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Hybrid Soybean Seed Production: Comparison of Three Methods

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Abstract

Improved methods to produce hybrid soybean [*Glycine max* (L.) Merr.] seed could augment several types of research. Two previously described methods, the traditional method and the dilution method, require insect-facilitated cross-pollination of ms ms nuclear male-sterile plants. The traditional method requires a substantial time investment during flowering to remove fertile siblings, and the dilution method requires a substantial amount of land and pollen-parent seed. Because time, land, and seed are limited, a more efficient method would be valuable. The cosegregation method was developed, utilizing close genetic linkage between the *W1* locus and the *Ms6* locus. The *W1* ____, seedling has a purple hypocotyl; the *w1 w1* seedling has a green hypocotyl. The ms6 ms6 plant is male sterile and female fertile. Approximately 97% of the purple-hypocotyl seedlings, *W1* ____, in a line segregating for the *w1* and *ms6* alleles in coupling phase will he fertile, *Ms6* ____, and can be removed as a pollen source at the first-trifoliolate stage. Our objective was to evaluate and compare the three methods of hybrid soybean seed production for seed yield, efficiency, and hybrid seed purity and quality. We used a randomized complete-block design (three replications per location, three locations, two years). The cosegregation method gave higher seed yield, better efficiency, and equal or better seed quality (percentage germination, 100-seed weight) than the other methods. Male-sterile plants yielded an average of 28.6 seeds plant^{-1} with the cosegregation method, 18.2 seeds plant^{-1} with the traditional method, and 9.5 seeds plant^{-1} with the dilution method. The cosegregation method will be useful in several research areas, including genetic control of complex traits, prediction of parental value, recurrent selection, and commercialization of hybrid soybean.

Keywords

NCRPIS, Zoology and Genetics

Disciplines

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Comments

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Hybrid Soybean Seed Production: Comparison of Three Methods

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ABSTRACT

Improved methods to produce hybrid soybean [*Glycine max* (L.) Merr.] seed could augment several types of research. Two previously described methods, the traditional method and the dilution method, require insect-facilitated cross-pollination of *ms ms* nuclear male-sterile plants. The traditional method requires a substantial time investment during flowering to remove fertile siblings, and the dilution method requires a substantial amount of land and pollen-parent seed. Because time, land, and seed are limited, a more efficient method would be valuable. The cosegregation method was developed, utilizing close genetic linkage between the *W1* locus and the *Ms6* locus. The *W1* ___ seedling has a purple hypocotyl; the *w1 w1* seedling has a green hypocotyl. The *ms6 ms6* plant is male sterile and female fertile. Approximately 97% of the purple-hypocotyl seedlings, *W1* ___, in a line segregating for the *w1* and *ms6* alleles in coupling phase will be fertile, *Ms6* ___, and can be removed as a pollen source at the first-trifoliolate stage. Our objective was to evaluate and compare the three methods of hybrid soybean seed production for seed yield, efficiency, and hybrid seed purity and quality. We used a randomized complete-block design (three replications per location, three locations, two years). The cosegregation method gave higher seed yield, better efficiency, and equal or better seed quality (percentage germination, 100-seed weight) than the other methods. Male-sterile plants yielded an average of 28.6 seeds plant^{-1} with the cosegregation method, 18.2 seeds plant^{-1} with the traditional method, and 9.5 seeds plant^{-1} with the dilution method. The cosegregation method will be useful in several research areas, including genetic control of complex traits, prediction of parental value, recurrent selection, and commercialization of hybrid soybean.

SOYBEAN is an autogamous crop species, and the production of hybrid seed has been tedious. Nevertheless, hybrid seed is important to certain types of research, including elucidation of the genetic control of complex agronomically important traits, determination of parental value of lines to improve these traits, population improvement for these traits through use of recurrent selection, and studies related to the commercialization of hybrid soybean. Some important aspects of this research require agronomic evaluation of the F1 generation. Replicated multirow evaluation of the F1 generation can require the production of hundreds or thousands of hybrid soybean seeds per parental combination.

Manual cross-pollination to produce large quantities of hybrid soybean seed is difficult and time consuming. The small size of the soybean flowers, the low success rate ($\approx$50% of the cross-pollinations result in a pod), and the few (average of one to two) seeds obtained per hybrid pod contribute to the difficulty of manually producing large quantities of hybrid seed (Fehr, 1987). An experienced breeder can pollinate 20 flowers per hour on an indeterminate cultivar (Fehr, 1980). Depending on the local environment, up to 6 h per day are available for cross-pollination (Fehr, 1980). Therefore, under nearly ideal conditions, an experienced person can produce about 120 hybrid seeds per day. Both manual cross-pollination and insect-mediated cross-pollination have been used successfully to intermate lines for soybean recurrent-selection programs. However, production of enough hybrid seed for replicated multirow agronomic trials generally has not been feasible.

Insect cross-pollination of male-sterile soybean plants facilitates the production of hybrid seed. Graybosch and Palmer (1988) evaluated relative yield of *ms1 ms1*, *ms2 ms2*, and *ms3 ms3* male-sterile plants and found that *ms2 ms2* plants had the greatest seed-set. Lines segregating for *ms2 ms2* male sterility have been used by several researchers to produce hybrid seed (Carter et al., 1983; St. Martin and Ehounou, 1989; Nelson and Bernard, 1984; Specht and Graef, 1992). Planting arrangements and pollen management methods varied among researchers.

Specht and Graef (1992) planted pod-parent lines segregating for *ms2 ms2* male sterility in short rows between pollen-parent lines. Fertile siblings in rows segregating for male sterility were identified at flowering by anther inspection and removed as a pollen source. Insect vectors carry pollen from the pollen-parent rows to the male-sterile plants in the pod-parent rows. This method is termed the traditional method.

There are several difficulties with the traditional method. Fertile siblings are removed at flowering, a time when soybean breeders concentrate their efforts on manual cross-pollinations. Roguing plants by inspecting anthers is time consuming. Male-sterile plants often are fertilized with pollen from fertile siblings before the fertile siblings can be removed. Finally, it is possible for male-sterile plants to be more susceptible to lodging after neighboring fertile siblings are removed.

A second method of F1 seed production, termed the dilution method, avoids these problems. Graef and Specht (1992) combined seed of a desired pollen parent with seed segregating for the *ms2 ms2* male sterile in the amounts of 4, 8, 16, and 32 times the number of fertile siblings expected in the pod-parent line. They reported percentages of desired pollinations to be 77.8, 75.0, 87.6, and 90.2% for the four treatments. The major advantage of the dilution method is that no roguing of fertile plants is required to manage pollen. Difficulties with the dilution method include the increased amount of land and pollen-parent seed required and the possible

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difficulty identifying male-sterile plants among male-fertile plants. Male-sterile plants usually are easy to identify at maturity because they bear fewer pods, some of which are parthenocarpic, and they are green when neighboring fertile plants have turned brown. Differences are less noticeable after a hard frost or if the amount of outcrossing onto male-sterile plants is high. Use of the dilution method requires that male-sterile plants be easily distinguishable from male-fertile plants at maturity.

A third method of F1 seed production avoids most of the difficulties of the traditional and dilution methods. The cosegregation method takes advantage of the close genetic linkage, ranging from 2 to 4% recombination, of the *W1* and *Ms6* loci (Skorupska and Palmer, 1989; Lewers and Palmer, 1993). The *W1* locus is involved in anthocyanin pigment production in several plant tissues. *W1* ___ plants have purple hypocotyls and flowers; *w1 w1* plants have green hypocotyls and white flowers. The *Ms6* locus affects pollen production (Skorupska and Palmer, 1989). *Ms6* ___ plants are fertile; *ms6 ms6* plants are female fertile but completely male sterile as a result of tapetal malfunction (Skorupska and Palmer, 1989). In a line segregating for the *w1* and *ms6* alleles in coupling phase, more than 92% of green-hypocotyl seedlings are expected to be male sterile.

The planting pattern for the cosegregation method is the same as for the traditional method. Shortly after emergence, purple-hypocotyl seedlings are removed manually, greatly reducing the number of fertile siblings. At flowering, the few white-flowered fertile siblings arising from recombination between the *W1* and *Ms6* loci are identified by anther inspection. These plants and any purple-flowered escapes are removed as a pollen source.

The land and seed use of the cosegregation method is identical to that of the traditional method, but the closely linked seedling marker should reduce the amount of time required to remove fertile siblings. Therefore, the cosegregation method may be more efficient than either the traditional or the dilution method. If so, the cosegregation method could improve production of hybrid soybean seed. Our objective was to compare the cosegregation method with the traditional and dilution methods for seed yield per plant, resource efficiency (time, seed, and land), and hybrid seed purity and quality.

MATERIALS AND METHODS

We compared three hybrid seed production methods (traditional, dilution, and cosegregation methods) using a randomized complete-block design for two years, with three locations (Ames, IA; Columbus, OH; and Harrow, Ontario, Canada) and three replications per location.

The pollen parent was 'Kenwood' (Cianzio et al., 1990), a Maturity Group II (MG II) cultivar with the genotype *W1 W1 Ms6 Ms6*. The pod parents were two sibling lines that differ from each other at the *W1* locus: (i) T295H (MG II) with the genotype *w1 w1 Ms6* ___ (Palmer and Skorupska, 1990) for the traditional and dilution methods and (ii) a sibling line with the genotype *W1* ___ *Ms6* ___ for the cosegregation method (Palmer and Skorupska, 1990). The *W1* locus was used as a genetic marker to measure the degree of cross-pollination among siblings in the pod-parent lines. The three methods were compared by means of the *ms6* male-sterility allele. T295H (*w1 w1 Ms6* ___) was increased and progeny tested at the Iowa State Univ. soybean research site at the Isabela Substation of the University of Puerto Rico near Isabela, PR. Remnant seed of white-flowered (*w1 w1*) entries segregating for male sterility were used as the pod parent in the traditional and dilution methods. Fertile plants (*Ms6* ___) from the T295H sibling line cosegregating for the *w1* and *ms6* alleles were harvested individually and progeny tested at the USDA Tropical Agriculture Research Station near Isabela, PR. Remnant seed of entries segregating for flower color and male sterility were used as the pod parent in the cosegregation method.

Seed was planted at the three locations, according to the following planting patterns. Each replication was surrounded by 15 m of bare ground to discourage insect-mediated pollination from outside the replication (Fig. 1), as recommended by J.E. Specht and G.L. Graef (1993, personal communication). Seeding rate for all methods was 16 seeds m^{-1} in 38-cm rows. Replications and methods within replications were randomized for each year and location. The number of pod-parent seeds planted for each replication of each method was 1250. Assuming a 90% germination rate and a segregation ratio for the pod parent of three fertile plants to one male-sterile plant, ≈281 male-sterile plants from each replication of each method were expected for harvest.

For the dilution method, 1250 seeds of T295H were combined with 11 000 pollen-parent seeds, an amount more than 10 times the expected number of fertile individuals in the T295H line, as recommended by G.L. Graef and J.E. Specht (1993, personal communication). The dilution method replications were not bordered with rows of pollen parent (Fig. 2). For the traditional and cosegregation methods, 305-cm-long rows of pod-parent lines (segregating for male sterility) were planted between pollen-parent rows of the same length, alternating in a checkerboard pattern (Fig. 2). At the ends of each set of five 305-cm rows was a 122-cm alley. At both ends of these series of rows was a 305-cm border of pollen parent (Fig. 2). Five rows of pollen parent bordered the sides of each replication of the traditional and cosegregation methods. One replication of the traditional or cosegregation method, including the border rows, was 29 m deep by 8 m wide, ≈57% of the area required for the dilution method. The number of pollen-parent seeds planted per method-replication was 8200, ≈75% of the number planted for the dilution method.

At Ames (1993 and 1994) and Harrow (1994), shortly after emergence, pod-parent rows in the cosegregation method were marked with wood stakes to facilitate roguing. Purple-hypocotyl seedlings at the first-trifoliolate stage were removed from the pod-parent rows in the cosegregation method. At flowering, remaining purple-flowered escapes were removed from the pod-parent rows in the cosegregation method. Fertile white-flowered plants were identified by anther inspection and removed from the pod-parent rows in the cosegregation and traditional methods. The time required to mark and rogue pod-parent rows was recorded for each method.

At Ames and Harrow, natural pollinator populations were augmented with honey bees (*Apis mellifera* L.) when Kenwood became an adequate source of nectar and pollen. Hives containing 80 000 to 160 000 honey bees were placed at least 90 m away from the replications to allow equal visitation to all replications and production methods. Buckfast strain honey bee (Osterlund, 1983), supplied by the North Central Regional Plant Introduction Station, was used at Ames; Italian race honey bee was used at Harrow. At Ames, in 1994, honey bees were counted in midafternoon on three dates between 29 July and 2 August. The number of honey bees observed visiting

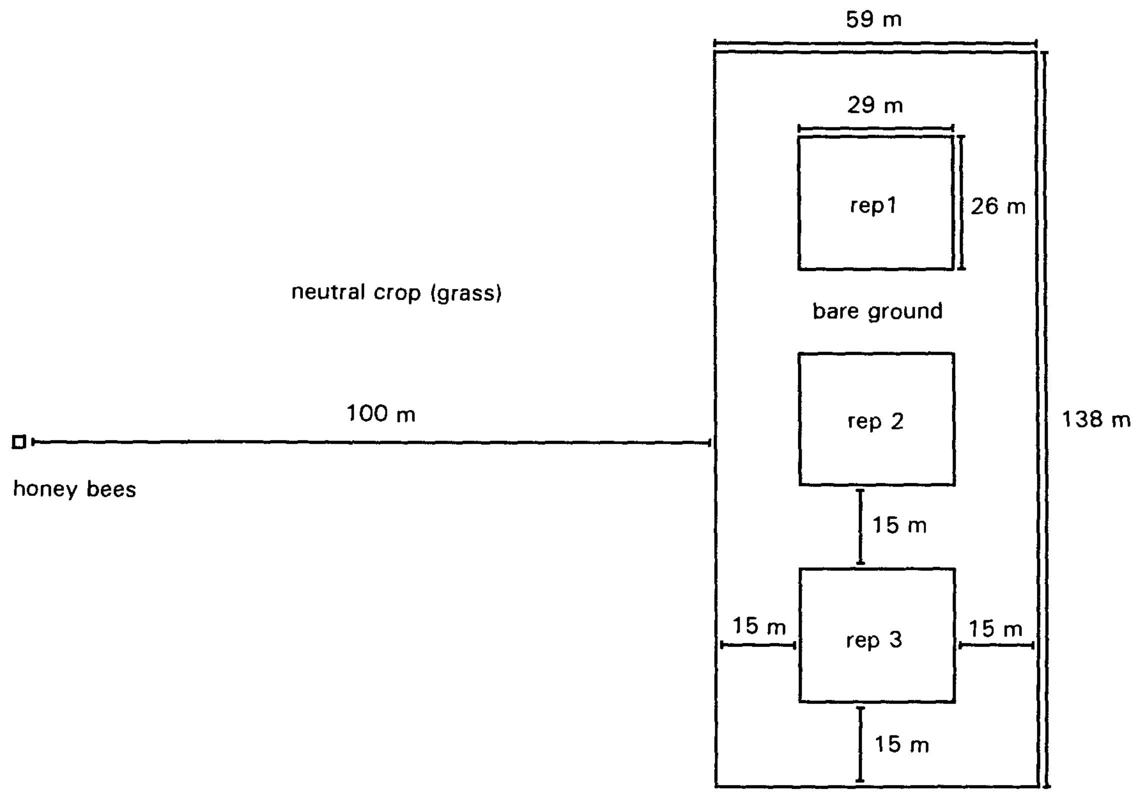

Fig. 1. Comparison of hybrid soybean seed production methods: arrangement of three replications (rep = replication).

plants in a five-row by 305-cm-long area in 30 s was recorded. Ten such observations were made on each date for each of the nine method-replications. The order in which method-replications were observed was randomized for each counting date.

At maturity, male-sterile plants can be identified because they bear fewer pods, some of which are parthenocarpic, and they are green when neighboring fertile plants have turned brown. Male-sterile plants were evaluated visually for lodging and given a score from 1 to 5, with a score of 1 representing no lodging and a score of 5 representing a prostrate plant. Male-sterile plants within each method and replication were harvested separately. Once pods turned brown, male-sterile plants were cut at soil level and tied together in bundles of 10 plants each. Each bundle was identified by method and replication and was dried. The number of male-sterile plants harvested from each method-replication was recorded (except at Harrow in 1994). The time required to harvest each method-replication also was recorded. The plants were threshed in bulk within method-replication. An unintentional deviation from this harvest procedure occurred at Columbus. For the traditional and cosegregation methods, all plants (male-sterile plants and their fertile siblings) remaining in a row designated male sterile were harvested.

All seed data were recorded after hand cleaning. Seed yield per male-sterile plant was calculated by dividing the total number of seeds for a method-replication by the number of plants from that method-replication. Hybrid seed was evaluated for the following characteristics: percentage moisture, 100-seed weight, percentage green or immature seeds, percentage diseased seeds, and percentage physiologically damaged seeds. Percentage moisture was determined by a seed-moisture analyzer. Hundred-seed weight was compared at 13% moisture and calculated from weight, in grams, of 500 seeds. Percentage green or immature seeds, percentage diseased seeds, and percentage physiologically damaged seeds were determined by visually identifying and counting the number of green or immature seeds, diseased seeds, or physiologically damaged seeds, respectively, in a dry 100-seed sample.

Seeds were germinated on trays (400 seeds per tray) with two sheets of germination towels and 825 mL of water per tray. The trays were spaced evenly in six germination carts, eight trays per cart. Seeds were germinated at 25°C, with 9 h light and 15 h dark, and grown for 7 d. For all but six entries (because of a shortage of seeds for those six entries), four replications of 100 seeds per entry were germinated. For the remaining six entries, the total number of seeds was divided into four even replications. Characters measured were percentage germination and percentage contamination. Percentage contamination was calculated as the average number of green-hypocotyl seedlings (*w1 w1*) in the four 100-seed replications. All hybrid seedlings of the desired parentage should have a purple hypocotyl (*W1 w1*).

At Ames, two of three replications were lost due to flood damage in 1993. Some data were collected on those two replications before the flood. Data from Columbus in 1994 were not included in any analyses of variance, because, due to the deviation from the intended procedure, many fertile siblings were accidentally harvested with the male-sterile plants. In addition, for Harrow in 1994, the number of seeds per plant cannot be reported, because the number of male-sterile plants harvested was not recorded.

Analyses of variance were made on all characters by using SAS software (SAS Institute, 1990). The model used was a randomized complete-block design, with two years and three locations. Years, locations, and replications were considered random; methods were considered fixed. Method × year, method × location, and method × year × location sources of variance were considered to be part of experimental error (D.F. Cox, 1995, personal communication).

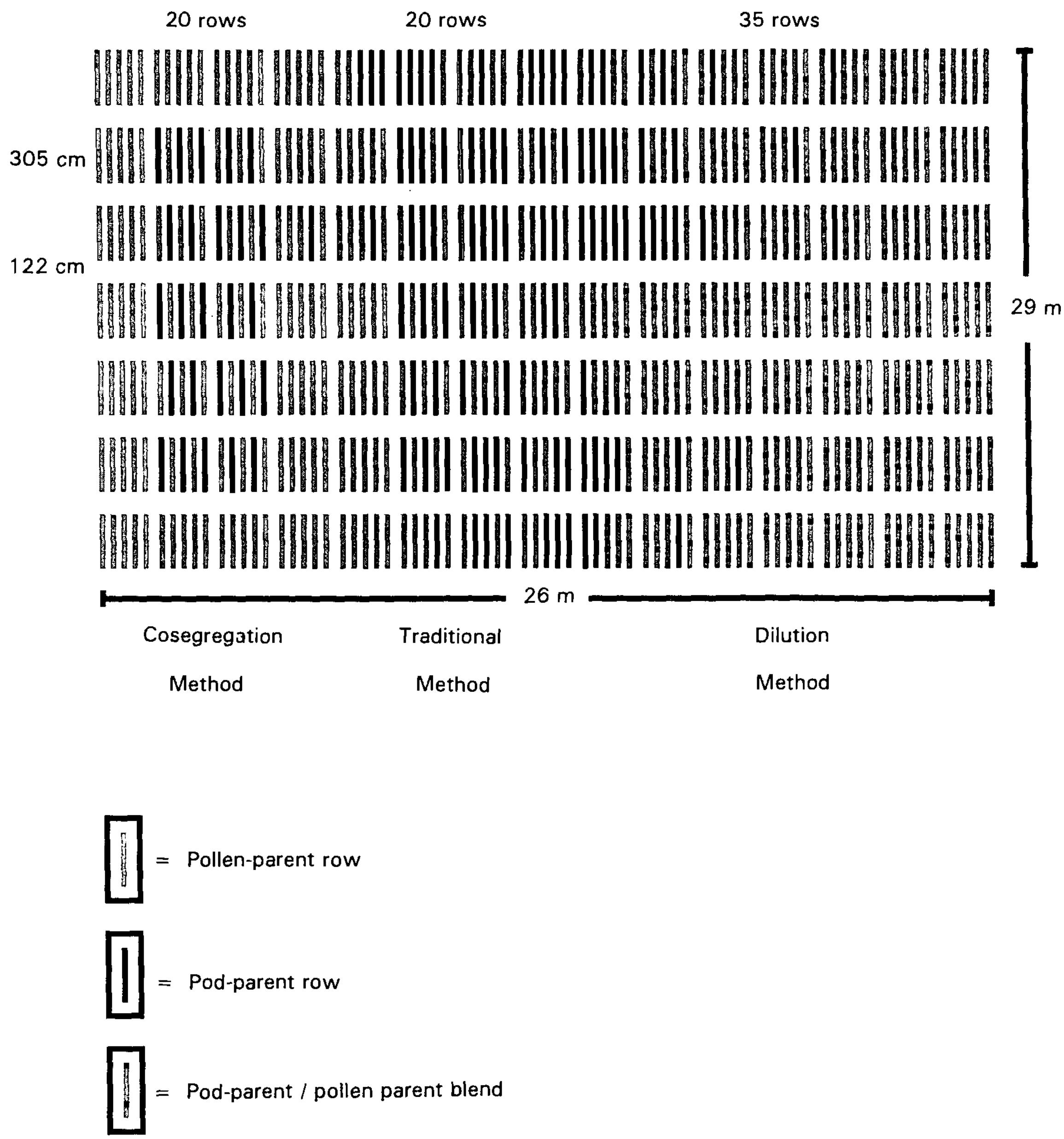

Fig. 2. Comparison of hybrid soybean seed production methods: one possible arrangement of methods within a replication.

RESULTS AND DISCUSSION

Hybrid Seed Yield

Seed yield per male-sterile plant varied considerably, depending on method, location, and year (Table 1). Significant differences were observed among hybrid seed production methods for seed yield (number of seeds per male-sterile plant; Table 2). The cosegregation method produced more seeds per plant (28.6 seeds plant^{-1}) than the traditional method (18.2 seeds plant^{-1}), which produced more seeds per plant than did the dilution method (9.5 seeds plant^{-1}; Table 2). The increased space and decreased competition around male-sterile plants, allowing increased growth and flower production, was an advantage of the traditional and cosegregation methods over the dilution method. The cosegregation method may have had an advantage over the traditional method because the roguing was done earlier, and the male-sterile plants grew with reduced competition for a longer time.

There were no significant differences among hybrid seed production methods in the number of honey bee visitations at Ames in 1994 (Table 2). Therefore, it is unlikely that yield differences among production methods were due to honey bee preference.

Table 1. Number of seeds per male-sterile plant using three methods of hybrid soybean seed production.

			Method		
Location	Year	*N*†	Traditional	Dilution	Cosegregation
Ames	1993	1	1.7	1.5	3.3
	1994	3	24.2	14.3	41.8
Columbus	1993	3	26.6	13.6	41.1
Harrow	1993	3	9.2	3.3	11.5

† *N* = number of observations.

Table 2. Means for three methods of hybrid soybean seed production using male-sterile soybean.

Character	*N*†	Method		
		Traditional	Dilution	Cosegregation
Number of plants harvested	10	134.6a‡	124.3a	157.5a
Number of seeds per male-sterile plant	10	18.2b	9.5c	28.6a
Number of bee visitations in one replication during a 5-min period	3	13.7a	14.7a	15.7a
Lodging scores of male-sterile plants	13	1.1a	1.0a	1.0a
Person-minutes required to rogue one replication	13	366.2a	0.0c	70.9b
Person-minutes required to harvest one replication	13	29.2a	37.8a	33.2a
Total person-minutes required for one replication	13	395.5a	37.8c	104.1b
Total person-minutes required per 100 seeds produced (one replication)	13	40.6a	6.9b	8.6b
Percentage seedlings with green hypocotyls	13	21.8a	14.8b	12.9b
Percentage germination	13	80.4a	82.0a	79.5a
Percentage diseased seeds	13	6.2a	6.8a	7.5a
Percentage physiologically damaged seeds	13	17.6a	16.7a	12.5a
Percentage green or immature seeds	13	0.9a	3.5b	1.5a
Grams per 100 seeds at 13% moisture	13	28.9a	27.0b	29.3a

† N = number of year-location replications.
‡ For each character, means followed by the same letter are not significantly different ($P \geq 0.05$).

Table 3. Year-location means for characters for which the analyses of variance detected differences among year-locations for three methods of hybrid soybean seed production.

Character	Year	Location		
		Ames	Columbus	Harrow
Number of seeds per male-sterile plant	1993	2.2a†	27.1b*b*	8.0a*a*
		N = 3‡	*N* = 9	*N* = 9
	1994	26.8b*b*	– §	– ¶
		N = 9	*N* = 0	*N* = 0
Lodging scores of male-sterile plants	1993	1.3b	1.0a*a*	1.0a*a*
		N = 3	*N* = 9	*N* = 9
	1994	1.0a*a*	–	1.0a*a*
		N = 9	*N* = 0	*N* = 9
Person-minutes required to rogue one replication	1993	100.0a	150.3a*a*	87.3a*b*
		N = 3	*N* = 9	*N* = 9
	1994	171.4a*a*	–	188.9b*a*
		N = 9	*N* = 0	*N* = 9
Person-minutes required to harvest one replication	1993	39.7b	28.9b*b*	31.0b*b*
		N = 3	*N* = 9	*N* = 9
	1994	50.4bc	–	21.1a*a*
		N = 9	*N* = 0	*N* = 9
Person-minutes required per 100 seeds (one replication)	1993	46.8a	13.9b*b*	16.2b*b*
		N = 3	*N* = 9	*N* = 9
	1994	4.0b*b*	–	31.4a*a*
		N = 9	*N* = 0	*N* = 9
Percentage of seeds with physiological damage	1993	0.0a	33.0b*b*	34.6b*b*
		N = 3	*N* = 9	*N* = 9
	1994	0.1a*a*	–	0.0a*a*
		N = 9	*N* = 0	*N* = 9

† For each character, means followed by the same letter and font are not significantly different ($P \geq 0.05$).
‡ N (number of observations) for a year-location is noted under the mean for that year-location-method replications.
§ Columbus, OH, 1994 data were not included.
¶ Number of seeds per male-sterile plant could not be calculated for the Harrow location in 1994, because number of male-sterile plants was not reported.

No differences among methods were observed for lodging of male-sterile plants; almost no lodging was observed for any method, year, or location (Tables 2 and 3). Year × location differences (Table 3) observed for lodging were the result of soil erosion from flooding at Ames in 1993.

Significant differences in seed yield also were observed among years and locations (Tables 1 and 4). Weather (temperature, cloud cover, moisture availability) greatly affects plant growth, flower abortion (Fehr, 1980, and references cited therein), honey bee activity (Jaycox, 1970b), and soybean attractiveness to honey bee (Jaycox, 1970a; Robacker et al., 1983), all of which are key factors in determining hybrid soybean seed yield. Palmer et al. (1983) reported environmental effects on the amount of outcrossing of male-sterile soybean.

Differences in local populations of bee species also may have affected seed yield differences among locations. Jaycox (1970a) lists several bee species found foraging on soybean near Urbana, IL: bumble bees, *Bombus impatiens* and *Bombus griseocollis*; parasitic bees, *Triepeolus* and *Coelioxys* spp.; and other solitary bees of the genera *Melissodes*, *Megachile*, *Calliopsis*, *Coletes*, *Halictus*, *Agapostemon*, and *Lasioglossum*. A few bumble bees were observed at Ames in 1994. Honey bee race or strain differences also may have affected seed yield differences among locations (Wilson and Collison, 1988). The Buckfast honey bees used at Ames were bred for industry and docility (Osterlund, 1983). Effectiveness of different bee species, races, and strains in producing hybrid soybean seed may be worthy of further investigation.

Land and Seed Use

The dilution method required ≈ 12% more land (Fig. 2) and 34% more pollen-parent seed than did the traditional or cosegregation methods to produce the same number of male-sterile plants. The traditional and cosegregation methods would become progressively more efficient (land and pollen-parent seed) as increasing amounts of the pod parent are planted. This is because the total size of the crossing block would increase, and the amount of land and seed required for the border rows would decrease in proportion to the total block size.

Each hybrid seed production method could be modified to increase the efficiency of land and seed utilization. First, maintaining the pod-parent line in a segregation ratio of one fertile to one male-sterile plant would double the number of male-sterile plants that could be grown in an area. Second, alleys could be omitted, reducing plot dimensions or increasing the number of plants that could be grown in an area. Alleys could be omitted for the traditional and cosegregation methods only if pod-parent rows could be planted between pollen-parent rows in continuous rows instead of alternating them in the checkerboard pattern. This can be done without decreasing insect cross-pollination if rows are narrowly spaced. We observed frequent honey bee movement across rows spaced 38 cm apart. In our crossing nursery, rows are spaced 102 cm apart, and honey bees tend to move along a row rather than across rows (common observation). Other researchers have reported similar

Table 4. Year and location means for characters for which the analyses of variance detected differences among year or locations, or both, for three methods of hybrid soybean seed production.

Character	Year 1993	Year 1994	Location Ames	Location Columbus	Location Harrow
	N = 21†	N = 18	N = 12	N = 9	N = 18
Number of seeds per male-sterile plant	15.4a‡	26.7b (N = 9)	20.6b	27.1a	8.0c (N = 9)
Person-minutes required to rogue one replication	116.2a	180.2b	153.7a	150.3a	138.1a
Person-minutes required to harvest one replication	31.3a	35.8a	47.8a	28.9b	26.1b
Total person-minutes required for one replication	147.5a	215.9b	201.4a	179.2a	164.2a
Percentage seedlings with green hypocotyls	19.0a	13.7b	14.3a	31.1b	10.7a
Percentage germination	76.1a	85.9b	87.1a	74.0c	79.7b
Percentage physiologically damaged seeds	29.0a	0.1b	0.1a	33.0c	17.3b
Percentage diseased seeds	9.1a	4.1b	1.8a	13.6c	6.8b
Percentage green or immature seeds	1.9a	2.0a	0.8a	1.7ab	2.8b
Grams per 100 seeds at 13% moisture	27.0a	30.0b	30.3a	21.6b	30.5a

† N (number of replications) for a year or for a location is the same for each character except number of seeds per male-sterile plant (in 1994 and at Harrow) where N is noted in parentheses under the mean for 1994 and under the mean for Harrow.
‡ For each character, means in the two year columns or means in the three location columns are not significantly different if followed by the same letter ($P \geq 0.05$).

observations (Chiang and Kiang, 1987). Third, the cosegregation method can be modified by planting the pod parent at a higher seeding rate because the seedlings would be thinned as part of the roguing process. Only the cosegregation method can be modified in this way, giving it the potential to be the most land- and seed-efficient method of the three.

Time Investment

Significant differences among methods were detected for the time required to rogue (Table 2). Roguing plants in the cosegregation method at the first-trifoliolate stage and again at flowering (to remove white-flowered fertile siblings arising from recombination and to remove late-germinating purple-flowered escapes) required much less time than roguing plants in the traditional method (Table 2). In addition, roguing plants in the traditional method by anther inspection at flowering is tedious and may interfere with concurrent cross-pollination efforts for other projects. Roguing was not required for the dilution method.

Significant differences among years were detected for the time required to rogue; roguing required more time in 1994 than in 1993 (Table 4). This was primarily caused by changes in roguing personnel. The time required to rogue plants in the traditional method is dependent on the degree of experience with manipulating soybean flowers and distinguishing *ms6 ms6* male-sterile plants from fertile plants. The time required to rogue plants in the cosegregation method was dependent on weather. The purple-hypocotyl of the *W1* ___ seedlings is bright after sunny conditions and pale after overcast conditions. Rainy weather slows roguing three ways: first, muddy conditions slow personnel movement through the plot; second, rain-splashed soil must be removed from each hypocotyl; and, third, the hypocotyl color fades under rain-splashed soil.

No significant differences were detected among methods for the time required to harvest (Table 2). Significant differences were detected among locations and year–locations for the time required to harvest. Harvesting at Ames required more time than at Columbus or Harrow, especially in 1994 (Tables 3 and 4). This is probably a reflection of plant size; the plants at Ames generally were larger (especially in 1994) than were plants at Columbus or Harrow, and larger plants are more cumbersome to harvest manually.

Harvest times would be greatly reduced if harvest were mechanized. Mechanized harvesting of the traditional and cosegregation methods would be practical if these methods were modified by planting pod-donor rows between pollen-donor rows in continuous rows instead of alternating them in the checkerboard pattern. Mechanized harvesting of the dilution method would be practical if all fertile plants could be chemically killed in the field shortly after flowering.

The traditional method required significantly more time per 100 seeds harvested than did the dilution or cosegregation methods (Table 2); no significant difference was detected between the dilution and cosegregation methods. The dilution method required less total time but yielded fewer seeds. Differences among year–locations for total time required per 100 seeds also were significant (Table 3). These differences are the effect of differences among year–locations for both time required and hybrid seed yield.

Hybrid Seed Purity

There were significant differences among methods for percentage contamination based on the percentage of green-hypocotyl seedlings (Table 2). The percentage contamination for the traditional method was much higher (21.8%) than for the other two methods. There was no significant difference in percentage contamination between the dilution method (14.8%) and the cosegregation method (12.9%). Percentage contamination was consistently below 10% for the cosegregation method at Ames and Harrow (Table 5).

Sources of contamination detected by percentage green-hypocotyl seedlings include accidental harvest of fertile white-flowered siblings and pollination of the male-sterile plants by pollen from fertile siblings or other white-flowered soybeans grown in the range of the insect pollinators. The effect of accidental harvest of fertile white-flowered siblings can be enormous as demonstrated by the traditional method data from Columbus in 1994 (98.8% contamination; Table 5). This is because a fertile plant can yield 10 times more than a male-sterile plant.

Table 5. Percentage *w1 w1* seedlings among hybrid seedlings in a comparison of three hybrid soybean seed production methods.

			Method		
Location	Year	*N*†	Traditional	Dilution	Cosegregation
Ames	1993	1	7.1	9.9	9.6
	1994	3	23.2	16.9	8.4
Columbus	1993	3	38.9	23.1	31.3
	1994	3	98.8‡	14.0	43.6
Harrow	1993	3	12.9	11.6	6.0
	1994	3	17.3	9.4	6.8

† *N* = number of observations.
‡ Columbus, OH, 1994 data were not included in the calculations of the means nor in the analysis of variance.

The effect of pollination of male-sterile plants by fertile siblings is not as large.

Significant year and location differences for percentage contamination also were observed (Tables 4 and 5). These differences are the effect of the high percentage contamination observed for seed from Columbus in 1993. Again, this large percentage of contamination is a result of misclassification followed by a harvest procedure deviation at Columbus: the harvest of fertile siblings with the male-sterile plants in rows designated male sterile.

Contamination was present with all three methods and requires management for these hybrid seed production methods to be most useful. Putative male-sterile plants can be harvested individually and progeny tested to confirm that the harvested plant was male sterile and not a misclassified fertile sibling. Progeny testing of putative hybrid seeds is much easier and requires fewer seeds with the cosegregation method because progeny can be evaluated based upon hypocotyl color instead of male sterility. In addition, hybrid seeds derived from sibmatings can be identified by hypocotyl color at the first-trifoliolate stage and removed from evaluation plots.

Hybrid Seed Quality

No significant differences among methods were detected for percentage germination, percentage diseased seeds, or percentage physiologically damaged seeds (Table 2). Percentage germination, influenced by disease and physiological damage, was dependent on environment; significant differences among years and locations were detected (Table 4). Significant differences among years, locations (Table 4), and year–locations (Table 3) were detected for percentage diseased seeds and percentage physiologically damaged seeds. Diseases visually observed on the seeds before germination include phomopsis (*Phomopsis* spp.), soybean mosaic virus, downy mildew (*Peronospora manshurica*), and purple stain (*Cercospora kikuchii*) (TeKrony et al., 1987). The physiological damage observed was similar to what is observed when mature dry seed is exposed to moist conditions, begins germination, and dries again.

Significant differences among methods were detected for percentage of green or immature seeds and for 100-seed weight (Table 2). The dilution method produced more green or immature seeds, with lower 100-seed weight, than did the traditional or cosegregation methods. This is probably an effect of the lower seed-set in the dilution method (Table 2); plants with low seed-set mature and dry slowly and unevenly. Differences among locations also were significant; hybrid seed from Ames had a significantly lower percentage green or immature seeds and a significantly higher 100-seed weight than hybrid seed from Harrow or Columbus (Table 4).

Uneven maturation and drying of male-sterile plants is detrimental to seed quality. Diseased seed, physiologically damaged seed, green immature seed, and low 100-seed weight were more common in seed from Columbus and Harrow in 1993 than in seed from Ames. The percentage of split seeds was not measured, but seed yield was reduced somewhat by seed splitting. It is very likely that these seed-quality problems are exacerbated by uneven maturation and drying of male-sterile plants. Until development of pod-parent lines yielding enough hybrid seeds to effect even maturation and drying, a desiccant can be used to accelerate drying (Whigham and Stoller, 1979). If a desiccant is used, seeds may be more susceptible to phomopsis infection (TeKrony et al., 1984) and should be harvested as soon as possible. As an alternative, hybrid soybean production fields could be treated with a fungicide as a preventative measure (Ellis and Sinclair, 1976).

CONCLUSIONS

The cosegregation method of hybrid soybean seed production was more efficient than either the traditional or the dilution method. With the cosegregation method, higher seed yield of better purity and quality was obtained by using fewer resources.

Use of the cosegregation method will allow efficient production and evaluation of experimental quantities of hybrid seed for genetic studies, germplasm evaluation, and recurrent selection. Replicated multirow agronomic evaluations can be made on the F1 generation. In addition, agronomic evaluation can be made on lines segregating for male sterility by overplanting at twice or four-thirds the desired stand (depending on segregation ratio) and removing green hypocotyl seedlings (*w1 w1 ms6 ms6*) at the first-trifoliolate stage. This practice will eliminate nearly all male-sterile individuals from the evaluation plots. Progeny testing can be conducted concurrently; if an entry contains nearly all green hypocotyl seedlings, the progenitor was probably not a male-sterile plant but rather a fertile sibling. Progeny from sibmatings (contamination) can be removed from the evaluation plots by overplanting at a rate of 5 to 10% and removing green-hypocotyl seedlings. The planting rate of the seed parent can be adjusted to maximize the number of seeds produced on a male-sterile plant for certain recurrent-selection methods. The best rate would be determined experimentally. Much past research on heterosis, combining ability, inbreeding depression, genetic control of quantitatively inherited characters, parental value, and germplasm evaluation and recurrent selection would have been enhanced by the ability to produce and agronomically evaluate large quantities of hybrid seed (Weiss et al., 1947; Nelson and Bernard, 1984; Burton, 1987, and references cited therein).

Burton (1987) stated that, "Additional research is needed to produce estimates of heterosis for a wider array of genotypes under commercial cultural conditions and to develop more information on the environmental stability of F1 hybrids relative to pure lines. Such research will provide a better assessment than now exists as to the economic advantages or disadvantages of F1 hybrids relative to pure lines." Use of the cosegregation method will enable some preliminary research regarding value, production, and cultivation of commercial hybrid soybean seed. Specific topics to be addressed include identification of ideal production locations, optimization of planting arrangement and ratio of seed parent to pollen parent, pollinator management, increasing seed quality and yield of hybrid seeds per plant, increasing heterosis, determination of economical feasibility of commercial hybrid soybean seed production, and comparison of optimal population density and soil fertility levels for hybrid vs. inbred lines.

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