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1) Seed yield on field-grown $ms_2$ $ms_2$ male-sterile plants.

Seed set on male-sterile plants is of general interest to geneticists. For the quantitative geneticist, high seed set allows greater flexibility in the design of basic genetic experiments, including selection and variance component estimation studies. For the plant breeder, high seed set raises a hope of hybrid soybean production. Hybrid production, to be economical, requires adequate seed yield on male-sterile plants grown in the field.

Seed yield on male-sterile plants is often quite low in soybean. When grown in isolation in a greenhouse, seed set is almost absent for male-sterile plants, especially those conditioned by $ms_1$, $ms_2$, or $ms_5$ sources of sterility. Outside, however, seed set on male-sterile plants can be either high or low, depending on the specific ms gene, the micro-environment, and insect population involved.

For example, sterile plants conditioned by the North Carolina $ms_1$ gene have a seed set that is always a small fraction of normal (25% or less). Even when all environmental conditions are apparently satisfactory, the plant is not very receptive to pollination. Problems in megasporogenesis have been implicated (Cutter and Bingham, 1977). In contrast, male-sterile plants conditioned by the $ms_2$ gene can be pollinated more successfully in the open environment. Carter et al. (1983) compared seed yield on male-sterile and male-fertile plants grown outside in pots. Both high and low seed set on $ms_2$ male-sterile plants were observed. It was noted in that study that, when environmental conditions are ideal (including adequate pollen vectors), seed yield can be quite high on $ms_2$ male-sterile plants grown in pots. Seed yield was only 8% less than that of control plants in the two years tested.

We have occasionally observed high seed set on field-grown male-sterile plants when insect populations are high, insecticides are not used, and moisture, light, and temperature conditions are favorable. Few data are available to document the upper limit of seed set on $ms_2$ male-sterile plants in the field, however. In this communication, we report on seed yield of male-sterile plants grown in the field under conditions that we consider nearly ideal for cross-pollination.

Materials and methods: The general approach each year was to grow about 2000 plants segregating for $ms_2$-conditioned male sterility in hill plots. Male-sterile plants were identified at flowering and then harvested individually at maturity. Seed yield was compared to the fertile control plants.

*Genotypes:* A randomly mating population segregating for male sterility was employed in this study. The population was developed in Raleigh, NC, by back-crossing the $ms_2$ gene twice into eight cultivars and lines: 'Gasoy 17', 'Jeff', 'Ransom', 'Johnston', 'Davis', 'Duocrop', and N79-1304. The $ms_2$ source was provided by R. L. Bernard and was a maturity group III line. Two random intermating cycles were completed, and the seed from sterile plants were used as planting seed in 1984. Seed from sterile plants in 1984 were used as planting seed in 1985. The population segregated about 55% fertile: 45% sterile in 1985.

*Environment:* The half-acre site was isolated from other soybean fields by over two miles. Insect pressure is typically low in this field and no control of insect infestation was attempted. Post-emergent herbicides were applied as needed in both years. A minimum of 12 honeybee hives were placed near the field each year. Actual distance from hives to male-sterile plants ranged from 30 to 150 yards. Moisture was adequate during pollination in both years, but light intensity was considerably lower in 1985 because of extended cloud cover. Plants flowered in the month of August, and no dramatic decreases in temperature were observed during pollination. The site was considered to be nearly ideal for cross-pollination. Plants were grown in a hill plot arrangement on 19-inch centers. Male-sterile and male-fertile plants were distributed randomly over the field because of the genetically segregating nature of the male sterility.

*Identification of sterile plants at flowering:* In our visual identification procedure, we classified plants only on those days when we knew that fertiles were in fact shedding pollen. If a single flower on a plant shed pollen, the plant was tagged fertile. Up to five fresh flowers per plant were checked for pollen shedding. If no flowers shed pollen, the plant was tentatively marked as sterile. On a different day, putative steriles were checked closely for pollen shedding and morphology. If no evidence of fertility was found, then the plant was labeled male-sterile. When time permitted, male-steriles were tested on three different days.

Although a pollen-germination test may have proved somewhat more reliable than our visual inspections, such a test was impractical for the large number

of plants we screened. Realizing that visual inspection is subject to occasional misidentification of plants, we carefully followed the protocol above in both years. In 1985, we used additional methods in the field to confirm our identification of steriles. The anthers of male-sterile plants were examined by magnifying lens. When the identity of a plant was in doubt, a dissection microscope was employed; shrunken anthers devoid of pollen were classified as sterile. Occasionally, anthers were crushed and stained with $I_2KI$ for a final determination. Fertile pollen grains stained a golden brown. Progeny tests were also conducted for plants grown in 1985. These tests consisted of growing progeny from sterile plants that had either white flowers or gray pubescence. Segregation for these traits was taken as evidence of seed set by hybridization. Progeny tests indicated that our error rate for identification of male steriles was about 5%.

*Harvest:* Individual plants were harvested by hand and threshed with either a Swanson or Brewer mechanical thresher. All plants in the progeny test were threshed with the Brewer electric thresher because cleanout of seed is superior and the chance of mixtures is quite small.

*Results:* Seed set on the $ms_2$ $ms_2$ male-sterile plants was quite high (Table 1). Sterile plants produced yields that were 85 and 76% of normal in 1984 and 1985, respectively. Seed on sterile plants were larger than those on fertile plants in general. The increase in seed size partially explains the tendency of male-sterile plants to yield higher than the human eye might predict. This is especially true for our study in 1985. In that year, sterile plants could be seen to have fewer pods than fertiles at maturity. Stems stayed green, and leaves were slow in senescing. We guessed that yield on sterile plants might be only one-third to one-half that of normal plants. Actual yield results indicated that our visual observations were inaccurate and that sterile plants yielded 76% of normal. In 1984, by contrast, pod set was very high on sterile plants, and it was quite apparent before harvest that yield on sterile plants would be nearly normal.

The results clearly show that male-sterile plants can produce seed yield high enough to suggest possibilities for $F_1$ hybrid seed production. Our growing environment was nearly ideal in this study. Adequate soil moisture and honeybee populations at flowering, high temperatures and high relative humidity all contributed to the seed yield we observed. Seed yield on steriles would probably have been even higher in 1985, except for extended cloud cover which tended to retard cross pollination.

Inferences from the data are limited somewhat, of course, regarding seed set in a commercial hybrid production system. Our genotypes were arranged randomly in hill plots, rather than in rows. In a commercial venture, male-sterile plants would likely be grown in pure stand with pollen sources grown in nearby rows. The full potential for seed yield in such a system is not known at present. No practical method has been reported for obtaining a pure stand of male-sterile plants that are also fully female-fertile. The *msp* gene has been used to develop a pure stand of male steriles, but we have noticed that female fertility is usually very low on *msp msp* male-sterile plants.

Even though seed production on sterile plants may be adequate for hybrid production, other problems remain. These additional barriers to hybrid seed production include 1) maintaining a proper production environment for cross pollination over a large area, 2) determining appropriate heterotic combinations, 3) obtaining a pure stand of male-sterile, female-fertile plants, and 4) developing economical quality control methods. At present, we maintain an open mind concerning the feasibility of $F_1$ hybrid production. Continued research may provide solutions to these latter problems.

Table 1. Yield and seed weight of $Ms_2$ male-sterile and male-fertile plants in Raleigh, NC, in 1984 and 1985

| Genotype | Year of evaluation | Seed yield | Seed weight | n[§] |
|---|---|---|---|---|
| | | (g/plant) | (g/100 seed) | |
| $Ms_2$[†] ____ | 1984 | 67.2* | 18.2* | 212 |
| $ms_2$ $ms_2$ | | 57.6 | 21.2 | 274. |
| $Ms_2$ ____ | 1985 | 38.5* | 15.2* | 199 |
| $ms_2$ $ms_2$ | | 29.6 | 20.8 | 215 |
| $Ms_2$ ____ | combined | 52.9*[‡] | 16.7* | 411 |
| $ms_2$ $ms_2$ | | 43.6 | 21.0 | 487 |

*Fertile and sterile genotypes significantly different in the 0.05 probability level.

[†]$Ms_2$____ = male fertile; $ms_2$ $ms_2$ = male sterile.

[‡]Each year given equal weight in average.

[§]Number of plants harvested.

References

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