Evaluation of insect-mediated seed set among soybean lines segregating for male sterility at the ms6 locus

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Abstract
Currently, there is no economical way to produce large quantities of F_1 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 2001–2003 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

Keywords
Soybean, Hybrids, Male-sterile lines, Pollinators

Disciplines
Agronomy and Crop Sciences | Plant Biology | Plant Breeding and Genetics

Comments

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Evaluation of insect-mediated seed set among soybean lines segregating for male sterility at the ms6 locus

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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 2001–2003 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.

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Keywords: Soybean; Hybrids; Male-sterile lines; Pollinators

1. 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 entomophilous 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 et al., 1996, 1998; Lewers and Palmer, 1997; 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 M. rotundata as pollinator during three growing seasons near Ames, Iowa.

2. Materials and methods

2.1. 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, PI533601 (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.

2.2. 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 2001–2003. 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 4 replications/male-sterile line. Plants were not thinned. At flowering, alfalfa leaf cutter bees (M. 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.

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

<table>
<thead>
<tr>
<th>Line</th>
<th>Flower color</th>
<th>Pubescence color</th>
<th>Source of pedigree information for the recurrent parent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line</td>
<td>Flower color</td>
<td>Pubescence color</td>
<td>Source of pedigree information for the recurrent parent</td>
</tr>
<tr>
<td>Ancestors of modern cultivars</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.K. Harrow</td>
<td>White</td>
<td>Gray</td>
<td>Selection from FC30761 from China</td>
</tr>
<tr>
<td>Mandarin (Ottawa)</td>
<td>Purple</td>
<td>Gray</td>
<td>Selection from PI36653 from China</td>
</tr>
<tr>
<td>Mandarin</td>
<td>Purple</td>
<td>Gray</td>
<td>PI36653 from China</td>
</tr>
<tr>
<td>Manchu</td>
<td>Purple</td>
<td>Gray</td>
<td>PI36593 from China</td>
</tr>
<tr>
<td>Richland</td>
<td>Purple</td>
<td>Gray</td>
<td>PI70502-2 from China, no. 8225</td>
</tr>
<tr>
<td>PI297544</td>
<td>White</td>
<td>Gray</td>
<td>USSR, no. II-2-269, Primorszkaja 529</td>
</tr>
<tr>
<td>PI370059</td>
<td>Purple</td>
<td>Gray</td>
<td>USSR, no. 5622</td>
</tr>
<tr>
<td>PI384474</td>
<td>Purple</td>
<td>Gray</td>
<td>USSR, no. 4987, VNIIMK7</td>
</tr>
<tr>
<td>PI472099</td>
<td>White</td>
<td>Gray</td>
<td>China, Jilin no. 3</td>
</tr>
<tr>
<td>PI91167</td>
<td>Purple</td>
<td>Gray</td>
<td>China, no.6622</td>
</tr>
<tr>
<td>PI261474</td>
<td>Purple</td>
<td>Gray</td>
<td>China, Kohoju</td>
</tr>
<tr>
<td>PI227333</td>
<td>White</td>
<td>Gray</td>
<td>Japan, Ohozyu</td>
</tr>
<tr>
<td>PI416941</td>
<td>Purple</td>
<td>Gray</td>
<td>Japan, no. H-020063, Houten Hakuby</td>
</tr>
<tr>
<td>PI417076</td>
<td>Purple</td>
<td>Gray</td>
<td>Japan, no. H-020169, Koushurei 224</td>
</tr>
<tr>
<td>Public lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hack</td>
<td>White</td>
<td>Gray</td>
<td>Nickell et al., 1985</td>
</tr>
<tr>
<td>Hardin</td>
<td>Purple</td>
<td>Gray</td>
<td>Fehr et al., 1983</td>
</tr>
<tr>
<td>M66m56W1W1</td>
<td>Purple</td>
<td>Gray</td>
<td>Palmer and Skorupska, 1990</td>
</tr>
<tr>
<td>M66m56W1w1</td>
<td>Purple</td>
<td>Gray</td>
<td>Palmer and Skorupska, 1990</td>
</tr>
<tr>
<td>T295H (M66m6W1w1)</td>
<td>White</td>
<td>Gray</td>
<td>Tachibana et al., 1977</td>
</tr>
<tr>
<td>BSR 101</td>
<td>Purple</td>
<td>Gray</td>
<td>Bernard and Cremeens, 1988</td>
</tr>
<tr>
<td>Corsoy 79</td>
<td>Purple</td>
<td>Tawny</td>
<td>Fehr and Bahrenfus, 1984</td>
</tr>
<tr>
<td>Century</td>
<td>Purple</td>
<td>Tawny</td>
<td>Wilcox et al., 1980</td>
</tr>
<tr>
<td>Hoyt</td>
<td>Purple</td>
<td>Tawny</td>
<td>Cooper et al., 1991</td>
</tr>
<tr>
<td>Private lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>82-378</td>
<td>White</td>
<td>Gray</td>
<td>Land O’ Lakes, Inc.</td>
</tr>
<tr>
<td>82-165</td>
<td>White</td>
<td>Tawny</td>
<td>Land O’ Lakes, Inc.</td>
</tr>
<tr>
<td>CX155</td>
<td>Purple</td>
<td>Gray</td>
<td>DeKalb Genetics Corp.</td>
</tr>
<tr>
<td>Glenn</td>
<td>Purple</td>
<td>Gray</td>
<td>ProFitSeed Inc.</td>
</tr>
<tr>
<td>A3307</td>
<td>White</td>
<td>Tawny</td>
<td>Asgrow Seed Co.</td>
</tr>
<tr>
<td>AX2858</td>
<td>Purple</td>
<td>Tawny</td>
<td>Asgrow Seed Co.</td>
</tr>
<tr>
<td>AG-020</td>
<td>Purple</td>
<td>Tawny</td>
<td>Asgrow Seed Co.</td>
</tr>
<tr>
<td>J201</td>
<td>Purple</td>
<td>Gray</td>
<td>Mycogen Seeds</td>
</tr>
<tr>
<td>SI346</td>
<td>Purple</td>
<td>Gray</td>
<td>Novartis Seeds Inc.</td>
</tr>
<tr>
<td>G3197</td>
<td>Purple</td>
<td>Gray</td>
<td>Novartis Seeds Inc.</td>
</tr>
<tr>
<td>P596-13</td>
<td>Purple</td>
<td>Gray</td>
<td>Pioneer Hi-Bred, Int’l.</td>
</tr>
<tr>
<td>P42257</td>
<td>Purple</td>
<td>Gray</td>
<td>Pioneer Hi-Bred, Int’l.</td>
</tr>
<tr>
<td>P301002</td>
<td>Purple</td>
<td>Tawny</td>
<td>Pioneer Hi-Bred, Int’l.</td>
</tr>
</tbody>
</table>
2.3. 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:

\[ Y_{ijk} = \mu + Y_i + B_{(ij)} + L_k + YL_{ik} + BL_{(ijk)} + C_l + YC_{il} + BC_{(ijkl)} + LC_{ilk} + YLC_{ilk} + BLC_{ijkl} \]

where \( \mu \) is the general mean; \( Y_i \) the effect of the \( i \)th year; \( B_{(ij)} \) 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; \( BL_{(ijk)} \) 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; \( BC_{(ijkl)} \) the interaction effect between the \( j \)th block nested in the \( i \)th year and the \( l \)th cytoplasm source; \( LC_{ilk} \) the interaction effect between the \( k \)th male-sterile line and the \( l \)th cytoplasm source; \( YLC_{ilk} \) the interaction effect between the \( i \)th year, the \( k \)th male-sterile line, and the \( l \)th cytoplasm source and \( BLC_{(ijkl)} \) is 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 3 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.

3. Results and discussion

3.1. 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 the cytoplasm genome. Significant interactions for year \( \times \) male-sterile line, and year \( \times \) male-sterile line \( \times \) 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 seeds/male-sterile plant in 2002, and 2.44 seeds/male-sterile plant 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 (Fig. 1). Frantz et al. (2004) reported that night respiration increased 4%/\(^{\circ}\)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 to August was less than one-half compared to 2002 (Fig. 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 plant’s growth and development. The poor performance of the lines in 2001, the year with the smallest amount of accumulated rain, seems to support this observation.

3.2. 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, PI297544, 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 Agrow 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; considerably less than that reported by Lewers et al. (1996) for lines cosegregating for the Mos allele (male sterility) and w1 (white flower) alleles. These authors have not been determined. According to Chiang and Kiang (1970; Nelson and Bernard, 1979; Handel, 1983; De Jong et al., 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), it 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 2001–2003, 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 and 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 (FaeGI 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 in 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 × male-sterile line interaction was present (Table 2); however, ranking of the lines for seed set was similar across years for the 10 highest-yielding lines and the 10 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.

3.3. 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 F5 derived line from Harcor × Elf (Cooper et al., 1991), has determinate growth habit, purple flowers, tawny pubescence, and plant height averaged 50 cm.

3.4. 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 (Fig. 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, 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 F5 derived line from Harcor × Elf (Cooper et al., 1991), has determinate growth habit, purple flowers, tawny pubescence, and plant height averaged 50 cm.

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 2001–2003 growing seasons

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Mean no. of seed/male-sterile plant/season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2001</td>
</tr>
<tr>
<td>Modern cultivars vs. ancestors</td>
<td>NS</td>
</tr>
<tr>
<td>Private vs. ancestors</td>
<td>NS</td>
</tr>
<tr>
<td>Public vs. ancestors</td>
<td>NS</td>
</tr>
<tr>
<td>Private vs. public</td>
<td>NS</td>
</tr>
<tr>
<td>Private vs. Chinese</td>
<td>*</td>
</tr>
<tr>
<td>Private vs. USSR</td>
<td>**</td>
</tr>
<tr>
<td>Private vs. Japanese</td>
<td>***</td>
</tr>
<tr>
<td>Chinese vs. USSR</td>
<td>NS</td>
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<tr>
<td>Chinese vs. Japanese</td>
<td>NS</td>
</tr>
<tr>
<td>Chinese vs. public</td>
<td>*</td>
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<tr>
<td>USSR vs. Japanese</td>
<td>*</td>
</tr>
<tr>
<td>USSR vs. public</td>
<td>*</td>
</tr>
<tr>
<td>Japanese vs. public</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = not significant.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

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 2001–2003 growing seasons

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Mean no. of seed/male-sterile plant/season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2001</td>
</tr>
<tr>
<td>Flower color</td>
<td></td>
</tr>
<tr>
<td>Purple-flowered lines vs. white-flowered lines</td>
<td>***</td>
</tr>
<tr>
<td>Mean no. of seed/ male-sterile plant</td>
<td></td>
</tr>
<tr>
<td>Purple-flowered lines</td>
<td>0.51</td>
</tr>
<tr>
<td>White-flowered lines</td>
<td>1.66</td>
</tr>
<tr>
<td>Pubescence color</td>
<td></td>
</tr>
<tr>
<td>Tawny-pubescence lines vs. gray-pubescence lines</td>
<td>**</td>
</tr>
<tr>
<td>Mean no. of seed/ male-sterile plant</td>
<td></td>
</tr>
<tr>
<td>Tawny-pubescence lines</td>
<td>0.9</td>
</tr>
<tr>
<td>Gray pubescence lines</td>
<td>0.67</td>
</tr>
</tbody>
</table>

** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Fig. 2. Flower color effect on mean number of seed per male-sterile line for the 34 pairs of near-isogenic lines in Ames, Iowa for the 2001–2003 growing seasons.
purple-flowered soybean lines did not significantly differ from white-flowered lines for yield, seed protein, and seed weight when evaluated as F₂.₅ 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 segregate 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 (Fig. 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 parent T₂95H Ms₆ms₆w₁w₁ (segregating white/purple flower color) and the donor parent T₂95H Ms₆ms₆W₁W₁ (white flower color). Male-sterile plants were expected to be male-sterile (Palmer et al., 1987) and genetic linkage between the Ms₆ and W₁ loci (3.14 ± 0.80% recombination).

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. Alalfa-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 (Fig. 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.

3.5. Pubescence color

Variation in seed set for pubescence color was observed (Fig. 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.

3.6. Cytoplasm source

The cytoplasm source effect was not significant among male-sterile lines. The effect of the interaction line × cytoplasm source was not significant, only the triple interaction; year × line × 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).

4. Summary

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.

Acknowledgments

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