NORTH CAROLINA STATE UNIVERSITY
Crop Science Department
USDA-ARS Plant Science
Raleigh, North Carolina 27607

1) Mechanical separation of seed from male-sterile and fertile plants by seed size.

In soybeans, there are at least four recessive genes (ms_1, ms_2, ms_3, ms_4) that impart unconditional male sterility. A plant homozygous for any of these genes sets seed almost exclusively as a result of pollen transfer from male-fertile plants. A consequence of male sterility is that seed from sterile plants tends to be larger than those from fertile plants (Burton et al., 1979; Carter et al., 1983). The reason for this effect is not well-understood. However, it is generally assumed to result from a reduced seed set per pod on male-sterile plants.

It occurred to us that this seed size differential might be used to simplify the annual maintenance of male-sterile and fertile isolines. A possible scheme for maintaining an isoline pair would include 1) a large "natural crossing block" which segregates for male sterility, and 2) insect vectors for adequate seed set on male-sterile plants. The block would be mechanically harvested in bulk at maturity. Seed from male sterile plants are separated out of the bulk based on large seed size. These large seed then become the planting seed for the next year's maintainer block.

Such a system requires less manual labor for harvest than our current system. In addition, this system requires no pollen identification of male-sterile types. Pollen identification would become necessary in our present system if yields of male-sterile plants should be high. For example, 'Williams' ms_2 isolines do not fit well in our current maintenance system because of high yields on sterile plants.

At this point, we should briefly review our current system for maintenance of male-sterile isolines. Typically, maintainer lines are half heterozygous and half homozygous recessive at the sterile loci and near isogenic at all other loci. To maintain the line it must be grown in an isolation block where insect pollen vectors will be available. At maturity, only the male-sterile plants are harvested in order to maintain the 1:1 fertile to sterile genotypic frequencies. A maintainer block is necessarily a random mixture of sterile and fertile plants because genotypically sterile seed cannot be distinguished from genotypically fertile seed at planting. Thus, sterile plants must be identified and removed individually from the block for threshing. For ms_1 isoline pairs, male-sterile plants are usually identified visually by low seed set. For other male-sterility genes (especially ms_2), pollen identification at flowering may be required.

It should be noted that Bradner (1975) patented an interesting method for hybrid seed production that involves seed size differential, although, in his system, a genotype with extruded floral stigmas rather than a male-sterile genotype serves as a female parent. (The extruded stigma presumably results in a high percentage of hybrid seed due to insect pollination and a low percentage of selfed seed.) To produce hybrid seed, a small-seeded female line is grown near a large-seeded male line. Because they result from pollination by the large-seeded male, hybrid seed are expected to be larger than selfed seed.

Bradner's system is dependent upon the existence of xenia for seed size. To date, there is little evidence of this effect in soybeans. Kilen (1980) found seed size to be mainly determined by the maternal parent. With no paternal influence, hybrid seed could not be distinguished from selfed seed in the system proposed by Bradner. Our system is different from that of Bradner's because his method depends on xenia for success. Ours does not.

It is clear that the feasibility of our system rests in large part on efficient mechanical separation of seed. With this idea in mind, we tested our ability to mechanically separate seed from male-sterile and fertile plants for two isoline stocks.

Materials and methods: Seven plants of Williams, a group 3 cultivar, and seven plants of its male-sterile (ms_2 ms_2) isoline were grown out-of-doors in pots at Raleigh, NC, in 1983. 'Forrest', a group 5 cultivar, was grown nearby as an additional pollen source. Seed from sterile and fertile plants were harvested separately at maturity to form 2 bulks.

N69-2774, a group 8 line, and its sterile (ms_1 ms_1) isoline were grown at Clayton, NC, in 1983. Male-sterile plants were bulk harvested from a maintainer block while fertile plants were bulk harvested from a separate block approximately 200 yards away.

Two hundred seed were selected at random from each bulk. Each sample of 200 seed was passed through a series of hand-held screens. In addition, 100 random seed from each sample were individually weighed in order to obtain a distribution for seed weight.

Results: Mean seed size for male-sterile and fertile isolines differed by 3.9 grams per 100 seed in each isoline stock. However, the fertile and sterile isolines show considerable overlap in seed-weight distributions (Fig. 1). This overlap indicates that mechanized separation of seed from male-sterile and fertile isolines may be difficult in practice.

The isolines also were passed over a series of screens to test the feasibility of mechanical separation (Table 1). Results show that seed from sterile plants can be recovered in relatively pure form if a large amount of seed is discarded, and if seed from sterile and fertile plants are present in equal quantities. For instance, a 50-50 mixture of seed from male-sterile (ms_2 ms_2) and fertile Williams plants could be screened to retain the largest 20% of the seed. This sample of largest seed would consist primarily of seed from male-sterile plants with only 2% contamination from fertile plants. A 50-50 mixture may, in fact, represent a "real life" situation for a bulk harvest of this isoline pair. (Yields of male-sterile plants are nearly equal to their fertile counterparts in some environments [Carter et al., 1983].)

By contrast, screen separation for male-sterile (ms_1 ms_1) and fertile isolines of N69-2774 is not encouraging. Retaining the largest 20% of seed from a 50-50 mixture results in a seed sample from male-sterile plants which contains 10% contamination from fertile plants. In practice, however, contamination would be much higher; a typical bulk from a natural crossing block is at best a 20-80 mixture of seed from male-sterile and fertile plants. (Seed set is always low on male-sterile plants conditioned by the North Carolina ms_1 gene.) In this more typical situation, contamination would reach over 30%.

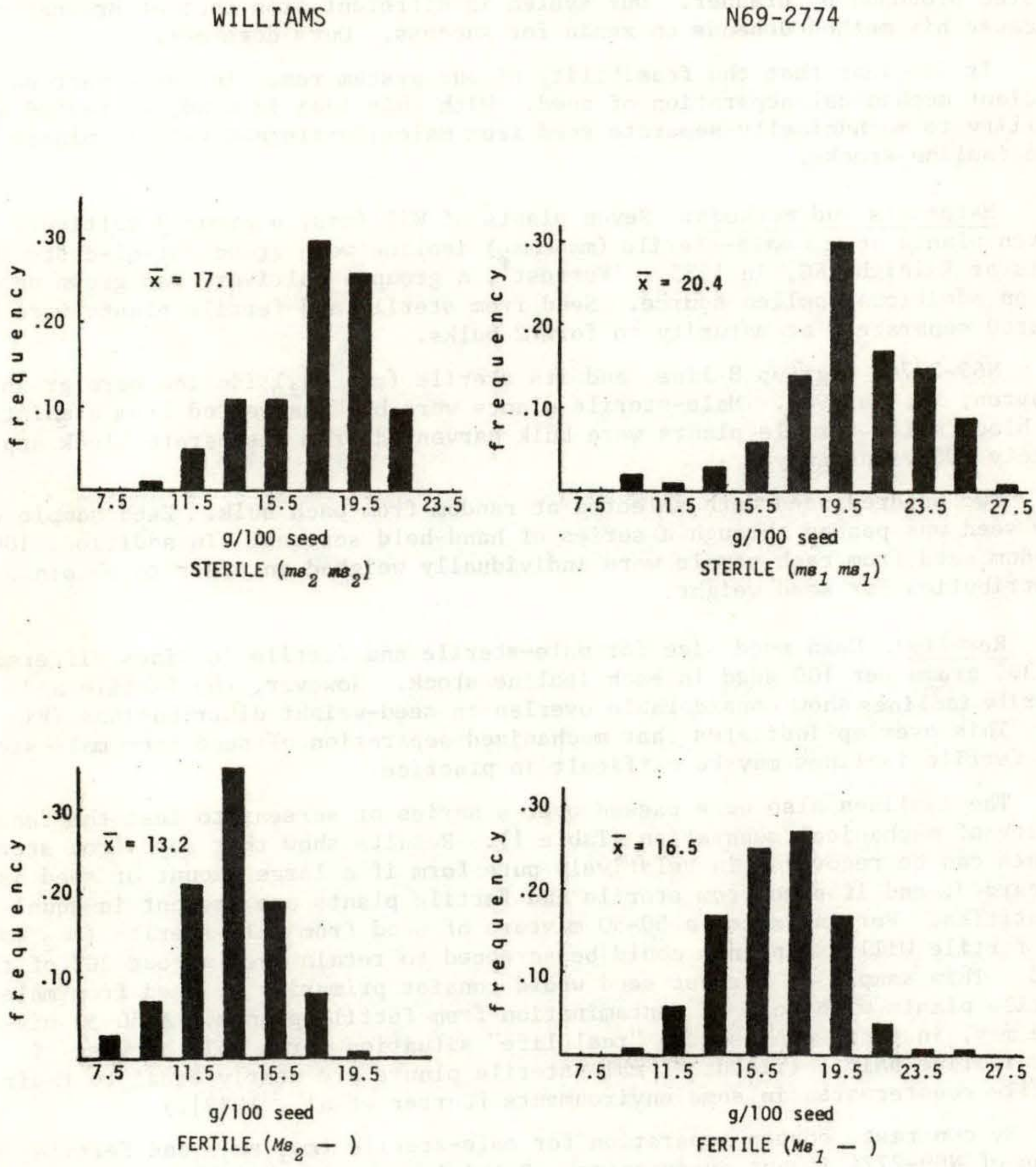

Figure 1. Seed weight distributions for Williams ms_2 isolines, and N69-2774 ms_1 isolines.

Table 1. Mechanical separation of seed samples from two sets of fertile and male-sterile isolines

Screen size	Williams Sterile (ms_2 ms_2)	Williams Fertile	N69-2774 Sterile (ms_1 ms_1)	N69-2774 Fertile
19[a]	0[b]	0	0	0
18	5	0	35	4
17	40	1	38	29
16	36	13	18	35
15	13	47	6	25
14	5	31	1	5
13	1	7	2	2
12	0	1	0	0
	100%	100%	100%	100%

[a]Diameter of round holes expressed in 64th of an inch.

[b]Percent of sample which rested on top of indicated screen but passed through the next larger screen.

In summary, the inferences from our results are limited at best. Both environment and genetic background seem to have a great effect on the ability to mechanically separate seed from male-sterile and fertile isolines. It may be safe to assume, however, that mechanical separation will not be easy in many cases. The ultimate utility of this method awaits further testing.

References

Burton, J. N., R. F. Wilson and C. A. Brim. 1979. Dry matter and nitrogen accumulation in male-sterile and male-fertile soybeans. Agron. J. 71:548-552.

Bradner, N. R. 1975. Hybrid soybean production. U.S. Patent No. 3,903,645. Off. Gaz. U.S. Patent Office 938:480.

Carter, T. E., Jr., J. N. Burton and Earl B. Huie, Jr. 1983. Implications of seed set on ms_2 ms_2 male-sterile plants in Raleigh. Soybean Genet. Newsl. 10:85-87.

Kilen, T. C. 1980. Parental influence of F_1 seed size in soybeans. Crop Sci. 20:261-262.

T. E. Carter, Jr.
J. W. Burton
E. B. Huie, Jr.