurrent parent used as male and the donor parent used as female (donor parent cytoplasm),

whereas the other line was developed with the donor parent used as male and the recurrent parent as female (recurrent parent cytoplasm). The two near-isogenic lines differed in their cytoplasm source. Genetic Type T295 was the donor of the *ms6* allele (Table 1). It is a white-flowered male-sterile, female-fertile soybean mutant registered as GS-1, PI 533601 (Palmer and Skorupska, 1990). The *Ms6* locus affects pollen production; *Ms6*_ plants are fertile, whereas *ms6ms6* plants are female-fertile and completely male-sterile due to tapetal abnormalities. Male sterility is inherited as a single-recessive gene (Skorupska and Palmer, 1989).

Seventy-one male-sterile lines were evaluated in this study (34 pairs, the donor parent, and its two isogenic lines). Nine pairs were white-flowered, segregating at the *Ms6* locus (*Ms6ms6 w1w1*), and 25 pairs were purple-flowered cosegregating at the *Ms6* and *W1* loci in coupling phase (*Ms6ms6 W1w1*). Nine of the recurrent parents were high-yielding accessions introduced into the United States from China, Japan, and the former USSR; five lines were ancestors of modern soybean cultivars in maturity group II and contributed more than 55% of the northern gene pool for cultivars released between 1971 and 1981 (Delannay et al., 1983); seven lines were important modern cultivars which represented five different public breeding programs, and 13 lines were commercial cultivars or breeding lines from eight different companies. All 34 near-isogenic pairs were similar to their respective recurrent parent for pubescence color, growth habit, pod-wall color, seed-coat color, and maturity.

Field studies

The near-isogenic male-sterile lines, the donor parent, Genetic type T295H (*Ms6ms6w1w1*), and its isogenic lines *Ms6ms6W1W1* and *Ms6ms6W1w1* were grown at the Bruner farm near Ames, IA, in the summers of 2001, 2002, and 2003. The experiments were conducted in fields with a Clarion-Nicollet loam soil type. Each year the lines were grown in four-row plots, 1.8 m long, spaced 76 cm between rows. Twenty seeds per meter were planted. A randomized complete block design (RCBD) was used with four replications per male-sterile line. Plants were not thinned. At flowering, alfalfa leaf cutter bees (*Megachile rotundata*) domiciles were placed in alley-ways surrounding the plots. Each domicile contained about 300 bee pupae. One domicile per 14 soybean plots was used. Flower and pubescence color were recorded. After flowering, the domiciles were removed. At maturity, male-sterile plants were identified visually within segregating rows (male-sterile plants produced fewer pods compared to male-fertile plants and stems remain green in most sterile plants). The number of male-sterile plants and number of seeds/male-sterile plant were recorded. The pollen source for the male-sterile plants was assumed to come from the fertile sibling plants within each segregating row or adjacent sibling rows. For each line, 40 male-fertile plants within segregating families were single-plant threshed and progeny tested the next season. The segregating progeny provided the male-sterile plants that were used for evaluation. Again 40 male-fertile plants within segregating families were single-plant threshed. They were progeny tested the next season. Soil temperature, air temperature, rainfall, relative humidity, and wind speed records were obtained throughout from the IEM (Iowa environmental

mesonet) No irrigation was applied. The same procedure was followed in summers 2002 and 2003.

Statistical analysis

Data were subjected to Analysis of Variance with PROC GLM of SAS (SAS 2003). A RCBD was used where the linear model was:

$$Y_{ijkl} = \mu + Y_i + B(i)j + L_k + YL_{ik} + BL(i)jk + C_l + YC_{il} + BC(i)jl + LC_{kl} + YLC_{ikl} + BLC(i)jkl$$

Where:

μ = the general mean; Y_i = the effect of the i^{th} year; $B(i)j$ = the effect of the j^{th} block nested in the i^{th} year; L_k = the effect of the k^{th} male-sterile line; YL_{ik} = the interaction effect between the i^{th} year and the k^{th} male-sterile line; $BL(i)jk$ = the interaction effect between the j^{th} block nested in the i^{th} year and the k^{th} male-sterile line; C_l = the effect of the l^{th} cytoplasm source; YC_{il} = the interaction effect between the i^{th} year and the l^{th} cytoplasm source; $BC(i)jl$ = the interaction effect between the j^{th} block nested in the i^{th} year and the l^{th} cytoplasm source; LC_{kl} = the interaction effect between the k^{th} male-sterile line and the l^{th} cytoplasm source; YLC_{ikl} = the interaction effect between the i^{th} year, the k^{th} male-sterile line, and the l^{th} cytoplasm source; $BLC(i)jkl$ = the interaction effect between the j^{th} block nested in the i^{th} year, the k^{th} male-sterile line, and the l^{th} cytoplasm source.

Replications and years were treated as random factors; male-sterile lines and cytoplasm source as fixed factors. The mean number of seeds per male-sterile plant across lines was compared for the three years. Selected orthogonal contrasts were computed for flower color, pubescence color, and origin of the recurrent parents. Effects were considered significant in all statistical calculations if $P < 0.05$. For the donor parent T295H (*Ms6ms6w1w1*), orthogonal contrasts for flower color were computed by comparisons with its isogenic lines *Ms6ms6W1w1* and *Ms6ms6W1W1*.

RESULTS AND DISCUSSION

Environmental conditions

The combined analysis showed significant differences in seed set for the effects of lines and years (Table 2). No significant effect for cytoplasm source was observed, suggesting that differences in seed-set were not related to the effects of cytoplasm genome. Significant interactions for year X male-sterile line, and year X male-sterile line X cytoplasm source were observed, so individual analyses were conducted for each year. The seed yield across male-sterile lines was 0.72 seeds/male-sterile plant in 2001, 3.48 in 2002, and 2.44 for 2003. The lowest seed set was observed in 2001, which could be due to climatic conditions. A clear pattern regarding differences in soil temperature, and wind speed records across years was not observed; however, air temperature fluctuation was slightly less in 2001, compared to 2002 and 2003. Warmer nights were observed during the critical period of flowering that was recorded between the first week of July and the first

week of August (Figure 1). Frantz et al. (2004) reported that night respiration increased 4% per °C in soybean which caused leaf mass to decrease significantly. Considerable reduction of leaf area along with increasing seed growth rate could limit the total photosynthate available for seed growth (Seddigh and Jolliff, 1984), which could result in more seed abortions. The proportion of soybean flowers developing into mature pods ranges from 20 to 70 % depending upon variety (Van Schaik and Probst, 1958), and environmental factors such as soil moisture (Swen, 1933), temperature, and photoperiod (Van Schaik and Probst, 1958).

Relative humidity in 2001 was higher and fluctuated more during the critical flowering period compared to 2002 and 2003. A large variation in total rainfall was observed across years. In 2001, total rainfall from July through August was less than one half compared to 2002 (Figure 1), the most favorable year for seed set. Water stress imposed during flowering reduces photosynthesis and the amount of photosynthetic assimilates allocated to floral organs, and thus might also increase the rate of flower abortion (Raper and Kramer, 1987). Since the plots were not irrigated, rainfall was considered a key factor affecting the plants growth and development. The poor performance of the lines in 2001, the year with the smallest amount of accumulated rain, seems to supports this observation.

Seed set on male-sterile lines

The effect of line on seed set was significant (Table 2). The male-sterile lines that presented the highest mean values were 82-165 from Land O' Lakes, PI

297544, an accession from the former USSR, A.K. Harrow, an ancestor of modern cultivars, and Hack, a public line (Table 3). The lowest means were observed in Hoyt, a public line, AX2858 from Asgrow Seed Co., P596-13 from Pioneer Hi-Bred International, and Richland, an ancestor of modern cultivars from China (Table 3). In general, the mean number of seeds/male-sterile plant observed across male-sterile lines was low; considerable less than that reported by Lewers et al. (1996) for lines cosegregating for the *ms6* allele (male sterility) and *w1* (white flower) alleles. These authors recorded 28.6, 18.2, and 9.3 seeds/male-sterile plant for the three plant spatial patterns they tested to evaluate seed set by using honey bees as a pollinator. In our experiment, the mean across lines was only 2.23 seeds/male-sterile plant. This difference could be due to differences in the methods used. Lewers et al. (1996) rouged the male-fertile sibling plants at the first trifoliolate stage. In our experiment, male-fertile sibling plants were not rouged, and spacing between plants was denser at flowering time when bees were carrying out the pollination. As a result of the higher plant density, any moisture stress would be magnified resulting in a reduction in seed-set on the male-sterile plants. Previous studies showed that the incidence of cross-pollination by insects in soybean is greatest among plants in close proximity and decreases with increased distance from the pollen source (Boerma and Moradshahi, 1975; Caviness, 1966; Jaycox, 1970; Nelson and Bernard, 1979; Handel, 1983; De Jong, 1993).

Another important aspect in this comparison between experiments is the pollinator used. In our experiment, the alfalfa leaf cutter bee was used as

the pollinator. Leaf cutter bee preferences related to plant spatial patterns in soybean have not been determined. According to Chiang and Kiang (1987), is possible to increase the rate of outcrossing and seed yield in soybeans by attracting bee visitation using a high density stand with each plant surrounded by a different genotype. In alfalfa, leaf cutter bees forage mainly on flowers exposed to the sun, missing the many shaded flowers in a dense stand (Pedersen et al., 1972). This could help to explain the poor seed set observed across male-sterile lines in years 2001, 2002, and 2003, when compared to the less dense plantings of Lewers et al. (1996). Another factor could be the threshold preference of nectar quality and volume content in soybean nectaries for alfalfa leaf cutter bees. For honey bees, response thresholds change with quality of sugar offered (10-50%), and the roles of foragers. Bees returning with pollen have response thresholds lower than those returning with nectar (Pankiw et al., 2001). Mean nectar sugar content in soybean has been reported between 37 to 45% (Erickson, 1975; Kettle and Taylor, 1979).

Plant-pollinator interaction is a very complex process. The effectiveness of pollination in plants is determined largely by three factors: 1) number of pollinators that visit the plant; 2) number of flowers each pollinator probes during its visit to the plant; and 3) effectiveness of the pollinator transferring appropriate pollen at each flower (Faegri and Van Der Pill, 1971; Cresswell and Galen, 1999). The 34 pairs of near-isogenic lines were selected as a random sample of high-yielding accessions from China, Japan, the former USSR, public sources, and from private companies.

Their attractiveness to pollinators had not been evaluated previously. The fact that their fertile recurrent parent was high-yielding did not necessarily correspond with high seed set in converted male-sterile near-isogenic lines. One reason could be that fertile soybean plants do not benefit from insect pollination (Rubis, 1970), whereas sterile plants need to be attractive to pollinators in order to produce seed-set. However, according to Erickson (1975) a significant yield increase in cv. Corsoy and cv. Hark was observed as result of pollination activity of honey bees. Yet, interactions between plants and pollinators are influenced also by both biotic factors (floral structure, timing of anthesis, quantity and quality of floral rewards, presence of others pollinator species) (Erickson 1975, Corbet, 1990), and abiotic factors (i.e., temperature, wind, solar radiation) (Corbet, 1990). Year X male-sterile line interaction was present (Table 2); however, ranking of the lines for seed set was similar across years for the ten highest -yielding lines and the lowest ten lowest-yielding lines.

Selected orthogonal contrasts were estimated for flower color, pubescence color, and origin of the recurrent parent. For the latter, lines were sub-grouped into ancestors, modern cultivars, public lines, private lines, and accessions from China, the former USSR, and Japan.

Recurrent parent origin

Differences in seed set between new cultivars and ancestors of modern cultivars were significant only in 2002, which was the best year for seed-set (Table

4). Lines from private companies yielded statistically significant less seed-set when compared to public, Chinese, former USSR, and Japanese lines. Differences in seed-set between Chinese and Japanese cultivars were not significant in any year (Table 4). Plant introductions or accessions introduced from Asia, selections from these introductions, or natural crosses that arose from these introductions, share a common gene pool (Sleper and Shannon, 2003). It was not unexpected that male-sterile lines with recurrent parents from the same geographical area would perform similarly. Although the private lines as a group were not high seed set lines, the line that presented the highest seed set in this study, 82-165, was one released by a private company (Land' O Lakes Inc., Table 1). 82-165 is a white-flowered line with indeterminate growth habit, tawny pubescence, and plant height of 81 cm. Hoyt, the lowest seed set line, was an F₋₅ derived line from Harcor X Elf (Cooper et al., 1991), has determinate growth habit, purple flowers, tawny pubescence, and plant height averaged 50 cm.

Flower color

Variation in seed set between purple-flowered and white-flowered lines was observed. Orthogonal contrasts showed significant differences among white and purple-flowered lines across years (Table 5). White-flowered lines outyielded purple ones (Figure 2), but the variation in seed-set across years for white-flowered lines was higher compared to purple-flowered lines (data not shown). Our results differed from the report by Gay et al. (1999) who found that overall, purple-flowered soybean

lines did not significantly differ from white-flowered lines for yield, seed protein, and seed weight when evaluated as $F_{2:5}$ families segregating for purple, pink, and white flower color.

When the donor parent, Genetic Type T295H *Ms6ms6w1w1* (white flower color) was compared with its isogenic line *Ms6ms6W1w1* (in which selfed progeny segregates for flower color producing plants with white-flower color and plants with purple-flower color), and *Ms6ms6W1W1* (in which selfed progeny produce only purple-flower color plants), the results did not support the general observation that white-flowered lines had more seed-set. *Ms6ms6W1W1* (isogenic line purple flower color) had higher seed-set (Figure 3) compared to *Ms6ms6W1w1* (segregating white/purple flower color) and the donor parent T295H *Ms6ms6w1w1* (white flower color). White and purple flower color in soybean are controlled by a single gene with purple (*W1*) being dominant (Bernard and Weiss, 1973). Homozygous recessive *w1w1* alleles produce pink flower color in soybean when in the presence of the non-allelic gene *W1* by modifying the expression of purple pigmentation (Stephens and Nickell, 1992).

One possible explanation for this difference is that there was a differential interaction of the *w1* and *W1* alleles with the genetic background of the donor line that favored the dominant homozygous condition *W1W1* over the heterozygous *W1w1* and the homozygous recessive *w1w1*. Skorupska and Palmer (1989) reported a pleiotropic effect of the *ms6* allele in flower size in families from Genetic type T295H *Ms6ms6w1w1*. Male-sterile plants (*ms6ms6w1w1*) had a smaller size

flower when compared to fertile, the purple flower *Ms6_W1W1* plants. Since there is a close genetic linkage between the *Ms6* and *W1* loci (3.14 ± 0.80 recombination value) more than 92% of *w1w1* white plants are expected to be male-sterile (Palmer et al., 1998).

The seed set observed in our experiment for Genetic Type T295H *Ms6ms6w1w1* was not significantly different from *Ms6ms6W1w1* (data not shown). This could be explained by the fact that in selfed progeny from the *Ms6ms6W1w1* isogenic line, male-sterile plants are likely to have white flower color because of the close coupling phase linkage of the *ms6* and *w1* loci. Whether a possible interaction between white flower color and small flower size influenced the seed-set through pollinator rewards (nectar quality and volume, volatiles, etc.) remains to be tested. An alternative explanation for the higher seed-set observed in the white-flowered near-isogenic lines is that differences were related to the genetic background of the lines regardless of the effect of the white flower color.

The highest lines for seed-set were quite diverse, but the common factor was that they were white-flowered lines, while the lowest seed-set lines were purple-flowered lines (Table 3). Robacker et al. (1983) stated that environmental conditions which promote greater flower production, larger flower size, more intensely colored flowers, and higher nectar secretion promote greater honey bee attractiveness. In soybean, honey bees seem to be equally attracted to white- and purple-flowered lines (Jaycox 1970; Mason 1979). Even though pollinator preference among petal color variants has been reported in several plant species (Levin, 1972; Waser and

Price, 1981; Brown and Clegg, 1984; Schoen and Clegg, 1985; Epperson and Clegg, 1987; Stanton et al., 1986, 1989; Levin and Brack, 1995), the literature presents contradicting reports depending on pollinator species and frequency of polymorphic phenotypes. Stanton et al. (1989) tested the effect of petal color on mating patterns in radish, *Raphanus raphanistrum* (L.). They reported that the cabbage white butterfly *Pieris rapae*, the most common pollinator, visited yellow flowers 50% more when compared to white flowers. Epperson and Clegg (1987) evaluated visitation patterns by pollinators in an *Ipomoea purpurea* population presenting white and purple color flowers. They reported that bumble bees, the most common pollinator, showed no preference when white-flower-plants were common, but discriminated against the white-flower color petal when it was rare. Our findings about the preference of alfalfa leaf cutter bees for male-sterile plants presenting white-color flower (such preference observed through a higher seed-set) were unexpected, since the literature reports indicate pollinator preference toward colored flowers.

White-flowered, male-sterile lines showed two-fold seed-set compared to purple-flowered lines (Table 5), even when white-flowered lines accounted for only 29% out of the total number of male-sterile lines tested. Severson (1983) reported significant differences between purple and white soybean varieties for fructose and glucose content, nectar volume and total carbohydrate content per flower. White-flowered varieties had a more uniform carbohydrate content per flower throughout the day than did purple-flowered lines. Alfalfa-leaf-cutter-bee color preference has not been reported in soybean. In alfalfa, Goplen (1970) observed that leaf cutter

bees preferred purple flowers to yellow flowers to a degree that influenced pod and seed set. One factor that could account for the variation observed among white- and purple-flowered lines is differences in the ultraviolet patterning. White and purple flowers of soybean lines were observed under UV light (265 nm range) but no visible pattern was observed that could serve as a guide for pollinators (unpublished data, 2005). Whether those white-flowered lines have traits associated with nectar quality and/or volume, or volatile production, remain to be tested.

Flower color affected cross-pollination in our studies, but it is difficult to separate the effect of flower color with the genetic background of the near-isogenic lines. When the effect of flower color in seed set was compared in a common genetic background (T295H and its isogenic lines), higher seed set was observed in purple-flowered plants (Figure 3). The effect of color also may be confounded by the effects of nectar quality and quantity, volatiles, etc, in pollinator reward, variables that were not determined in this study.

Pubescence color

Variation in seed set for pubescence color was observed (Figure 4). Orthogonal contrasts showed significant differences among lines with gray pubescence and tawny pubescence (Table 5). One effect of trichome color is to regulate temperature. In soybean, pubescence color may influence the microclimate of the canopy and, consequently, yield. Morrison et al. (1994) evaluated yield, stability, and general adaptability in soybean lines differing in pubescence color.

They reported that seed yield in tawny lines was higher in years or locations receiving < 2600 CHU (Corn Heat Units) of accumulated temperature compared to gray lines. The effect of dark trichomes is related with improvement in heat absorption during the day. In our study, lines with gray pubescence had more seed-set compared to lines with tawny pubescence. The advantage observed for gray lines could be related to the fact that the location and the years where the lines were tested did not present a cool-season climate where tawny lines possibly would perform better. Although gray lines performed better than tawny ones (Table 5), the role of pubescence color and its effect on seed-set in male-sterile lines remains elusive. The influence of pubescence color on seed set could be indirect. It might affect leaf-surface reflectance, and modify the microclimate of the plants by altering the canopy heat load (Morrison et al., 1994), and not be directly related to pollination reward.

Cytoplasm source

The cytoplasm source effect was not significant among male-sterile lines. The effect of the interaction line x cytoplasm source was not significant, only the triple interaction; year X line X cytoplasm source was significant (Table 2). This could be as result of differential seed set observed among cytoplasm sources only in the year when the highest seed set was observed (2002).

CONCLUSIONS

This study evaluated the feasibility of hybrid soybean seed-production on a group of soybean lines segregating for male sterility at the *ms6* locus. Since a large number of lines were tested, some factors involved directly in pollinator attraction and/or reward could not be evaluated. Preferential pollination was present among male-sterile lines observed through seed set, suggesting that selection among male-sterile, female-fertile lines can be made in order to obtain female parents suitable to produce hybrid soybean seed. The effect of flower color in seed set was significant. White-flowered lines had more seed set than purple-flowered lines, which was unexpected. Since the seed set observed was not suitable for commercialization, more research needs to be conducted on male-sterile lines to determine which traits are involved in insect cross-pollination. The important effect of year on seed set suggested that the influence of environmental conditions on seed set among male-sterile lines was of paramount importance to plant-pollinator interactions and needs to be assessed in order to establish an efficient hybrid soybean program.

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Table 1. Male-sterile lines and donor parents evaluated for seed-set. Flower color, pubescence color and origin of the recurrent parents are listed.

Line	Flower color	Pubescence color	Pedigree information for the recurrent parent
Ancestors of modern cultivars			
A.K. Harrow	White	gray	Selection from FC 30761 from China
Mandarin (Ottawa)	Purple	gray	Selection from PI 36653 from China
Mandarin	Purple	gray	PI 36653 from China
Manchu	Purple	tawny	PI 30593 from China
Richland	Purple	gray	PI 70502-2 from China, no. 8225
Plant introductions from the former USSR			
PI 297544	White	gray	USSR, no. II-2-269, Primorszkaja 529
PI 370059	Purple	gray	USSR, no. 5622
PI 384474	Purple	gray	USSR, no. 4987, VNIIMK7
Plant introductions from China			
PI 427099	White	gray	China, Jilin no. 3
PI 91167	Purple	gray	China, no.6622
PI 261474	White	gray	China, Kohoju

Table 1. (continued).

Line	Flower color	Pubescence color	Pedigree information for the recurrent parent
Plant introductions			
from Japan			
PI 227333	White	gray	Japan, Ohozyu
PI 416941	Purple	gray	Japan, no. H-020063, Houten Hakuby
PI 417076	Purple	gray	Japan, no. H-020169, Koushurei 224
Public lines			
Hack	White	gray	Nickell et al., 1985
Hardin	Purple	gray	Fehr et al., 1983
<i>Ms6ms6W1W1</i>	Purple	gray	Palmer and Skorupska, 1990
<i>Ms6ms6W1w1</i>	Purple	gray	Palmer and Skorupska, 1990
T295H	White	gray	Palmer and Skorupska, 1990
<i>(Ms6ms6w1w1)</i>			
BSR 101	Purple	gray	Tachibana et al., 1987
Corsoy 79	Purple	gray	Bernard and Cremeens, 1988
Elgin	Purple	tawny	Fehr and Bahrenfus, 1984
Century	Purple	tawny	Wilcox et al., 1980
Hoyt	Purple	tawny	Cooper et al., 1991
Private lines			
82-378	White	gray	Land O' Lakes, Inc.

Table 1. (continued).

Private lines	Flower color	Pubescence color	Pedigree information for the recurrent parent
82-165	White	tawny	Land O' Lakes, Inc.
CX155	Purple	gray	DeKalb Genetics Corp.
Glenn	Purple	gray	ProfiSeed, Inc.
A3307	White	tawny	Asgrow Seed Co.
AX2858	Purple	tawny	Asgrow Seed Co.
AG-020	Purple	tawny	Asgrow Seed Co.
J201	Purple	gray	Mycogen Seeds
S1346	Purple	gray	Novartis Seeds, Inc.
G3197	Purple	gray	Novartis Seeds, Inc.
P596-13	Purple	gray	Pioneer Hi-Bred Int'l.
P422-57	Purple	gray	Pioneer Hi-Bred Int'l.
P3010-02	Purple	tawny	Pioneer Hi-Bred Int'l.

Table 2. Combined analysis of variance for seed-set on male-sterile lines in Ames, Iowa for the 2001, 2002, and 2003 growing seasons.

Source of variation	Degrees of freedom	Mean squares
Year	2	545.12***
Male-sterile line	33	49.60***
Year X male-sterile line	66	12.96***
Cytoplasm source	1	0.36NS
Male-sterile line X cytoplasm source	33	2.08NS
Year X male-sterile line X cytoplasm source	66	1.76**

** , *** Significant at the 0.01, and 0.001 probability levels respectively; NS = not significant.

Table 3. Ranking of male-sterile lines across years for seed-set and mean values, in Ames, Iowa for the 2001, 2002, and 2003 growing seasons.

Lines	Flower color	Mean no. seed /male-sterile plant /season			
		2001	2002	2003	Mean
82-165	White	4.13	6.96	5.09	5.39
PI 297544	White	1.21	9.45	5.18	5.28
A.K. Harrow	White	1.46	9.47	3.52	4.82
Hack	White	2.31	5.22	6.36	4.63
PI 91167	Purple	0.86	8.47	4.2	4.51
PI 261474	White	0.59	7.33	4.83	4.25
PI 227333	White	0.68	8.17	3.02	3.96
Hardin	Purple	0.56	3.72	5.32	3.20
PI 370059	Purple	0.29	4.85	4.31	3.15
W1W1 (isoline of T295H)	Purple	0.46	6.28	2.48	3.07
BSR 101	Purple	0.34	4.9	3.89	3.04
Corsoy 79	Purple	1	3.2	3.78	2.66
CX155	Purple	0.96	4.1	2.28	2.45
G3197	Purple	0.95	3.09	2.98	2.34
Mandarin	Purple	0.45	2.88	3.57	2.30
Elgin	Purple	0.86	2.57	2.79	2.07
A3307	White	0.39	3.03	1.77	1.73
PI 417076	Purple	0.11	2.74	2.33	1.73
Glenn	Purple	0.77	2.15	2.16	1.69
PI 427099	White	0.49	3.18	1.07	1.58

Table 3. (continued).

Lines	Flower color	Mean no. seed /male-sterile plant /season			
		2001	2002	2003	Mean
S1346	Purple	0.49	2.34	1.8	1.54
W1w1 (isoline of T295H)	Purple	0.63	2.05	1.61	1.43
Century	Purple	0.54	1.95	1.74	1.41
Mandarin (Ottawa)	Purple	0.47	1.88	1.46	1.27
82-378	White	0.48	2.57	0.74	1.26
PI 416941	Purple	0.05	2.46	1.25	1.25
T295H (w1w1)	White	0.43	1.84	1.34	1.20
P 422-57	Purple	0.82	1.82	0.94	1.19
P3010-02	Purple	0.73	1.66	0.73	1.04
J201	Purple	0.39	1.89	0.65	0.98
AG-020	Purple	0.57	1.51	0.79	0.96
Manchu	Purple	0.35	1.55	0.77	0.89
PI 384474	Purple	0.08	1.25	1.33	0.89
Richland	Purple	0.18	0.84	1.51	0.84
P596-13	Purple	0.58	0.71	1.09	0.79
AX2858	Purple	0.12	0.55	0.23	0.30
Hoyt	Purple	0.38	0.14	0.29	0.27
Mean		0.72	3.48	2.44	
Maximum		4.12	9.47	6.36	
Minimum		0.05	0.14	0.23	
LSD ($\alpha=0.05$)		0.42	1.11	1.18	

Table 4. Statistical significance of orthogonal contrasts for the origin of recurrent parents in seed-set for the 34 pairs of near-isogenic lines in Ames, Iowa for the 2001, 2002, and 2003 growing seasons.

Contrast	Mean no. seed /male-sterile plant /season		
	2001	2002	2003
Modern cultivars vs			
ancestors	NS	*	NS
Private vs ancestors	NS	***	**
Public vs ancestors	NS	**	***
Private vs public	NS	**	***
Private vs Chinese	***	***	***
Private vs USSR	**	***	***
Private vs Japanese	***	***	*
Chinese vs USSR	NS	***	**
Chinese vs Japanese	NS	NS	NS
Chinese vs public	***	***	**
USSR vs Japanese	*	*	***
USSR vs public	**	***	NS
Japanese vs public	***	***	***

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels respectively; NS = not significant.

Table 5. Statistical significance and means for seed-set for flower color and pubescence color contrasts for the 34 pairs of near-isogenic lines in Ames, Iowa for the 2001, 2002, and 2003 growing seasons

Contrast	Mean no. seed /male-sterile plant /season		
	2001	2002	2003
Flower color	***	***	***
Mean no. seed /male sterile plant			
Purple-flowered lines	0.51	2.22	1.73
White-flowered lines	1.66	5.74	3.38
Pubescence color			
Mean no. seed /male sterile plant	**	***	***
Tawny-pubescence lines	0.9	2.22	1.58
Gray pubescence lines	0.67	3.91	2.73

**** , ***** Significant at the 0.01, and 0.001 probability levels respectively

Figure 1. Total accumulated rainfall from June 1 to August 31 in Ames Iowa for the 2001, 2002, and 2003 growing seasons.

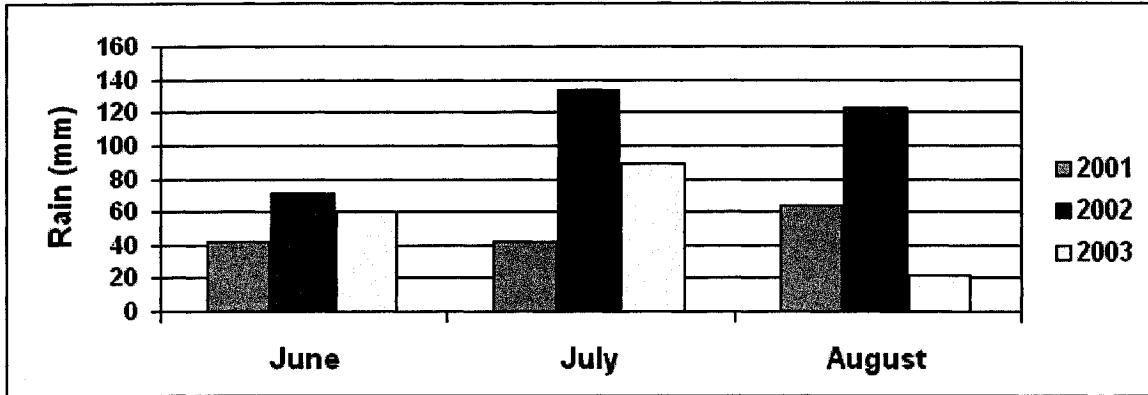


Figure 2. Flower color effect on mean number of seed per male-sterile line for the 34 pairs of near-isogenic lines in Ames, Iowa the 2001, 2002, and 2003 growing seasons.

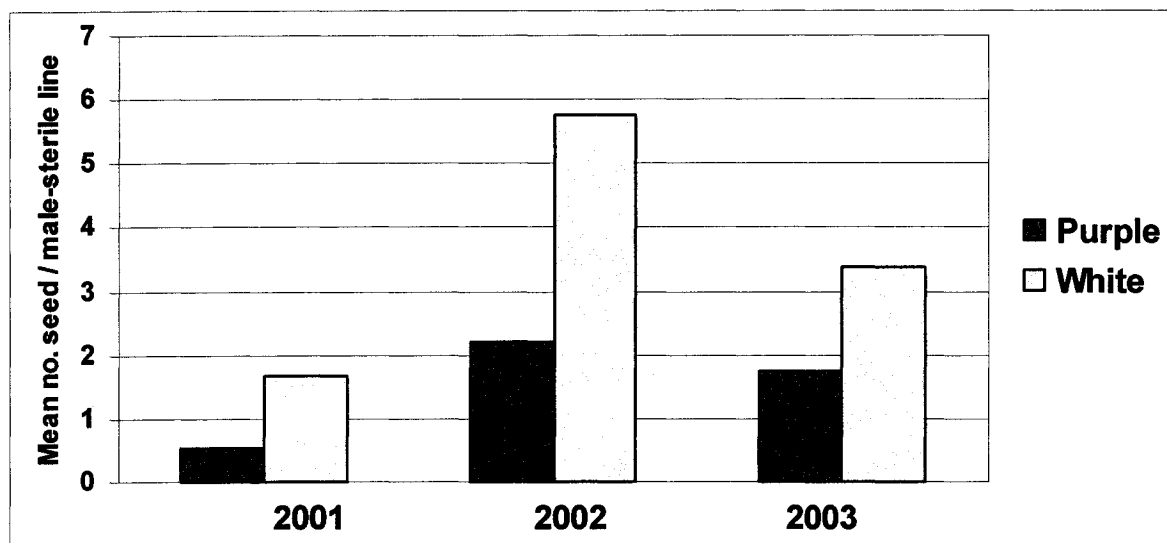
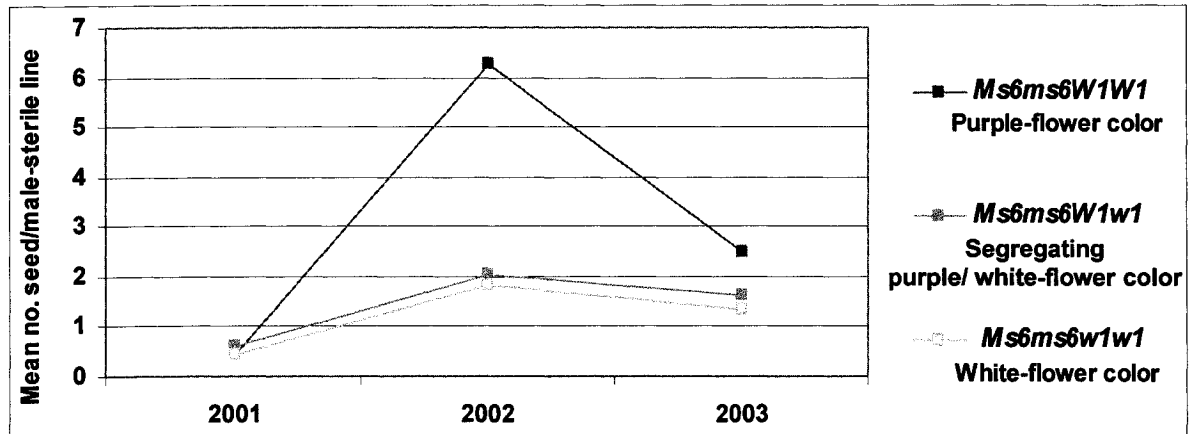
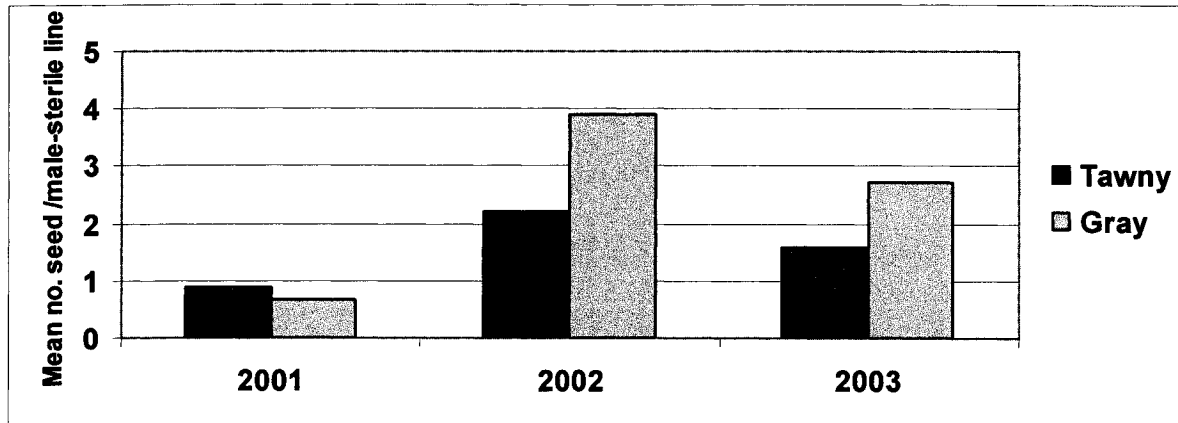


Figure 3. Mean comparison for seed-set among the donor parent Genetic Type T295H *Ms6ms6w1w1*[§] and its isogenic lines *Ms6ms6W1w1*[§] and *Ms6ms6W1W1*[§] In Ames, Iowa for the 2001, 2002, and 2003 growing seasons.



[§]Close genetic linkage between the *Ms6* and *W1* loci ($3.14 \pm 0.80\%$ recombination).

Figure 4. Pubescence color effect on mean number of seed per male-sterile line for the 34 pairs of near-isogenic lines, growing seasons in Ames, Iowa for the 2001, 2002, and 2003 growing seasons.



**INSECT-MEDIATED CROSS-POLLINATION IN SOYBEAN [*Glycine max* (L.)
MERRILL]: I. HETEROSIS EVALUATION**

A paper to be submitted to Crop Science

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ABSTRACT

Manual cross-pollination to produce large quantities of F_1 hybrid soybean seed for yield trials is difficult to obtain and time-consuming. This has been one of the reasons why parental combinations that produce heterosis levels superior to the best pure-line cultivars, (i.e., identification of heterotic patterns) have not been identified in soybean. Insect-mediated cross-pollination has been shown to produce large quantities of hybrid soybean seed and should facilitate the identification of heterotic patterns. The objective of this study was to compare F_1 hybrid soybean yield and agronomic traits from single-crosses, three-way crosses, and backcrosses (BC_1F_1) produced by male-sterile lines as female parents and a selected group of male parents. Bees from the families Megachilidae, Halictidae, Anthophoridae, and Andrenidae were the pollinator vector. In 2003, F_1 seed of biparent crosses and their parental lines were evaluated for agronomic traits at three locations in replicated four-row plots. Also in 2003, BC_1F_1 and three-way cross seed were produced. Evaluation of BC_1F_1 , the three-way crosses, and their parental lines was done in 2004 at one location. High-parent heterosis values (HPH) for single-crosses for grain yield ranged

from -65.70% to +16.17%; for seed-protein content, HPH from -4.34 % to +3.53%, and for seed-oil content from -13.22% to -0.84%. For three-way and BC₁F₁ crosses, grain yield HPH ranged from -25.21% to 41.97 %, for seed-protein content HPH from -2.72% to +1.92 %, and for seed-oil content HPH ranged from -5.87% to +2.22%. Our results indicated that yield heterosis greater than 10% is attainable. To evaluate possible commercial production of hybrid soybean, extensive agronomic research in multiple environments needs to be conducted to determine parental combinations that will produce high heterosis levels.

INTRODUCTION

Exploitation of heterosis or hybrid vigor, i.e., the difference between the hybrid and the mean of the two parents (Falconer and Mackay, 1996) has been studied extensively in allogamous crops and those with mixed breeding systems, including maize, sorghum, pearl millet, rapeseed, onion, sunflower, and cotton, and tomato. Usually, heterosis has been attributed to dominance interactions and masking of deleterious recessive alleles, although other causes of heterosis are possible such as additive X additive epistasis from multiplicative gene action (Schnell and Cockerman, 1992).

Using F₁ hybrids in commercial production realized considerable yield increases, along with other benefits. Earlier maturity and uniformity of the harvested product results in more market potential for farmers. Hybrids offer additional

advantages in crops where important traits are controlled by dominant genes.

Stacking of several useful traits such as disease and herbicide resistance is common in hybrid production systems (Palmer et al., 2003). However, benefits of hybrids are not limited to traits *per se*. One important issue is the protection of parental inbreds used in the production of elite hybrids (Wehner, 1999). Intellectual property rights systems have facilitated commercialization of hybrids by offering legal protection for seed companies discouraging seed saving by farmers. In some cases, the protection is offered by the hybrid itself because of segregation of undesirable traits beyond the F₁ generation.

For self-pollinated crops such as rice, wheat, tobacco, and soybean, use of hybrids has been considered impractical because of strict self-pollination mechanisms that greatly reduce cross-pollination (Palmer et al. 2001). However, in recent years, efforts to overcome the challenge of hybrid seed production in self-pollinated crops have been successful; i.e., hybrid rice in China covers more than 50 % of the planted area (Virmani, 1997, 1999), hybrid wheat achieved yield increases in many countries (Jordaan et al. 1999); and hybrid pigeonpea cultivars have been developed (Plant Breeding News IV, edit. 107, Nov 1999). Unfortunately hybrid production for legumes other than pigeonpea has received little attention. In soybean [*Glycine max* (L.) Merrill], a self-pollinated legume species, manual cross-pollination to produce large quantities of hybrid seed for yield trials is not practical, i.e., not cost effective. Thus, parental combinations that produce heterosis levels superior to the

best pure-line cultivars, (i.e., identification of heterotic patterns) have not been identified in soybean.

The use of nuclear male sterility in soybean has provided some information on levels of out-crossing and heterosis (Graybosch and Palmer, 1988). Male-sterile, female-fertile lines have been used to produce F_1 seed to test for heterosis. Average mid-parent yield heterosis for 2, 8, 27, 18, and 18 hybrid combinations were 28%, 13%, 8%, 2%, and 8%, respectively (Brim and Cockerham, 1961; Hillsman and Carter, 1981; Nelson and Bernard, 1984; Lewers et al., 1996). Most heterosis studies in soybean have been done with spaced F_1 and parental plants, so that application to commercial production is limited.

In soybean, one of the first requirements to establish a successful hybrid program is to develop a stable male-sterile, female-fertile system; however, one important limiting factor is the efficient transfer of pollen from the male to the female parent (Palmer et al., 2001). Although soybean is a self-pollinated crop, it possesses most of the characteristics of an entomophilus plant species (Juliano, 1976; Erickson and Garment, 1979; Arroyo, 1981; Erickson, 1983; Delaplane and Mayer, 2000; Horner et al., 2003). Hence, insect pollen vectors may be the most practical mean by which pollen can be transferred. Insect-mediated cross-pollination has been shown to produce large quantities of hybrid soybean seed (Lewers and Palmer, 1997; Lewers et al., 1996, 1998; Ortiz-Perez et al., 2004), which would facilitate seed production and the identification of heterotic patterns. For hybrid seed

production, single-cross combinations are typically the most productive type of hybrid, followed by modified single-cross, and double-modified single cross. Three-way crosses are considered less productive and less uniform because of the genetic segregation from three parents (Fehr, 1991).

Productivity of single crosses, three-way crosses and backcrosses in soybean has been evaluated with contradicting results. According to Thorne and Fehr (1970a), three-way crosses have shown significant higher mean seed protein, seed oil, and protein + oil content as well as higher grain yield (Thorne and Fehr, 1970b). Cober and Voldeng (2000), however, did not find significant differences for grain yield and protein content among single-crosses and backcross-derived lines. In these studies hybrid seed was produced by manual cross-fertilization.

The objective of this study was to compare F_1 hybrid soybean yield and agronomic traits from single-crosses, three-way crosses, and backcrosses (BC_1F_1) produced by male-sterile lines as female parents and a selected group of male parents. The pollen mediator vectors used were bees from families Megachilidae, Halictidae, Anthophoridae, and Andrenidae.

MATERIALS AND METHODS

Plant materials

Soybean lines with nuclear male-sterile mutations were used as female parents. The selected female parents were male-sterile lines segregating for *ms2*

(L75-0587) (Bernard et al., 1991), *ms2* (A00-39) (Cervantes-Martinez et al., 2005), *ms3* (T284) (Chaudhari and Davis, 1977), *ms6* (T295H) (Skorupska and Palmer, 1989), *ms8* (T358) (Palmer, 2000), and *ms9* (T359) (Palmer, 2000). The lines were selected for high and low seed-set from a group of male-sterile lines that had been evaluated in a three-year experiment at Ames, IA. (unpublished data). Eight male-fertile, female-fertile lines were used as male parents. The male-parents were chosen based on their attractiveness to pollinator insects and/or agronomic characteristics. Female and male parent lines and their combinations are shown in Table 1.

Single-cross hybrid seed production

Single-cross hybrid seeds were produced by using a randomized complete block design (RCBD) with five replications and eight entries. Each entry was the combination of one segregating male-sterile, female-fertile line (female parent) and one male-fertile, female-fertile line (male parent). Each plot was comprised of six rows, the first and the sixth were males and the four center rows were the segregating male-sterile lines. Each row was 4.8 m long, spaced 76 cm between rows and 1.2 m among plots, and the planting rate was 14 seeds per meter. The eight single-combinations were cross-pollinated using alfalfa leaf cutter bees (*Megachile rotundata* F.) as a pollinator in the summer of 2002 at Plainview, Texas (Table 1). At the beginning of flowering, alfalfa leaf cutter bee pupae were placed in a container in the base of a bee board, which consisted of a wooden board with

10.6-cm by 10.6-cm wood cylinders, about 1.2 m. long, with closely spaced holes 0.47 cm in diameter, and 8.89 cm deep. Approximately 10, 000 pupae were placed per bee board, and only one bee board was placed in the center of the experimental plots. The bees emerged as adults in two days. At flowering, the male-sterile plants were identified by their lack of pollen production and were labeled. The male-fertile siblings were rogued. At maturity, number of pods and number of seeds per male-sterile plant were recorded. The two sister lines segregating for *ms2* (A00-39) (Cervantes-Martinez et al., 2005) were cross-pollinated to a commercial high-yielding line in November 2002 at Massai, Chile. The same insect pollinator species and procedures used in Texas also were used in Chile to obtain the hybrid seed.

Three-way crosses and BC₁F₁ hybrid seed production

A seed sample of the F₁ seed obtained at Texas 2002 was planted in an off-season nursery in October 2002, at Isabela, Puerto Rico, under natural photoperiod, and each individual plant was harvested. Fifty F₂ seeds from plants with the most seed within each entry were selected to plant in the same location in January 2003 under controlled photoperiod, which consisted on exposure to continuous light for 15 d after emergence, to 14.5 h days for an additional 35 days, and to natural day length thereafter. Only fertile plants were harvested. The progeny of individual fertile plants were considered as a family. Twenty-four F_{2:3} families per each combination were insect-mediated cross-pollinated to their male-fertile, female-fertile recurrent parents and also to a common parent in the summer of 2003 at Plainview,

Texas (Table 1). Native bees from the families Megachilidae, Halictidae, Anthophoridae, and Andrenidae were observed to carry out the pollinations during the summer of 2003 at Plainview, Texas.

A randomized complete block design (RCBD) with 6 replications and 16 entries was used to produce the seed. Each plot was six rows, the first and the sixth rows were males and the four center rows were the segregating male-sterile lines. Each row was 4.8 m long, spaced 76 cm between rows and 1.2 m among plots, the planting rate was 14 seeds per meter. Each entry was the combination of one segregating male-sterile, female-fertile line and one male-fertile, female-fertile line. At flowering, male-sterile plants were identified and labeled, and male-fertile siblings removed. At maturity, the number of pods and number of seeds per male-sterile plant were recorded.

Field Trials

Single-crosses

F₁ hybrid seed produced in Texas and Chile from each combination was bulked. The cross A00-73 *ms9* X Raiden was not included in field trials in 2003; however, the F₁ seed produced in this cross was advanced to produce the three-way and BC₁F₁ crosses. F₁ hybrid seed from every combination, except one, their parents, and three commercial lines as checks were grown at the Bruner farm near Ames, IA (42°03'N, 93°61'W, altitude 288 m.), Gilbert, IA (42°13'N, 93°65'W, altitude 291 m.),

and Otterbein, IN (40°61'N, 87°32'W, altitude, 253 m.) in 2003. A RCBD design was used with two replications per combination; each replication was a single plot. Each plot consisted of four rows, 5.1 m long, spaced 76 cm between rows and 90 cm among plots. Twenty seeds per meter were planted. At maturity, the two middle rows of each plot were harvested to measure grain yield and lodging, height, seed-protein, and seed-oil content were also determined. Grain yield was estimated based on total weight of the two harvested rows. For plant height, two plants per plot were measured and averaged. For lodging score, all plants in the plot were considered. A scale from 1-5 was used (1 = erect to 5 = prostrate). Seed-protein content and seed-oil content (hereafter referred as protein content and oil content) were analyzed by near infrared reflectance spectroscopy (NIR) at the National Center for Agricultural Utilization Research (NCAUR), Peoria, IL.

Three-way and BC₁F₁ crosses

The female plants from single crosses provided enough hybrid F₁ seed only for the 2003 evaluation. It was not possible to evaluate hybrid seed from single crosses in 2004, only their female and male parents together with the three-way crosses and BC₁F₁ were evaluated. The three-way crosses, BC₁F₁, all the parents and two commercial lines as checks were grown in the field at the Bruner farm near Ames, IA in summer 2004. A RCBD design with two replications per combination and 16 combinations was used; each replication was a single plot. Each plot consisted of four rows, 5.1 m long, spaced 76 cm between rows and 90 cm among plots. Twenty

seeds per meter were planted. At maturity, the two middle rows of each plot were harvested to measure grain yield, protein, and oil content. Grain yield was estimated based on total weight of the two harvested rows. Protein and oil content were analyzed by NIR, at the NCAUR.

Statistical analysis

Analyses of variance were performed for each trait using PROC GLM of SAS v. 9 (SAS Institute, 2003). For the single-cross evaluation, replications and locations were considered random effects, genotypes as fixed effects. For the three-way and BC₁ crosses, replications were considered random effects, and genotypes fixed.

Mid-parent heterosis (MPH) was calculated as:

$$\text{MPH} = \frac{(F_1 - \text{MP})}{\text{MP}} \times 100$$

Where, F_1 is the mean of the F_1 hybrid performance and $\text{MP} = (P_1 + P_2)/2$ in which P_1 and P_2 are the means of the inbred parents for the single crosses, and for the three-way-crosses and BC₁ F_1 crosses, $\text{MP} = [(P_1 + P_2)/2 + P_3]/2$, in which P_1 and P_2 and P_3 are the means of the inbred parents for the single-crosses and the mean of the common parent or recurrent parent, respectively.

High-parent heterosis (HPH) was calculated as:

$$\text{HPH} = \frac{(F_1 - \text{HP})}{\text{HP}} \times 100$$

Where HP= highest parent, ie., the mean of the best parent.

Pearson phenotypic correlation coefficients were calculated to detect associations between heterosis expression for grain yield and either grain yield, plant height, and protein, and oil content using PROC CORR of SAS v. 9.

RESULTS

Hybrid and parental performance

Single crosses

The combined analysis of variance for grain yield showed significant differences for location, lines, and location X lines for single crosses and their parental lines (data not shown). However, when parents and hybrids were analyzed separately, the effect of location was not significant for parents, but it was for hybrids (Table 2). Grain yield mean values for parental lines ranged from 2065.14 kg ha⁻¹ in Ames; 2185.72 kg ha⁻¹ in Otterbein, and 2225.30 kg ha⁻¹ in Gilbert. For hybrids, mean values ranged from 1790.23 kg ha⁻¹ in Ames, to 1797.54 in Gilbert, and 2381.05 kg ha⁻¹ in Otterbein (Table 3). Across locations, the hybrids cross ID 1 and 2, showed the highest grain respectively. The commercial line DSR 11939 was the parent with the highest mean value for grain yield (Table 3).

For plant height, significant differences were observed among and within hybrids and parents; although the effect of location and the interaction location X lines was not significant for either hybrids or parents (Table 2). The combined analysis for lodging score showed a location effect. Significant differences for the interaction

location X lines were observed only among parents; hybrids were more homogeneous in their response to lodging (Table 2). For protein content, significant differences were not observed for either parents or hybrids. Mean values among parental lines and their hybrids were very similar (Table 3), although the mean value for hybrids (396.90 g kg^{-1}) was higher than for parental lines (387.99 g kg^{-1}). For parental lines, the highest value (403.00 g kg^{-1}) was observed in the line A94-20x19 (*ms6*); the lowest value (376.40 g kg^{-1}) was for the line A00-39 (ARS-10-483) (Table 3). The hybrid cross ID 9 and ID 1 showed the highest protein content (411.40 g kg^{-1} and 407.20 g kg^{-1} , respectively), and the lowest value, (384.00 g kg^{-1}), was observed in cross ID 8 (Table 3).

The hybrid cross ID 1 and the commercial line DSR 11939 showed the highest grain yield without a reduction in protein content (Table 3). Oil content, was not significantly different among hybrids, only among parental lines. Contrary to the observation for protein content, the mean for oil content among parents was slightly higher when compared to hybrids (Table 3). The commercial check line DSR 218 had the highest oil content (220 g kg^{-1}), and the lowest value for parents was observed in the line A00-68 *Ms3* (179.2 g kg^{-1}); for hybrids, the highest mean value was observed in the cross ID 3, (204.40 g kg^{-1}), and the lowest value was for the cross ID 9 (179.30 g kg^{-1}) (Table 3). The mean value for oil content for parental lines was 202.24 g kg^{-1} compared to 195.6 g kg^{-1} for hybrids. Although differences among hybrids and parents for oil and protein content were very small, hybrids generally presented higher protein content, and lower oil content than did the parents.

Three-way and BC₁F₁ crosses

Significant differences for grain yield were observed only among hybrids for the BC₁F₁ crosses. The mean for their parental lines was 2349.06 kg ha⁻¹; for BC₁F₁ crosses, 2659.46 kg ha⁻¹ (Table 5). The highest yielding parental line was the commercial line DSR 11939 which averaged 3038.1 kg ha⁻¹, the lowest mean was showed by the parent line A00-68 Ms3 (1924.1 kg ha⁻¹) (Table 4). For hybrids, the BC₁F₁ cross ID 23 showed the highest mean (3155.11 kg ha⁻¹). The lowest value (2000.08 kg ha⁻¹) was observed in the BC₁F₁ cross ID 22 (Table 4).

Significant differences for protein content were observed only in BC₁F₁ crosses. Numerically, for parental lines the highest mean was observed in cv. Hark (418.81 g kg⁻¹), whereas the hybrid with the highest mean was the BC₁F₁ cross ID 26 (420.51 g kg⁻¹) (Table 4). Oil content was significantly different only for parental lines for the three-way crosses (Table 5). The parental commercial line DSR 11939 had the highest mean oil content (190.61 g kg⁻¹); the lowest value was observed in the parental line A94-20x19 (Ms6) (172 g kg⁻¹) (Table 4). For hybrids, the three-way cross ID 13 presented the highest mean value (188.32 g kg⁻¹). The lowest value was observed for the BC₁F₁ ID cross 20, that averaged 177.33 g kg⁻¹. The results observed for protein and oil content in the three-way and BC₁F₁ crosses were very similar to those observed for single crosses. That is, there were no significant differences among hybrids for protein content and for oil content; significant differences were noted only among parents.

Heterosis

Single crosses

Positive and negative heterosis values for grain yield were observed in the combined analysis of variance for single crosses. Across locations Mid-parent heterosis values (MPH) for grain yield were observed ranging from -59.19 to +37%, whereas high-parent heterosis values (HPH) were from -65.70 to +16.17%. The hybrid ID cross 2 showed the highest average MPH value across locations (9.71 %) (Table 6). The hybrid ID cross 9 showed the lowest average MPH and HPH (-34.30% and -41.11%, respectively). All average HPH values were negative. For plant height, average MPH ranged from -3.63% to +20.05%, average HPH values were from -11.53 % to +10.11% (Table 5). The highest value was observed in the hybrid cross ID 2 for both MPH and HPH. The lowest MPH, was observed for the hybrid ID cross 4.

For protein content, MPH values were from -0.62% to +5.80%; HPH ranged from -4.34% to +3.53% (Table 5). The hybrid cross ID 1 showed the highest value for both MPH and HPH (Figure 1), the hybrid cross ID 4, the lowest value for both MPH and HPH (Figure 1). Oil content MPH values ranged from -9.39% to +0.70%, HPH values ranged from -13.22% to -0.84% (Table 5). The hybrid cross ID 7 showed the only positive value for MPH (Figure 1).

Three-way and BC₁F₁ crosses

Positive and negative heterosis values for grain yield were observed for three-way and BC₁F₁ crosses (Table 5). The BC₁F₁ cross ID 23 showed the highest HPH value (+41.97 %). The lowest value (-25.21 %) was observed in the three-way cross ID 12 (Table 6).

Heterosis values for protein content were observed within a narrow range when compared to grain yield (Table 5). The three-way cross ID 15 showed the highest MPH and HPH value (+3.8% and +1.92 %, respectively) (Figure 2). The three-way cross ID 11, showed the lowest value (-2.72 %) (Figure 2). Only 14% of the three-way crosses showed positive heterosis compared to 28% from the BC₁F₁ crosses. The same trend for heterosis values reported for protein content was observed for oil content, that is, the heterosis values were small and within a narrow range (Table 5). The BC₁F₁ cross ID 26, showed the highest value (+4.97% MPH, and +2.22% HPH) (Figure 2), the lowest HPH value (-5.87 %) was observed in the three-way cross ID 12 (Figure 2).

Phenotypic correlations

For single crosses, heterosis for grain yield was significantly correlated with protein content and grain yield (Table 7). Pair-wise correlations among protein and grain yield, grain yield and oil content, and protein and oil content were not significant (Table 7). For the three-way and BC₁F₁ crosses, heterosis for grain yield was significantly correlated with grain yield only in the BC₁F₁ crosses. Oil content was significantly correlated with grain yield only in the three-way crosses (Table 7).

DISCUSSION

Grain yield among single crosses was variable; however, the ranking for the top yielding crosses was very similar across locations (data not shown). The fact that location was significant only for hybrids may reflect their ability to respond to different environmental conditions compared to their parental pure lines which showed more stable yield across locations (Table 3). A significant genotype X environment interaction was observed, but seemed that the best hybrids were only slightly affected by such an interaction, since the hybrids that were better in high yielding environments, also performed good in the low yielding environment. Genetic stability, that is, small G X E interactions in response to environmental variation (biotic and abiotic stresses) is one of the ideal characteristics when breeding for either pure lines or hybrids (Fehr, 1991).

The contribution to yield of the commercial male parent line DSR 11939 (which presented the highest grain yield in the experiment) to their hybrids was observed. The crosses ID 1 and ID 2, respectively, also presented the highest mean for grain yield (Table 3). Their female parents, lines A00-39 and A00-41 are different single plant selection from ARS-10-483. The same trend was observed for the lowest yielding hybrid ID 9. Its male parent line A00-68 *Ms3*, represented the lowest mean for grain yield as well. The observation that the commercial line DSR 11939 produced the best hybrids for grain yield when crossed to A00-39 and A00-41 suggests that more hybrid combinations involving that commercial line as male parent should be evaluated. MPH and HPH observed for single-crosses (Table 5),

were in the range that has been reported in the literature, where high-parent heterosis values from -39% (Weber et al., 1970), +8% (Paschal and Wilcox, 1975), +26% (Chauhan and Singh, 1982), and + 48% (Tain, 1981) have been reported. Manjarrez-Sandoval et al. (1997) reported MPH for yield ranging from +0.8 to +15% for F_2 plants.

The superiority of some hybrids for yield over their mid-parent value suggested that gene action other than additive was present. Quantitatively, heterosis effects can be observed when genetic divergence between parents is present. The vigor showed for such hybrids maybe due to the nullification of epistatic effects of recessive major genes (Miranda-Filho, 1999). The parental combinations evaluated were not chosen based on combining ability tested previously, but rather on traits of the parents *per se* related to pollinator attraction in one parent (mainly the female parent, but also in some of the male parents), and agronomic traits in the other. Although observed heterosis values were within the range reported in the literature, the general mean of all the hybrids was inferior to that of checks and the parental lines. The single-cross combinations were backcrossed to their male parent (high-yielding recurrent parent), and to the commercial line DSR 11939 to form BC_1F_1 and three-way crosses. The observed heterosis values for grain yield of the BC_1F_1 were higher when compared to single-crosses and three-way crosses; however, the general mean for BC_1F_1 crosses was not significantly different when compared to the means of three-way crosses.

Predicting parental combinations that will produce hybrids with superior performance is difficult to achieve, although it is generally believed that maximizing the genetic divergence of the parents will produce the most heterosis (Palmer, et al., 2001). The fact that the BC₁F₁ ID 23 was the highest yielding with the most HPH and numerically superior to the best parent (common male DSR 11939) was unexpected. However, the results in this study are supported by evidence of no significant association between seed yield heterosis and genetic distance between parents in soybean (Cerna et al., 1997). Additive gene action has been reported as a main component in the genetic variance for soybean (Brim and Cockerham, 1961). Models to explain heterosis in self-pollinated crops based on additive x additive epistasis have been proposed (Compton, 1977) and confirmed in peanut (Isleib et al., 1978) and oat (Pixley and Frey, 1991). F₂ generations were not evaluated in our studies because nuclear male-sterility would segregate. However, the fact that the BC₁F₁ crosses showed heterosis suggested that additive x additive epistasis, which does not contribute to inbreeding depression (Compton, 1977; Lamkey and Edwards, 1999) could be present.

The values observed for plant height heterosis are in agreement with reports in the literature where heterosis for plant height has been observed in a very wide range, from -2% to +0.2% (Weber et al., 1970), -50% to +63 % (Raut et al., 1988), -64.6% to +30.3% (Gadag and Upadhyaya, 1995) and -7% to +18.54% (Lewers, 1996). Heterosis for protein and oil content has been observed to be non-significant (Nelson and Bernard, 1984; Loiselle et al., 1990; Lewers, 1996), or mainly negative (Loiselle et al., 1990; Gadag and Upadhyaya, 1995; Sabbouh, 1987). In our study

some of the single-crosses, three-way crosses, and BC₁F₁ crosses, showed positive heterosis values for protein content, which was very important since protein content is one of the major objectives in soybean breeding programs. Although significant positive heterosis for oil has been reported (Leffel and Weiss, 1958; Sabbouh, 1987), for single crosses in our study, heterosis for oil content was the only variable for which 88% of the crosses showed negative values for both MPH and HPH (Figure 1). The parental lines were not selected for high oil content; however, some favorable epistatic interactions present in the parental lines could be broken during recombination which would result in the negative values observed. In contrast, some of the three-way and BC₁F₁ crosses did show significantly positive heterosis values for oil content.

In the presented study, for single crosses, phenotypic correlations between heterosis for grain yield and grain yield, heterosis for grain yield and protein content, and grain yield with plant height were positive and significant. We did not observe significant pair-wise correlations between any of these parameters in the three-way and BC₁F₁ crosses. The highest yielding hybrid BC₁F₁ ID 23, also presented the highest HPH for grain yield, without any significant decrease in protein and oil content. The three-way ID cross12, with the lowest mean for grain yield, also showed the lowest HPH for grain yield, the lowest mean for oil content, and the lowest HPH for oil content. These results suggest that in these crosses, alleles without the expected pleiotropic effect of low grain yield and high protein content, and low protein content with high oil content reported in pure line cultivars, were not

present. Estimates of genetic correlations between yield and protein content in breeding populations are usually negative (Burton, 1985, 1987). Although positive correlations for traits of interest are preferred, the fact that non-significant correlations were observed between heterosis for grain yield and grain yield, heterosis for grain yield and protein content, grain yield and protein content and protein content and oil content was of interest. The results in the presented study are not in agreement with the observations by Wilcox and Cavins (1995), who reported a moderate to strong inverse relationship between seed yield and protein content ranging from $r = -0.23$ to -0.86 . Burton (1987) also reported this inverse relationship. Because of the negative correlation between these traits, methods to select and improve them simultaneously typically require large populations from many different parental combinations.

CONCLUSIONS

Evaluation of agronomic data revealed that positive heterosis was present in some of the crosses tested. Although heterosis is a dynamic attribute strongly affected by the environment, some promising parental combinations were found. Our results revealed that hybrid vigor was present for seed yield. In some combinations the best hybrids yielded between 9.94% and 41.97% over the highest parent. Additionally, these hybrids did not have significant differences in protein and oil content when compared to the best parent. We are now using high-yielding agronomic male parents in insect-mediated crosses in attempts to increase our yield

levels. Then, heterosis levels could be adequately evaluated for their economic significance. A limiting factor for developing efficient hybrid seed production in soybean has been the transfer of pollen from the male parent to the female parent. Insect-mediated cross-pollination has been an economical and efficient option to overcome this barrier. We have used insect pollinators to produce large quantities of hybrid seed and have replicated yield tests in multiple locations. However, caution must be taken. Extensive research in different environments must be conducted to determine what parental combinations will produce the highest heterosis levels

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Table 1. Parental combinations for single-crosses, three-way crosses, and BC₁F₁ crosses. Ames, Iowa 2003 and 2004.

ID number	Single crosses	
	Female	Male
1	A00-39 (ARS-10-483)	DSR 11939
2	A00-41 (ARS-10-483)	DSR 11939
3	A00-39 (ARS-10-483)	Corsoy 79
4	A94-20x19 (<i>ms6</i>)	A00-39 (ARS-10-483)
5	A00-41 (ARS-10-483)	A00-73 <i>Ms9</i>
6	A00-68 <i>ms3</i> (T284)	A00-41 (ARS-10-483)
7	A00-63 <i>ms2</i> (Beeson)	A00-61 Wells
8	A00-39 (ARS-10-483)	Hark
9	A00-72 <i>ms8</i> (T358)	A00-68 <i>Ms3</i>
10	A00-73 <i>ms9</i> (T359)	Raiden
Three-way crosses		
11	A00-39 (ARS-10-483) X Corsoy 79	DSR 11939
12	A94-20x19 (<i>ms6</i>) X A00-39 (ARS-10-483)	DSR 11939
13	A00-41 (ARS-10-483) X A00-73 <i>Ms9</i>	DSR 11939
14	A00-68 <i>ms3</i> X A00-41 (ARS-10-483)	DSR 11939
15	[A00-63 <i>ms2</i> (Beeson) X A00-61 Wells]	DSR 11939
16	A00-39 (ARS-10-483) X Hark	DSR 11939
17	A00-72 <i>ms8</i> X A00-68 <i>Ms3</i>	DSR 11939
18	A00-73 <i>ms9</i> X Raiden	DSR 11939

Table 1. (continued).

ID number	BC ₁ F ₁ crosses	
	Female	Male
19	A00-39 (ARS-10-483) X Corsoy 79	Corsoy 79
20	[A94-20x19 (ms6) X A00-39 (ARS-10-483)]	A00-39 (ARS-10-483)
21	A00-41 (ARS-10-483) X A00-73 Ms9	A00-73 Ms9
22	A00-68 <i>ms3</i> X A00-41 (ARS-10-483)	A00-41 (ARS-10-483)
23	[A00-63 <i>ms2</i> (Beeson) X A00-61 Wells]	A00-61 Wells
24	A00-39 (ARS-10-483) X Hark	Hark
25	A00-72 <i>ms8</i> X A00-68 Ms3	A00-68 Ms3
26	A00-73 <i>ms9</i> X Raiden	Raiden

Table 2. Combined analysis of variance for grain yield, plant height, and plant lodging at Ames, Iowa, Gilbert, Iowa, and Otterbein Indiana 2003.

		Mean squares		
	Source of variation	Grain yield (kg ha ⁻¹)	Plant height (cm)	Plant lodging ^{***}
Hybrids	Location	1,230,016.82**	87.29NS	13.76**
	Lines	919,599.87**	139.59**	0.23NS
	Location X hybrids	206,289.61**	9.62NS	0.17NS
	Error	1,153,080	21.96	0.09
Parents				
	Location	54189NS	565.17NS	8.51*
	Lines	1094942.40**	251.19*	0.80NS
	Location X parents	247619.37**	61.75NS	0.72**
	Error	2207024.28	46.22	0.1158

*, ** Significant at the 0.05 and 0.01 probability levels respectively; NS = not significant.

***Plant lodging score based on a scale from 1-5 (1 = erect to 5 = prostrate).

Table 3. Mean values for grain yield, protein content, oil content, plant height, and plant lodging for parents, commercial checks, and single crosses at Ames, Iowa, Gilbert, Iowa, and Otterbein Indiana 2003.

	Mean grain yield				Mean			
	(kg ha ⁻¹)				Protein content		Oil content	
					(g kg ⁻¹)		(g kg ⁻¹)	
	Ames	Gilbert	Otterbein	Mean				
Parents and commercial checks							Plant height (cm)	Plant lodging*
DSR 11939	2608.29	2649.11	3035.80	2764.40	393.30	214.50	76.11	1.00
DSR 9389	2593.70	3187.60	2427.00	2736.10	382.40	209.90	78.99	1.00
DSR 180	2915.30	2708.90	2359.70	2661.30	392.85	205.75	75.72	1.00
DSR 218	2339.30	2813.80	2427.00	2526.70	394.05	220.00	87.95	1.00
Corsoy 79	1894.81	3010.65	1988.38	2297.95	392.60	210.50	97.97	2.25
A94-20x19 (Ms6)	2117.04	NT	NT	2117.04	403.00	188.75	96.01	3.00
A00-72 Ms8	2060.00	2164.60	2031.20	2085.27	379.00	182.30	70.05	1.60
A00-63 (Beeson)	2070.00	1824.40	2105.35	1999.92	378.50	201.00	86.85	2.00

Table 3. (continued).

	Mean grain yield				Mean			
	(kg ha ⁻¹)				(g kg ⁻¹)			
	Ames	Gilbert	Otterbein	Mean	Protein content	Oil content	Plant height	Plant lodging*
Parents and commercial checks								
Hark	1903.58	1839.20	2222.31	1988.36	396.40	206.35	88.01	2.12
A00-61 Wells	1693.10	2292.72	1695.97	1893.93	390.10	194.85	80.54	1.66
A00-41 (ARS-10-483)	1614.10	1404.32	2310.03	1776.15	386.20	216.30	90.30	1.75
A00-73 Ms9	1915.28	1688.02	1345.08	1649.46	386.30	200.00	71.62	1.50
A00-68 Ms3 (T284)	1511.75	1474.10	1783.70	1589.85	380.80	179.20	88.83	2.37
Mean	2065.14	2225.30	2185.72	2156.23	387.99	202.24	84.51	1.72
LSD ($\alpha=0.05$)	651.44	1555.90	527.11	166.68	23.80	12.70	10.89	0.54
C.V	13.71	29.57	10.20	17.83	2.59	2.58	7.95	19.15
Single crosses								
ID number								

Table 3. (continued).

Single crosses	Mean grain yield					Mean		
	(kg ha ⁻¹)					Protein content (g kg ⁻¹)	Oil content (g kg ⁻¹)	Plant height (cm)
	Ames	Gilbert	Otterbein	Mean				
ID number								
1	2274.94	2426.53	2894.85	2532.11	407.20	203.80	99.01	1.50
2	2210.61	2575.78	2748.65	2511.68	398.00	203.35	97.01	1.63
3	1818.94	2609.87	2427.00	2285.27	391.70	204.45	93.87	2.00
4	1824.63	1965.93	2748.65	2179.74	385.50	191.60	92.22	2.25
5	2160.90	1249.29	2514.72	1974.97	397.20	188.75	96.98	2.12
6	1365.55	1731.76	2046.86	1714.72	392.50	187.70	87.93	2.00
7	1646.26	1329.06	2222.31	1732.54	399.20	199.30	85.44	2.00
8	1739.83	1103.90	1900.66	1581.46	384.00	200.65	93.72	2.12
9	1093.61	742.37	1812.94	1216.31	411.40	179.35	79.06	2.50
Mean	1790.23	1797.54	2381.05	1993.11	396.90	195.66	92.25	1.98
LSD ($\alpha=0.05$)	442.22	793.89	467.67	334.74	22.10	18.48	7.85	0.51
C.V.	10.16	16.89	6.35	11.13	2.3	3.88	5.07	15.28

Table 3. (continued)

NT= not tested.

**Plant lodging score based on a scale from 1-5 (1 = erect to 5 = prostrate).

Table 4. Mean values for grain yield, protein content, and oil content for parents and commercial checks, three-way crosses, and BC₁F₁ crosses. Ames, Iowa 2004.

Parents and commercial checks	Mean		
	Grain yield	Protein content	Oil content
	(kg ha ⁻¹)	(g kg ⁻¹)	(g kg ⁻¹)
DSR 11939	3038.13	402.89	190.61
GH 4190	2727.20	396.10	188.16
GH 4189	2666.50	393.61	185.21
Raiden	2534.60	413.55	173.36
Hark	2503.00	418.81	184.21
A00-41 (ARS-10-483)	2368.50	403.33	181.28
Corsoy 79	2274.90	397.76	184.13
A00-39 (ARS-10-483)	2263.30	416.13	181.89
A94-20x19 (<i>Ms6</i>)	2222.30	399.20	172.00
A00-63 (Beeson)	2222.30	378.00	182.20
A00-61 Wells	2198.90	404.93	181.17
A00-73 <i>Ms9</i>	2146.30	404.42	179.59
A00-68 <i>Ms3</i>	1924.10	413.81	176.97
Mean	2406.48	404.15	181.97
LSD ($\alpha=0.05$)	789.91	23.07	9.24
C.V.	13.88	2.41	2.14

Table 4. (continued).

Mean			
Three-way crosses	Grain	Protein	Oil
ID number	yield	content	content
	(kg ha ⁻¹)	(g kg ⁻¹)	(g kg ⁻¹)
11	2503.02	411.37	182.05
12	2272.02	411.29	179.41
13	2833.45	402.91	188.32
14	2716.48	402.49	183.00
15	2576.13	412	182.87
16	2865.61	409.22	187.88
17	2901.28	411.45	181.15
18	2716.48	403.24	186.45
BC₁F₁ crosses			
ID number			
19	2672.62	405.45	185.55
20	2754.5	408.09	177.33
21	2558.58	401.5	180.46
22	2000.08	409.59	177.33
23	3155.1	408.45	182.82
24	2587.82	414.55	181.82
25	2760.35	419.91	181.29
26	2786.66	420.51	183.50

Table 4. (continued).

Mean			
BC ₁ F ₁ crosses	Grain	Protein	Oil
ID number	yield	content	content
	(kg ha ⁻¹)	(g kg ⁻¹)	(g kg ⁻¹)
Mean	2666.26	409.5	182.69
LSD ($\alpha=0.05$)	659.05	21.19	21.19
C.V	11.65	1.91	2.15

Table 5. Mean values for grain yield, plant height, protein content, oil content, mid-parent heterosis(MPH) and high-parent heterosis (HPH) ranges among single crosses, three-way crosses, and BC₁F₁ crosses. Ames, Iowa, Gilbert, Iowa, and Otterbein, Indiana 2003 and 2004.

Trait	Single crosses			
	Parents	Hybrids	MPH	HPH
Grain yield (kg ha ⁻¹)	2156.23**	1993.11**	-59.19 to +37.00NS	-65.7 to +16.17**
Plant height (cm)	85.24*	92.25***	-3.63 to +20.05*	-11.53 to +10.01NS
Seed protein content (g kg ⁻¹)	38.91NS	39.69NS	-0.62 to +5.80*	-4.34 to +3.53NS
Seed oil content (g kg ⁻¹)	20.38**	19.56NS	-9.39 to +0.70**	-13.22 to -0.84**
Three-way crosses				
Grain yield (kg ha ⁻¹)	2406.48NS	2648.57NS	-22.97 to +10.02*	-25.21 to -4.50*
Seed protein content (g kg ⁻¹)	389.11NS	408.01NS	-0.78 to +3.80NS	-2.72 to +1.92NS
Seed oil content (g kg ⁻¹)	203.80*	183.80NS	-2.51 to +1.58NS	-5.87 to -1.20**
BC ₁ F ₁ crosses				
Grain yield (kg ha ⁻¹)	2349.06NS	2659.46*	-6.81 to +42.00*	-15.65 to +41.97*
Seed protein content	404.26NS	411.01*	-0.92 to +2.71NS	-1.94 to +1.30NS
Seed oil content	181.19NS	181.49NS	-0.98 to +4.97NS	-2.47 to +2.22NS

Table 5. (continued).

* , ** , *** Significant at the 0.05, 0.01 and 0.001 probability levels respectively; NS = not significant.

Table 6. Mid-parent value (MPV), high parent value for grain yield and percent heterosis in single crosses, three-way crosses, and BC₁F₁ crosses. Ames 2003 and 2004.

	Grain yield (kg ha ⁻¹)		Heterosis %	
	MPV	HPV	MPH	HPH
Single crosses				
ID number				
1	2377.85	2764.40	+4.77	-8.62
2	2270.27	2764.40	+9.71	-9.15
3	2144.63	2297.95	+7.62	-5.49
4	2054.17	2117.04	-7.76	-13.81
5	1712.8	1776.15	+1.88	-1.43
6	1683	1776.15	+5.89	-3.53
7	1946.92	1999.92	-8.96	-18.98
8	1989.83	1991.31	-13.47	-23.11
9	1837.56	2085.27	-34.30	-41.11
Three-way crosses				
ID number				
11	2653.61	3038.13	-5.67	-17.61
12	2640.45	3038.13	-13.95	-25.2
13	2647.76	3038.13	+7.01	-6.73
14	2592.2	3038.13	+4.79	-10.58
15	2624.37	3038.13	-1.83	-15.2
16	2710.63	3038.13	+5.71	-5.67
17	2500.2	3038.13	+16.04	-4.5

Table 6. (continued).

Three- way crosses ID number	Grain yield (kg ha ⁻¹)		Heterosis %	
	MPV	HPV	MPH	HPH
18	2689.28	3038.13	+1.01	-10.58
BC₁F₁ crosses				
ID number				
19	2272.01	2274.94	+17.63	+17.48
20	2253.01	2263.25	+22.25	+21.7
21	2201.84	2368.52	+16.2	+8.02
22	2257.4	2368.52	-11.39	-15.55
23	2216.46	2222.31	+42.34	+41.97
24	2443.07	2503.6	+5.92	+3.36
25	1962.12	2000.2	+40.68	+38
26	2437.52	2534.6	+14.32	+9.94

Table 7. Pearson phenotypic correlation coefficients among heterosis for grain yield, protein content, and oil content for single crosses, three-way crosses, and BC₁F₁ crosses. Ames, Iowa, Gilbert, Iowa, and Otterbein, Indiana 2003 and 2004.

Single crosses			
Traits	Seed protein content	Seed oil content	Grain yield
Heterosis for grain yield	0.93**	0.04NS	0.74***
Seed protein content		-0.05NS	0.53*
Seed oil content			0.17NS
Three-way crosses			
Traits	Seed protein content	Seed oil content	Grain yield
Heterosis for grain yield	0.04NS	-0.29NS	-0.25NS
Seed protein content		0.60NS	-0.38NS
Seed oil content			0.65*
BC ₁ F ₁ crosses			
Traits	Seed protein content	Seed oil content	Grain yield
Heterosis for grain yield	0.35NS	0.11NS	0.65*
Seed protein content		0.14NS	0.14NS
Seed oil content			0.37NS

* ,*** Significant at the 0.05 and 0.001 probability levels respectively; NS = not significant.

Figure 1. Heterosis values in percent in the x-axis for seed protein content and seed oil content among single crosses. The numbers indicate their corresponding ID number in Table 1.

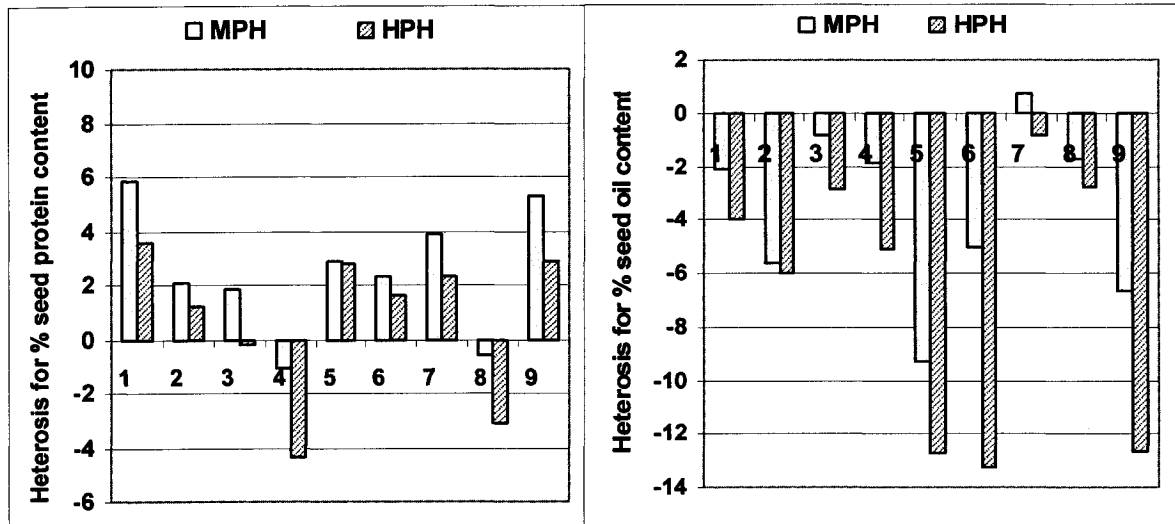
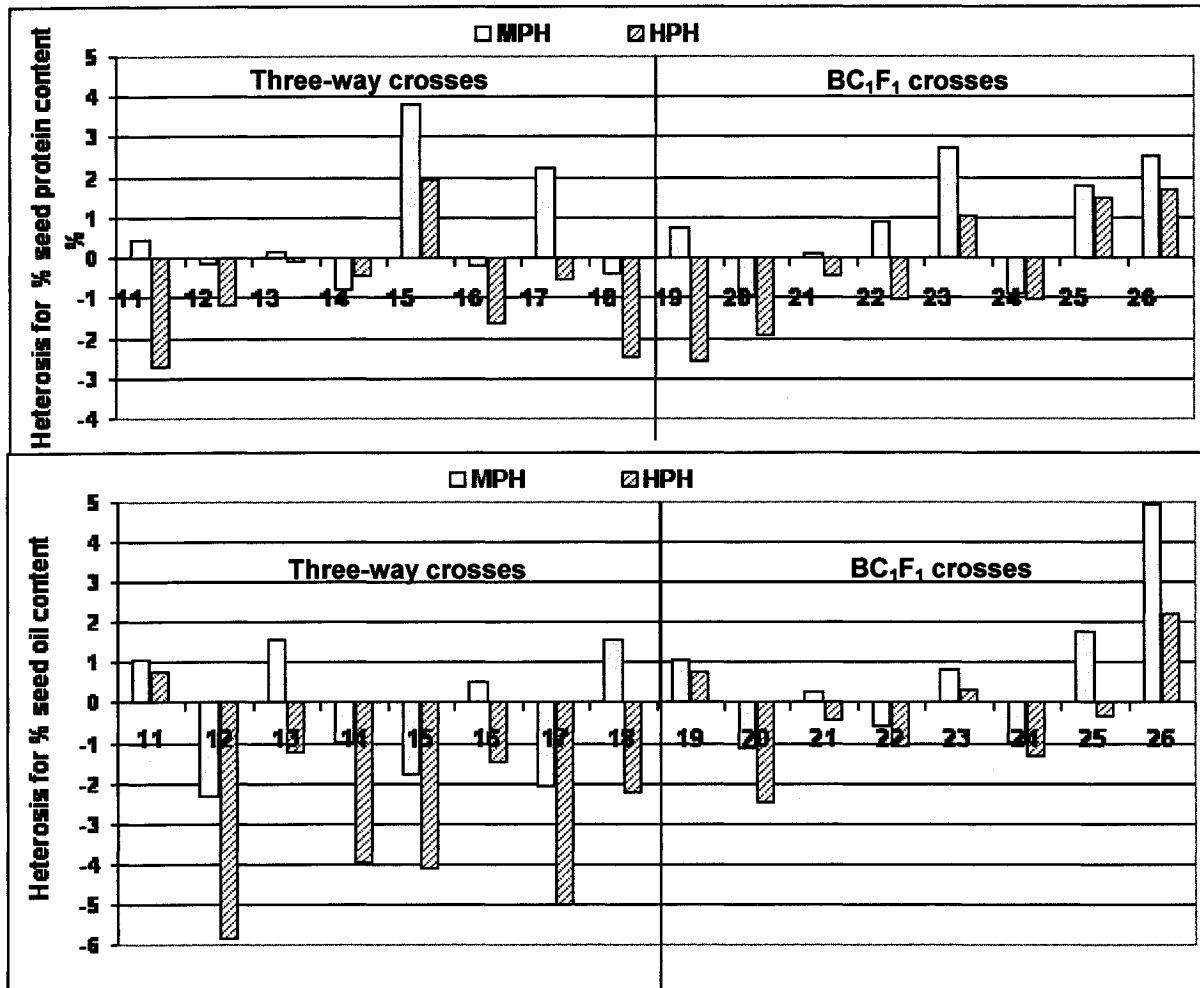


Figure 2. Heterosis values in percent for seed protein content, and seed oil content for three-way and BC₁F₁ crosses. The numbers indicate their corresponding three-way and BC₁F₁ ID number in Table 1.



**INSECT-MEDIATED CROSS-POLLINATION IN SOYBEAN [*Glycine max* (L.)
MERRILL]: II. RECURRENT PHENOTYPIC SELECTION**

A paper to be submitted to Crop Science

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Preface

The data presented in this study are part of a recurrent selection program for increased seed set on a group of selected male-sterile lines, which was started in summer 2002. Since the selection program is currently ongoing, the final evaluation including all the cycles in multiple locations has not been conducted, only data for each cycle, and for each year are included.

ABSTRACT

Recurrent selection is a method for population improvement used in soybean [*Glycine max* (L.) Merrill] to improve traits such as grain yield, seed-protein content, seed-oil content, iron-deficiency chlorosis, and seed size on male-sterile plants. Nuclear male-sterility with insect-mediated cross-pollination has been successfully used in recurrent selection schemes in soybean. However, little attention has been given to selection to increase the seed-set on male-sterile plants *per se*. The objective of this study was to evaluate the response to phenotypic recurrent selection for increased seed set on male-sterile, female-fertile soybean lines segregating for male-sterile alleles *ms2* (low seed set), *ms2* (high seed set), *ms3*,

ms6, *ms8*, and *ms9* using a selected group of males. Bees from families Megachilidae, Halictidae, Anthophoridae, and Andrenidae were the pollinator vectors. The high seed set we observed in this study indicated that selection in a favorable environment was successful in increasing the number of seeds per male-sterile plant. Although differential response was observed among the lines, the seed-set observed would justify the use of some of these improved genotypes as female parents in a hybrid soybean seed-production system.

INTRODUCTION

Recurrent selection (RS) is a method of population improvement, designed to improve population performance. This is accomplished by increasing the frequency of favorable alleles for quantitatively inherited traits, and without decreasing the genetic variability for continued selection (Hallauer, 1985; Fehr, 1991; Lewers and Palmer, 1997). RS includes the systematic selection of desirable individuals from a population followed by recombination of the selected individuals to form a new population. Phenotypic recurrent selection is practiced on an individual plant or the progeny of the plant (vegetatively propagated) in single or replicated plots. Thus, improved populations or lines can be used as cultivars *per se*, as parents of hybrids, inbred lines, clonal cultivars or parents of a synthetic cultivar (Fehr, 1991). In soybean [*Glycine max* (L.) Merrill], RS schemes have been used to improve traits such as grain yield (Kenworthy and Brim, 1979; Piper and Fehr, 1987; Holbrook et

al., 1989; Burton et al., 1990; and Werner and Wilcox, 1990), seed-protein content (Brim and Burton, 1979; Miller and Fehr, 1979; Holbrook et al. 1989; and Xu and Wilcox, 1992), seed-oil content (Burton and Brim, 1981), iron-deficiency chlorosis (Prohaska and Fehr, 1981), and seed size in male-sterile plants (Tinius et al., 1993). The most common used methods for RS in soybean are mass selection and S_n (self pollinated to the n^{th} generation) (Lewers and Palmer, 1997).

Nuclear male-sterility with insect-mediated cross-pollination has been successfully used in recurrent selection schemes in soybean. Wilson et al. (1981) implemented three cycles of mass recurrent selection for increasing the percentage of seed oleic acid in a population derived from $F_{3:4}$ progeny of hybrids between two exotic lines segregating for the *ms1* male-sterile allele. Burton and Brim (1981) reported an increase in seed-oil content percentage using high oil content lines as male parents to insect-mediated pollinated male-sterile plants segregating for the *ms1* allele. Werner and Wilcox (1990) and Xu and Wilcox (1992) used a type of mass selection called S_0 recurrent selection to improve grain yield, maturity, and seed protein content where intermating was insect-mediated and facilitated by using the *ms2* male-sterile allele.

Recurrent selection strategies in soybean mainly have been used to increase traits directly related to agronomic performance. However, selection to increase the seed set on male-sterile plants *per se* has received minimal attention. Graybosch and Palmer (1988) used *ms1ms1*, *ms2ms2*, and *ms3ms3* male-sterile plants to

evaluate seed-set by using alfalfa leaf cutter bees and honey bees as pollinators with two planting dates. Seed set on male-sterile plants as a percentage of male-fertile plants ranged from 1.6% to 32.6%. Roumet and Magnier (1993) evaluated seed-set on male-sterile plants segregating for the *ms2* allele in caged plots with alfalfa leaf cutter bees as pollinators. The seed-set observed represented 60% of their fertile counterpart.

Although insect-mediated cross-pollination has been used to produce seed on male-sterile plants, traits influencing preferential pollination on male-sterile, female-fertile soybean lines have not been clearly elucidated. Previous studies indicated that seed set on male-sterile, female-fertile plants is a good indicator of insect attraction (Lewers and Palmer, 1997; Lewers et al., 1996, 1998; Ortiz-Perez et al., 2004). Preferential pollination observed through seed set suggested that selection on male-sterile plants for high seed-set can be attained. Thus selected male-sterile, female-fertile lines could be suitable to produce larger amounts of hybrid soybean seed. The objective of this study was to evaluate the response to phenotypic recurrent selection for increased seed-set on male-sterile, female-fertile soybean lines segregating for male-sterile alleles *ms2*, *ms3*, *ms6*, *ms8*, and *ms9* by using several bee species as pollinator vectors.

MATERIALS AND METHODS

Cycle 0

Parental lines

Male-sterile mutant lines segregating for single recessive nuclear genes were selected from a group of male-sterile lines evaluated in a three-year experiment at Ames, IA (Unpublished data). Male-sterile, female-fertile lines segregating for *ms2* (L75-0587) (Bernard et al., 1991), *ms2* (Cervantes-Martinez et al., 2005), *ms3* (T284) (Chaudhari and Davis, 1977), *ms6* (T295H) (Skorupska and Palmer, 1989), *ms8* (T358) (Palmer, 2000), and *ms9* (T359) (Palmer, 2000) were grown in the field and evaluated. The selection criterion was seed set on male-sterile plants. Among these, high and low seed-set male-sterile lines were selected. These lines were used as female parents to be insect-mediated cross-pollinated by eight male-fertile, female-fertile soybean lines, which were the male parents. The male-parents were chosen based on either their attractiveness to pollinator insects or agronomic characteristics. Eight single-cross combinations were established (Table 1).

Male-sterile lines field evaluation

Seed set on male-sterile, female-fertile lines was evaluated using a randomized complete block design (RCBD) with five replications and eight entries. Each entry was the combination of one segregating male-sterile, female-fertile line (female parent) and one male-fertile, female-fertile line (male parent). Each plot consisted of six rows; the first and the sixth were males and the four center rows were the segregating male-sterile lines. Each row was 4.8 m long, spaced 76 cm between rows and 1.2 m among plots, the planting rate was 14 seeds per meter. The eight single-combinations were cross pollinated by alfalfa leaf cutter bees

(*Megachile rotundata* F.) as pollinators in the summer of 2002 at Plainview, Texas. At the beginning of flowering, alfalfa leaf cutter bee pupae were placed in a container in the base of a bee board, which consisted of a wooden board with 10.6-cm by 10.6-cm wood cylinders long, with closely spaced holes 0.47 cm in diameter and 8.89 cm deep. Approximately 10, 000 pupae were placed per bee board, and only one bee board was placed in the middle of the experimental plots. The bees emerged as adults in two days. At flowering, the male-sterile plants were identified by their lack of pollen production and labeled. The male-fertile siblings were removed. At maturity, the number of pods per male-sterile plant and number of seeds per male-sterile plant were recorded. Selection for high seed set within each cross-combination was made. A 15 % selection intensity was applied resulting in 56 selected plants, 7 plants per combination.

Cycle 1

Parental lines

F₁ seed from male-sterile plants selected in Texas in 2002 was grown in an off-season nursery in Fall 2002, at Isabela, Puerto Rico. A composite of 25 hybrid seeds per cross-combination was grown under natural photoperiod. At harvest, each plant was threshed individually. Fifty F₂ seeds from three single plants within each cross-combination were selected to plant in the same location in January 2003 with controlled photoperiod, which consisted of exposure to continuous light for 15 d after emergence, to 14.5 h days for an additional 35 days, and to natural day length

thereafter Only fertile plants were harvested. The progeny of individual fertile plants was considered as a family. Twenty-four $F_{2:3}$ -derived families per cross-combination were obtained.

Male-sterile lines field evaluation

The $F_{2:3}$ derived families from each cross-combination were grown in the field in the summer of 2003 at Plainview, Texas, where they were insect-mediated cross-pollinated to their eight male-fertile, female-fertile recurrent parents (BC_1 crosses) and to a common parent (three-way crosses) (Table 1). Sixteen cross-combinations were established (Table 2). A RCBD with 6 replications and 16 entries was used. Each entry was the combination of one segregating male-sterile, female-fertile line (female parent) and one male-fertile, female-fertile line (male parent). Each plot was established in the same manner as for cycle 0. At flowering, male-sterile plants within each segregating row were identified and labeled; the male-fertile siblings were removed. Solitary native bees from the families Halictidae, Anthophoridae, Andrenidae, and Megachilidae were observed carrying out the pollinations. At maturity, the number of pods and number of seeds per male-sterile plant were recorded. Selection for the highest seed-set in male-sterile plants within each segregating $F_{2:3}$ derived families was made. A 15% selection intensity was applied resulting in 128 selected plants, 8 plants per combination. Thus, three-way and BC_1F_1 seed were produced from selected high-seed-set male-sterile plants, and used to start cycle 2.

Cycle 2

Plant materials

Twenty seeds from each single-plant selection from Texas 2003 were grown in an off season nursery in January 2004, at Isabela, Puerto Rico under controlled photoperiod, which consisted on exposure to continuous light for 15 d after emergence, to 14.5 h days for an additional 35 days, and to natural day length thereafter. Selection for the three-way and BC_1F_1 plants with the highest seed-set was made. At harvest, each plant was threshed individually. Three-way F_2 and BC_1F_2 seeds were produced. Twenty-four three-way F_2 and BC_1F_2 derived families were selected.

Male-sterile lines field evaluation

Twenty-four selected three-way- F_2 and BC_1F_2 derived families were grown in the field in summer 2004 at Plainview, Texas, where they were insect-mediated cross-pollinated to their eight male-fertile, female-fertile recurrent parents to produce BC_2F_1 seed, and to a common parent, to produce four-way- F_1 seed (Table 1). Sixteen cross-combinations were established (Table 3). A RCBD with six replications and 16 entries was used. Each entry was the combination of one segregating male-sterile, female-fertile line (female parent) and one male-fertile, female-fertile line (male parent). Each plot was constituted in the same manner as for cycle 0 and 1. At flowering, the process to identify male-sterile plants was the same as for cycles 0 and 1. The bees observed carrying out the pollinations

belonged to the same families observed pollinating the male-sterile plants in cycle

1. At maturity, plant height, number of pods, and number of seed per male-sterile plant were recorded. Only plants with more than 80 pods and at least 50 cm tall were selected for harvest. A selection intensity of 15% was applied

Statistical analysis

For cycle 0, analyses of variance were performed using PROC GLM of SAS (SAS Institute Inc., Cary, NC 2003). Replications were treated as random factors, two-way crosses, as fixed factors. A RCBD was used where the linear model was:

$$Y_{ijkl} = \mu + C_i + B_j + CB_{ij}$$

Where:

μ = the general mean; C_i = the effect of the i^{th} two-way cross; B_j = effect of the j^{th} block; CB_{ij} = the interaction effect of the i^{th} two-way cross and the j^{th} block.

For cycles 1 and 2, analyses of variance were performed using PROC GLM of SAS (SAS Institute, 2003). Replications and families within crosses, were treated as random factors; crosses, as fixed factors. A RCBD was used where the linear model was:

$$Y_{ijkl} = \mu + C_i + F(i)j + B_k + CB_{ik} + FB(i)jk$$

Where:

μ = the general mean; C_i = the effect of the i^{th} cross; $F(i)j$ = effect of the j^{th} family nested in the i^{th} cross; B_k = effect of the k^{th} block; CB_{ij} = the interaction effect of the i^{th} cross and the j^{th} block, $FB(i)jk$ = the interaction effect of the i^{th} family nested in the i^{th} cross with the j^{th} block.

Each cycle was analyzed separately to determine differences among cross-combinations for the evaluated traits. A regression model was conducted where the cycle was considered as an independent variable. The numbers of seeds and number of pods were considered as dependent variables. The regression model was:

$$Y_i = \beta_0 + \beta_1 x_i + \varepsilon_i$$

Where :

Y_i = predicted response of the i^{th} cycle; β_0 = intercept; β_1 = regression slope; x_i = value from the i^{th} cycle ε_i = random error related to the i^{th} observation.

RESULTS AND DISCUSSION

Cycle 0

The combined analysis of variance for seed set showed significant differences among single combinations. The two-way cross that presented the

highest mean values for seed-set was the population ID 7 (120.66 seeds/male-sterile plant). The lowest values were observed in population ID 8 and ID 4, which averaged 58.30, and 66.90 seeds/male-sterile plant respectively (Table 2). For pod number, differences among two-way crosses also were observed. The rank for the crosses for number of pods/male-sterile plants was the same as for seed-set, indicating that number of pods (rather than number of seeds per pod) influenced the total seed-set per male-sterile plant.

The highest seed set observed for the population ID 7 was consistent with the highest seed set observed when male-sterile plants from the line A00-73 *ms9* were evaluated in previous experiments (unpublished data). The same trend was observed for the lowest seed-set, population ID 4; that is, when A00-63 *ms2* Beeson and A00-61 *ms2* Wells were evaluated for seed set, both lines presented the lowest seed-set (unpublished data). These results are in contrast with those reported by Graybosch and Palmer (1988). They found that *ms2* carrying lines produced more seeds per male-sterile plant than did male-sterile lines carrying the *ms1* and the *ms3* alleles. PI's and commercial lines were used as male parents; honey bees and alfalfa leafcutters were the pollinators. One of the lines carrying the *ms2* allele was T259H (Graybosch et al., 1984), the line carrying the *ms3* allele was T273H (Palmer et al., 1980). The *ms3* allele used in the present study came from T284H. The effect of the genetic background on the mutations could explain the differences observed, where interaction with the environment also could influence not only the performance of the lines but also pollinator activity.

The male-sterile lines used as females were evaluated and selected for high and low seed set, thus the rank observed for seed set among male-sterile lines was not totally unexpected, but rather the overall high seed set observed across combinations. Differences in floral abortion among the male-sterile lines could cause a difference in seed-set, although failure in fertilization has been reported to be negligible in contributing to soybean flower abortion (Abernethy et al., 1977). It is possible that asynchrony factors such as phenological differences between the female and adjacent male parent, could cause differences in seed set among the two-way crosses. However, according to Suso et al. (2005), differences in out-crossing rate caused by asynchrony among parents did not explain most of the variation observed in inter-crossed fava bean (*Vicia fava* L.) accessions. These authors attributed such variation to differences in floral traits. The differences observed among cross-combinations for seed-set in the soybean male-sterile plants could reflect differences in the way each male-sterile allele affected traits involved in floral attractiveness; these include nectar quality and volume, volatiles, etc. However, since these traits were not evaluated in the experiment, we could not determine their influence in the rate of out-crossing, and consequently, seed set.

Cycle 1

The analysis of variance for number of seeds per male-sterile plant showed significant differences among crosses and among families from the same cross (Table 3). The mean for number of seeds per male-sterile plant for $F_{2:3}$ segregating families sharing a common male parent (three-way crosses) was significantly

different from that observed in the advanced $F_{2:3}$ families using their recurrent male parent (BC_1 crosses), suggesting an effect of the male parent on seed set. The population ID 31 presented the highest seed-set among the BC_1 crosses; for the three-way crosses, the population ID 10 had the highest mean for seed-set (Table 4).

The general mean for seed set in three-way crosses (89.41 seed/male sterile plant) (Table 4) and BC_1 crosses (75.11 seeds /male sterile plant) was very similar to the observed in cycle 0 (81.85 seeds/male sterile plant) (Table 2). A key observation was the large variation within crosses among $F_{2:3}$ families (Table 5). Among families from the same cross, extreme variation was observed for some of the crosses that yielded the highest seed-set (Figure 1). Such variation was not observed in crosses that presented the lowest seed set (Figure 2). The BC_1 cross population ID 28 and ID 12, performed similarly (Table 4), being both in the lowest rank for seed-set. This also was observed in the two-way cross in cycle 0, which might imply a very low frequency of genes (alleles) in those lines for traits favoring insect-mediated cross-pollination. The results also suggested that both parents contributed genes (or alleles) that were different for pollinator attraction or reward, irrespective of whether the recurrent parent or a common was used as male parent

Cycle 2

The analysis of variance for seed-set showed significant differences among advanced BC_2 crosses and four-way crosses (Table 3). F_2 segregating families using

a common male parent, (four-way crosses) were significantly different from those observed in the advanced F_2 families using their recurrent male parent (BC_2 crosses). Four-way crosses presented the highest mean for seed-set when compared to BC_2 crosses (Table 6).

The four-way cross population ID 17 presented the highest seed-set. Among the BC_2 crosses, population ID 37 had the highest mean for seed-set (Table 6). Variation among families from the same cross also was observed, and was very extreme in some crosses (Table 7). For some four-way crosses, variation for seed-set among families was evident, but to a lesser degree (Figure 3). The population ID 19, ID 21, and ID 24 presented three-fold variation for the highest family mean when compared to the lowest family mean (Table 7). For BC_2 crosses, the same pattern for variation among families was observed (Figure 4). The population ID crosses 35, and ID 39 showed the most extreme values for the highest family mean as compared to the lowest family mean (Table 7), although less intra-family variation for number of seeds was observed. For BC_2 crosses this was expected, since the additive genetic variation within lines is reduced to a half of that present in the preceding generation (Fehr, 1991).

The fact that both three-way crosses and four-way crosses out-yielded the BC crosses in each cycle was of special interest. A different common male parent for the three- and four-way crosses was chosen based on agronomic characteristics and previous reports about their attractiveness to pollinators. The assumption was

that adding new genetic material to the original cross-combinations could cause a change in the mean for seed-set compared to the BC populations. If that assumption was correct, then we could expect that these male parents would bring a different assortment of alleles (since the commercial lines used as male parents are unrelated) for traits related to insect-mediated-cross-pollination. This was the opposite for the BC crosses, where the same male parent was used to create the new population.

Gain per cycle

The mean values for the selection cycles indicated a linear increase for number of pods and number of seeds with recurrent selection from cycle 0 to cycle 2 for the backcrossing strategy (Figures 5, 6). Estimators of the regression parameters were significant for the effects of cycle on number of seeds and on number of pods per male-sterile plant (Table 8), although the regression coefficients for both variables were very low, indicating a weak prediction of the model.

The cross-combinations responded differently to selection. Derived BC₁ and BC₂ families from the crosses ID 5 and ID 8 were the only crosses that had a positive increase from cycle 0 to cycle 2 (Table 9). We observed a negative change from cycle 0 to cycle 1 in most of the cross-combinations (Table 9), which could be related to the fact that from cycle 0 to cycle 1, the evaluated lines were F_{2:3} derived families. After two selfing generations, variability for the traits associated with pollinator activity in some of the lines could play a factor in the decrease of seed set

among the crosses. From cycle 1 to cycle 2, F_2 families were evaluated, where we could observe a positive increase for all the crosses (Table 9).

CONCLUSIONS

The results of this study indicated that phenotypic recurrent selection in a favorable environment can be used to increase the number of seeds per male-sterile soybean plant. The seed set observed would justify the use of some of these selections as female parents in a hybrid soybean seed production system. Mean seed-set per family as high as 304 seeds per male-sterile plant was observed after just two selection cycles. This suggests that very few genes with major effects may be regulating the traits related to pollinator preference and out-crossing. A differential response was observed among the cross-combinations, suggesting there is variability for those traits among the parental lines. Currently, experiments are being conducted to evaluate the selected lines in relation to their fertile parental line; i.e., percent of seed set on male-sterile plants compared to fertile normal plants from the same background.

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Table 1. Parental combinations for two-way crosses, three-way crosses, four-way crosses, BC₁, and BC₂ crosses. Plainview, Texas 2002, 2003 and 2004.

Population		
Two-way crosses		
ID number	Female	Male
1	A00-39 (ARS-10-483)	Corsoy 79
2	A00-39 (ARS-10-483)	Hark
3	A00-41 (ARS-10-483)	A00-73 Ms9
4	A00-63 ms2 (Beeson)	A00-61 Wells
5	A00-68 ms3 (T284)	A00-41 (ARS-10-483)
6	A00-72 ms8 (T358)	A00-68 Ms3
7	A00-73 ms9 (T359)	Raiden
8	A94-20x19 (ms6)	A00-39 (ARS-10-483)
Three-way crosses		
9	A00-39 (ARS-10-483) X Corsoy 79	DSR 11939
10	A00-39 (ARS-10-483) X Hark	DSR 11939
11	A00-41 (ARS-10-483) X A00-73 Ms9	DSR 11939
12	A00-63 ms2 Beeson X A00-61 Wells	DSR 11939
13	A00-68 ms3 X A00-41 (ARS-10-483)	DSR 11939
14	A00-72 ms8 X A00-68 Ms3	DSR 11939
15	A00-73 ms9 X Raiden	DSR 11939
16	A94-20x19 (ms6) X A00-39 (ARS-10-483)	DSR 11939
Four-way crosses		
17	[A00-39 (ARS-10-483) X Corsoy 79] X DSR 11939	GH4190

Table 1. (continued).

Population		
Four-way crosses		
ID number	Female	Male
18	[A00-39 (ARS-10-483) X Hark] X DSR 11939	GH 4190
19	[A00-41 (ARS-10-483) X A00-73 <i>Ms9</i>] X DSR 11939	GH 4190
	[A00-63 <i>ms2</i> (Beeson) X A00-61 Wells] X DSR	
20	11939	GH 4190
21	[A00-68 <i>ms3</i> X A00-41 (ARS-10-483)] X DSR 11939	GH 4190
22	(A00-72 <i>ms8</i> X A00-68 <i>Ms3</i>) X DSR 11939	GH 4190
23	A00-73 <i>ms9</i> X Raiden X DSR 11939	GH 4190
	[A94-20x19 (<i>ms6</i>) X A00-39 (ARS-10-483)] X DSR	
24	11939	GH 4190
BC ₁ crosses		
25	A00-39 (ARS-10-483) X Corsoy 79	Corsoy 79
26	A00-39 (ARS-10-483) X Hark	Hark
27	A00-41 (ARS-10-483) X A00-73 <i>Ms9</i>	A00-73 <i>Ms9</i>
28	A00-63 <i>ms2</i> (Beeson) X A00-61 Wells	A00-61 Wells
	A00-68 <i>ms3</i> X A00-41 (ARS-10-483)	A00-41 (ARS-10-
29		483)
30	A00-72 <i>ms8</i> X A00-68 <i>Ms3</i>	A00-68 <i>Ms3</i>
31	A00-73 <i>ms9</i> X Raiden	Raiden
	[A94-20x19 (<i>ms6</i>) X A00-39 (ARS-10-483)]	A00-39 (ARS-10-
32		483)

Table 1. (continued).

Population		BC ₂ crosses	
ID number	Female		Male
33	[A00-39 (ARS-10-483) X Corsoy 79] X Corsoy 79		Corsoy 79
34	[A00-39 (ARS-10-483) X Hark] X Hark		Hark
35	[A00-41 (ARS-10-483) X A00-73 Ms9] X A00-73 Ms9		A00-73 Ms9
	[A00-63 ms2 (Beeson) X A00-61 Wells] X A00-61		
36	Wells		A00-61 Wells
	[A00-68 ms3 X A00-41 (ARS-10-483)] X A00-41		A00-41 (ARS-10-
37	(ARS-10-483)		483)
38	(A00-73 ms9 X Raiden) X Raiden		Raiden
	[A94-20x19 (ms6) X A00-39 (ARS-10-483)] X A00-39		A00-39 (ARS-10-
40	(ARS-10-483)		483)

Table 2. Mean values for number of pods and number of seeds per male-sterile line for two-way crosses. Plainview, Texas 2002.

Population ID	Mean no. pods per male-sterile plant	Mean no. seeds per male-sterile plant
1	45.41	90.80
2	50.87	101.70
3	52.50	104.90
4	33.425	66.90
5	35.00	73.80
6	35.80	71.60
7	60.30	120.66
8	29.15	58.30
Mean	43.30	81.85
LSD ($\alpha=0.05$)	8.91	17.90
C.V	28.80	30.87

Table 3. Analysis of variance for number of seeds per male-sterile plant for three-way crosses, four-way crosses, BC₁, and BC₂ crosses. Plainview, Texas, 2003, and 2004.

Source of variation		Mean squares
Three-way crosses		
Block		16205.92*
Cross		49512.25***
Family(cross)		8127.15***
Block*family(cross)		3156.97NS
Four-way crosses		
Block		20778.55*
Cross		49236.02**
Family(cross)		19919.50***
Block*family(cross)		22718.33***
BC₁ crosses		
Block		18608.24**
Cross		26378.02***
Family(cross)		10194.28***
Block*family(cross)		952.36NS
BC₂ crosses		
Block		38957.01***
Cross		27363.81***
Family(cross)		16407.88***
Block*family(cross)		21738.59***

Table 3. (continued).

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels respectively; NS= not significant

Table 4. Mean values for number of pods and number of seeds per male-sterile plant for three-way crosses and BC₁ crosses. Plainview, Texas 2003.

Three-way crosses		
Population	Mean no. pods	Mean no. seeds
ID number	per male-sterile plant	per male-sterile plant
9	46.78	94.04
10	61.13	122.89
11	60.02	118.85
12	19.6	40.39
13	43.24	95.57
14	51.19	101.88
15	46.49	107.40
16	24.34	48.21
Mean	44.09	89.41
LSD ($\alpha=0.05$)	11.3	24.54
CV	57.23	60.37

BC₁ crosses		
Population	Mean no. pods	Mean no. seeds
ID number	per male-sterile plant	Per male-sterile plant
25	43.31	83.6
26	44.05	90.32
27	40.1	78.61

Table 4. (continued).

BC₁ crosses		
Population	Mean no. pods	Mean no. seeds
ID number	per male-sterile plant	per male-sterile plant
28	23.56	49.49
29	39.18	82.17
30	14.66	34.17
31	50.6	100.19
32	40.14	82.3
Mean	36.95	75.11
LSD ($\alpha=0.05$)	7.22	15.97
CV	57.8	59.99

Table 5. Mean values for number of pods and number of seeds per male-sterile line for three-way crosses and BC₁ crosses. Plainview, Texas 2003.

Three-way crosses			
Population ID number	No. families	Highest mean no. seeds per family	Lowest mean no. seeds per family
9	3	174.50	61.70
10	15	316	57.03
11	14	195.38	27.2
12	12	84.38	8.5
13	6	187.1	49.78
14	3	159.7	68.71
15	12	173.11	6.23
16	9	104.11	24.8
BC₁ crosses			
Population ID number	No. families	Highest mean no. seeds per family	Lowest mean no. seeds per family
25	13	198.29	17.59
26	10	219.38	33.2
27	11	142.67	35.17
28	12	95.07	18.17
29	13	176.33	28.14
30	4	45.25	26.83
31	11	234.84	13.08
32	16	137.01	33.29

Table 6. Mean values for number of pods and number of seeds per male-sterile plant for four-way crosses and BC₂ crosses. Plainview, Texas 2004.

Four-way crosses		
Population	Mean no. pods per	Mean no. seeds per
ID number	male-sterile plant	male-sterile plant
17	152.6	304.73
18	87.46	174.79
19	111.71	223.03
20	131.3	262.5
21	92.29	184.17
22	120.46	240.60
23	73.87	156.08
24	84.65	168.87
Mean	95.2	189.58
LSD ($\alpha=0.05$)	33.8	39.54
C.V	60.29	60.78
BC₂ crosses		
33	70.44	140.59
34	99.31	198.36
35	84.52	168.73
36	35.77	71.5
37	103.59	204.63
38	68.09	135.88

Table 6. (continued).

BC₂ crosses		
Population	Mean no. pods per	Mean no. seeds per
ID number	male-sterile plant	male-sterile plant
39	65.52	130.87
Mean	75.48	150.52
LSD ($\alpha=0.05$)	16.56	33.13
C.V	50.76	50.89

Table 7. Number of families, highest and lowest number of seed per family within four-way crosses and BC₂ crosses. Plainview, Texas 2004.

Four-way crosses			
Population		Highest mean no. seeds	Lowest mean no. seeds
ID number	No. families	per family	per family
17	6	465.15	244.33
18	8	330.53	6.11
19	13	337.2	94.55
20	3	297.46	228.43
21	11	263.77	78.27
22	5	329.60	147.05
23	17	266.25	38.33
24	6	232.22	63.56
BC ₂ crosses			
Population		Highest mean no. seed	Lowest mean no. seed
ID number	No. families	per family	per family
33	10	201.11	49.07
34	13	271.83	73.11
35	17	269.22	3.40
36	5	140.15	36.33

Table 7. (continued).

BC₂ crosses			
Population		Highest mean no. seed	Lowest mean no. seed
ID number	No. families	per family	per family
37	13	482.00	75.23
38	11	241.50	67.31
39	16	245.00	10.00

Table 8. Regression parameters for number of pods per male-sterile plant and number of seeds per male-sterile plant after two selection cycles. Plainview, Texas 2001, 2002, and 2003.

	Source of variation	Mean squares	F value	Pr > F
No. pods/male-				
sterile plant	Regression	177033.30	126.96	<.0001
	Residual	1394.39		
	R ²	0.08		
			Standard	
		Coefficients	error	t value
	Intercept	33.53	1.71	19.65
	Cycle	15.78	1.40	11.27
	Source of variation	Mean squares	F value	Pr > F
No. seed/male-				
sterile plant	Regression	803231.31	145.37	<.0001
	Residual	5525.37		
	R ²	0.09		
			Standard	
		Coefficients	error	t value
	Intercept	63.78	3.39	18.79
	Cycle	33.64	2.79	12.06

Table 9. Differences in percent for number of pods and number of seeds among cycles for two-way cross populations. Plainview, Texas 2002, 2003, and 2004.

Population	Cycle 0	BC ₁	BC ₂
ID number	Mean no. pods/ male-sterile plant	% relative to cycle 0	% relative to cycle 1
1	45.41	-4.62	+62.64
2	50.87	-13.41	+125.44
3	52.50	-23.62	+110.77
4	33.43	-29.51	+51.82
5	35.00	+11.94	+164.39
6	35.80	-59.05	*
7	60.30	-16.09	+34.56
8	29.15	+37.70	+63.22

Population	Cycle 0	BC ₁	BC ₂
ID number	Mean no. seeds/ male-sterile plant	% relative to cycle 0	% relative to cycle 1
1	90.80	-7.92	+68.16
2	101.70	-11.19	+120.01
3	104.90	-25.06	+114.64
4	66.90	-26.02	+44.47
5	73.80	+11.34	+149.03
6	71.60	-52.27	NT
7	120.60	-16.50	+35.37

Table 9. (continued).

Population	Cycle 0	BC1	BC2
ID number	Mean no. pods/ male-sterile plant	% relative to cycle 0	% relative to cycle 1
8	58.30	+41.16	+59.01

NT= not tested

Figure 1. Mean values for seed number per male-sterile plant for two contrasting families from the BC₁ cross (A00-73 X Raiden) X Raiden. Plainview, Texas 2003.

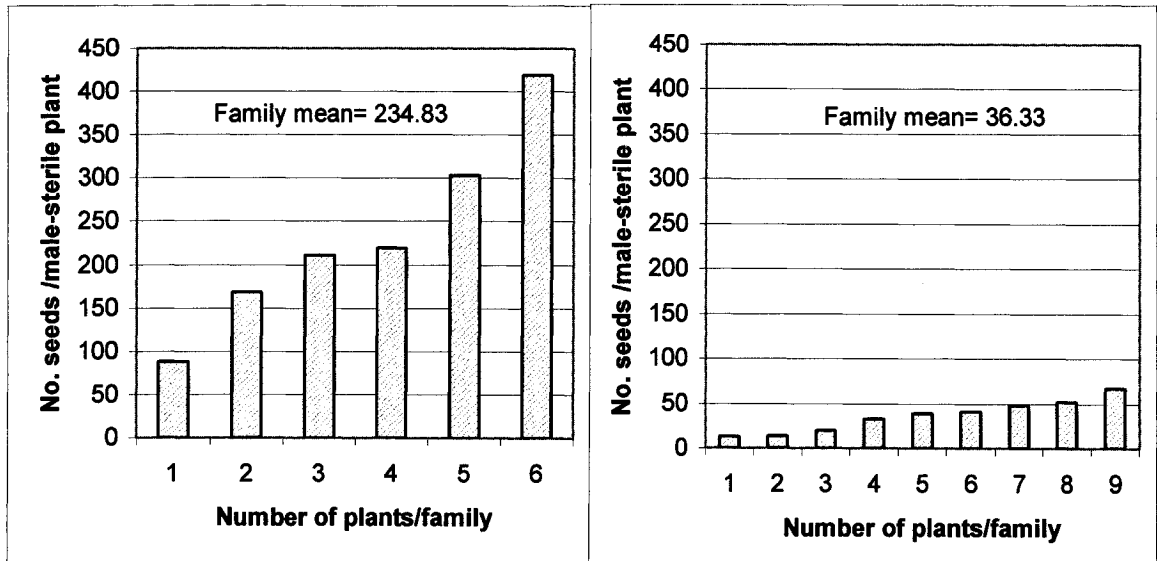


Figure 2. Mean values for number of seeds per male-sterile plant for two families from the BC₁ cross [A00-63 *ms2* (Beeson) X A00-61 Wells] X A00-61 Wells. Plainview, Texas 2003.

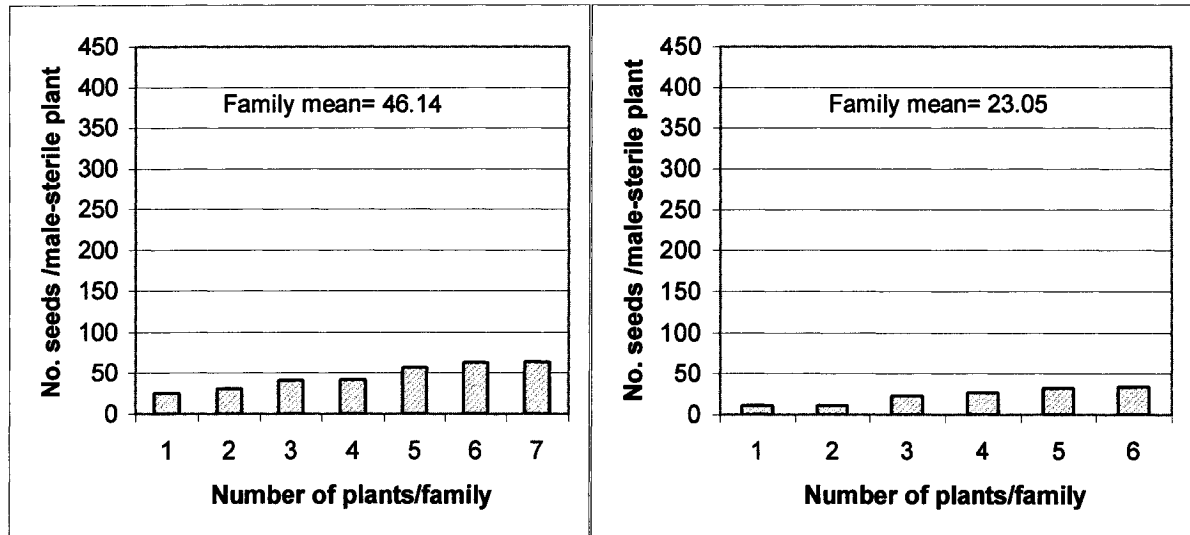


Figure 3. Mean values for number of seeds per male-sterile plant for two families from the four-way cross [A00-41 (ARS-10-483) X A00-73 *Ms9*] X DSR 11939 X GH 4190

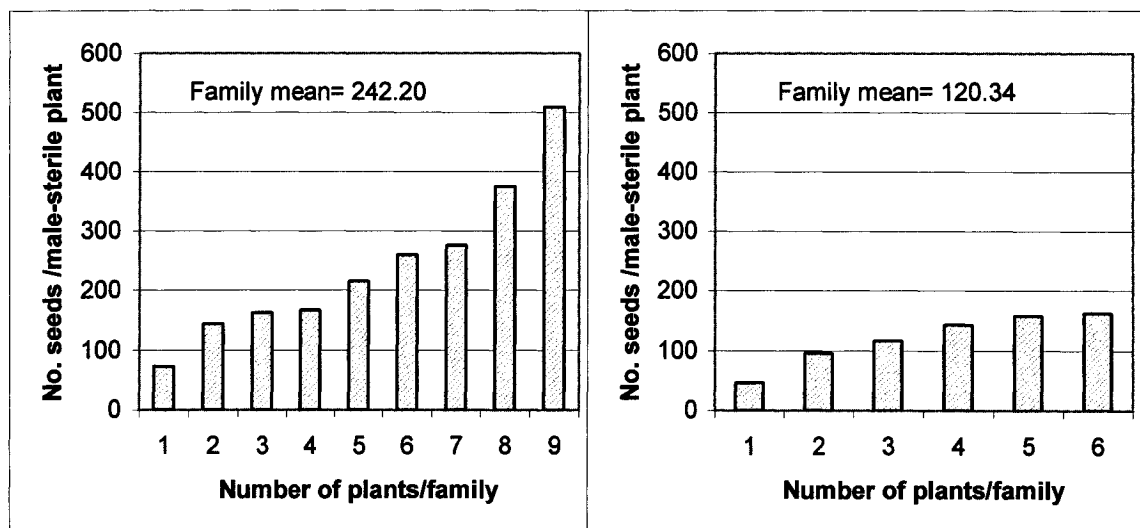


Figure 4. Mean values for number of seeds per male-sterile plant for two contrasting families from the BC₂ cross [A00-39 (ARS-10-483) X Hark] X Hark X Hark. Texas 2004.

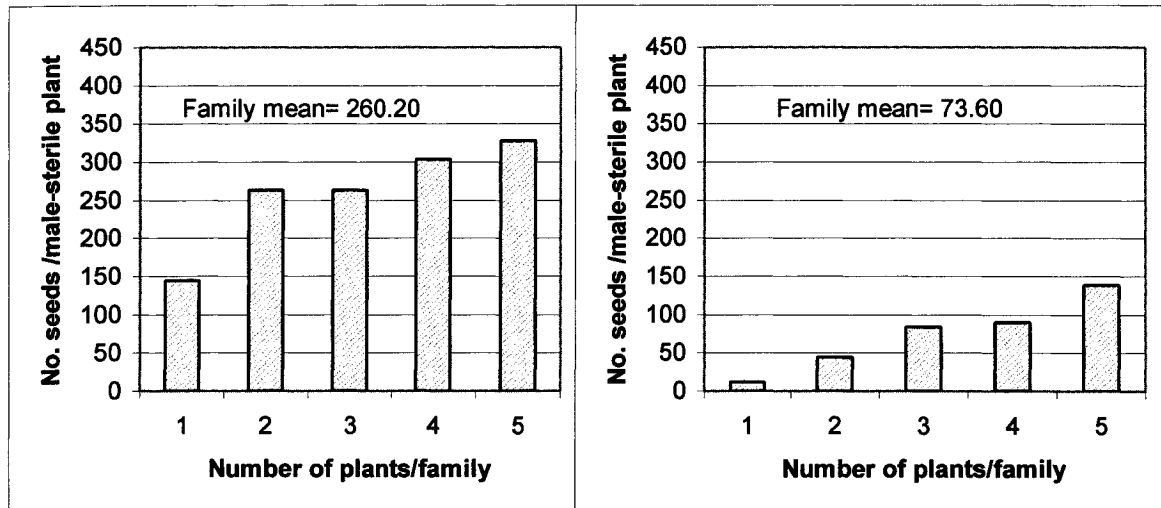


Figure 5. Predicted and observed response to selection for number of pods after two selection cycles. Plainview, Texas. 2002, 2003, and 2004.

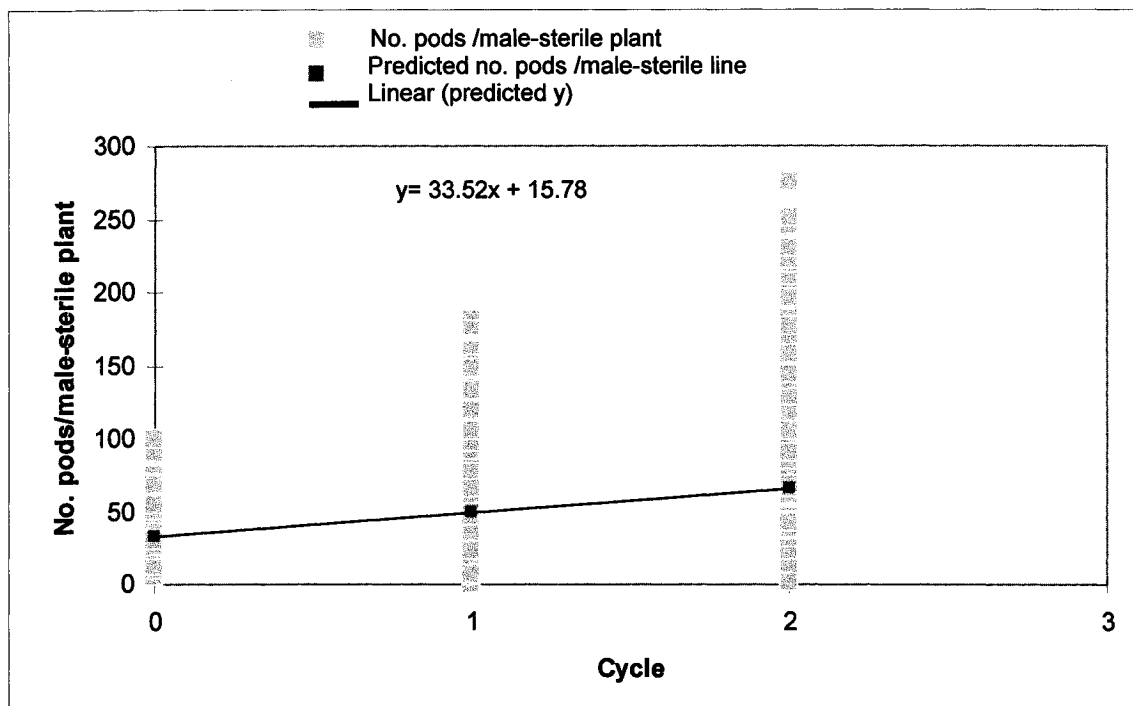
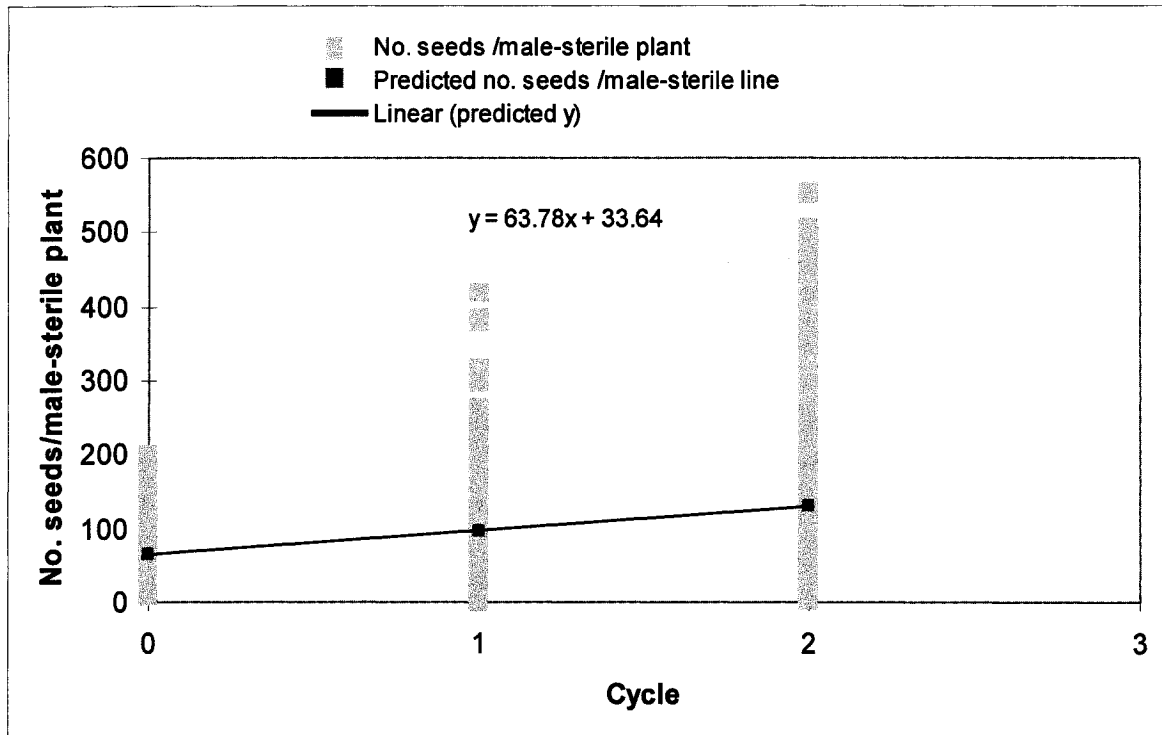


Figure 6. Predicted and observed response to selection for number of seeds after two selection cycles. Plainview, Texas 2002, 2003, and 2004.



GENERAL CONCLUSIONS

The first study evaluated the feasibility of hybrid soybean seed-production on a group of converted male-sterile lines segregating for the *ms6* allele. Since a large number of lines were tested, some factors involved directly in pollinator attraction and/or reward could not be evaluated. Some traits that affected cross-pollination were determined. Differential seed set was observed among the evaluated lines, indicating that preferential pollination was present, which could suggest that selection among male-sterile, female-fertile lines can be made in order to obtain female parents suitable to produce hybrid soybean seed. The effect of flower color in seed-set was significant. White-flowered lines produced more seeds than did purple-flowered lines, which was unexpected. Since levels of seed production were not suitable for commercialization, more research needs to be conducted on male-sterile lines to determine which traits are involved in insect cross-pollination. It is crucial to identify the optimal paramount importance is to identify the growing conditions for male-sterile lines and suitable insect pollinator species used to optimize the out-crossing rate and consequently seed-set.

For the second study, evaluation of agronomic data revealed that positive heterosis was present in some of the crosses tested. Although heterosis is not a static attribute and is strongly affected by the environment, some promising parental combinations were found. My results identified hybrid vigor seed yield. In some combinations, the best hybrids yielded between 9.94% and 41.97% over the highest parent. Additionally, these hybrids did not have significant differences in

protein and oil content when compared to the best parent. Ongoing research is focused in the use of high-yielding agronomic male parents in insect-mediated crosses in attempts to increase our yield levels. Then, heterosis levels can be adequately evaluated for economic significance. A factor limiting the development of an efficient hybrid seed production system in soybean has been pollen transfer from the male parent to the female parent. Insect-mediated cross-pollination has been an economical and efficient option to produce large quantities of hybrid seed in replicated yield test in multiple locations (Lewers et al., 1996; Lewers and Palmer, 1997; 1998; Ortiz-Perez et al., 2004). However, caution must be exerted. Extensive research in different environments must be conducted to determine what parental combinations will consistently produce the highest heterosis levels.

In the third study, results indicated that phenotypic recurrent selection in a favorable environment was successful in increasing the number of seed per male-sterile soybean plant. Mean seed-set per family as high as 304 seeds per male-sterile plant was observed after just two selection cycles. This suggested that very few genes with major effects may regulate the traits related to pollinator preference and out-crossing. A differential response was observed among the cross-combinations, suggesting variability for those traits among the parental lines. Currently, experiments are being conducted to evaluate the selected lines in relation to their fertile parental line; i.e., percent of seed set on male-sterile plants compared to fertile normal plants from the same background. The pollen transfer from the male parent to the female parent has been the most challenging barrier to develop

an efficient hybrid system in soybean. We have used insect pollinators to produce large quantities of hybrid seed in male-sterile plants. The high seed set observed would justify the use of some of these selections as female parent in a hybrid soybean seed production system.