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Feasibility of Producing F_1 Hybrid Cotton Seed Through Radiation-Induced Pollen Sterility[1]

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HETEROSIS is exhibited by F_1 hybrids of cotton *(Gossypium hirsutum* L.) for seedling vigor, yielding ability, date of maturity, and other agronomic traits[3] (7, 9, 13, 15, 20). The high degree of uniformity found in F_1 hybrids for various fiber properties (17) is desirable from the standpoint of fiber processing. Thus breeders, producers, and processors are interested in developing F_1 hybrid cotton varieties, but as yet there are no practical means of producing commercial quantities of seed.

Although the cotton plant has perfect flowers, it is commonly classified as often cross-fertilized (1). However, Kohel and White (11) recently presented data characterizing cotton as being more nearly like a self-pollinating species. Pollen grains are too heavy and sticky to permit wind dispersal, so the extent of natural outcrossing is proportional to the population density of pollinator insects (3, 8, 10, 18). The variation in natural outcrossing in cotton ranges from very little to approximately 50% among areas of the United States cotton belt (3, 10, 16, 18). Brown and Ware (1) concluded that the amount of natural outcrossing varies from year to year and from one location to another.

Various methods have been suggested and tried as means of producing large quantities of F_1 hybrid cotton seed. Simpson (15) suggested either (a) make initial hand pollinations to produce a limited amount of hybrid seed and permit random outcrossing in multiplications fields, or (b) plant multiplication fields with a mechanical mixture of seeds from two varieties and allow natural outcrossing to occur. Stroman (17) suggested hand pollination to produce as many F_1 seeds as possible, then utilizing both ratooning and vegetative reproduction of F_1 crop plants to produce a larger second-year crop. Duncan et al. (5) considered the utilization of geographical areas with known high incidence of natural outcrossing as synthetic hybrid production centers. Selective gametocides and male sterility have been used to increase the amount of outcrossing, but they reduced yield severely[3,4,5,] (6, 10, 14). Justus[6] re-

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[5] Pate, J. B., and Duncan, E. N. Yield and other characteristics of experimental cotton hybrids. Proceedings of Thirteenth Annual Cotton Improvement Conference, 51–56. 1961.

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ported significant progress towards the production of F_1 hybrid cotton by means of mechanical seed separation; F_1 seeds show heterosis or partial dominance for size. Natural outcrossing was considered by Cross and Richmond (3) as offering the only practical means of producing hybrid seed in quantity. Kohel and Richmond (10) summarized efforts directed at this problem as follows, "Neither the present stage of technical knowledge . . . , nor the practical aspects of the problem are encouraging from the standpoint of producing hybrid cotton seed in commercial quantities."

Highly self-fertilized plants grown from irradiated seeds show an increase in percent of outcrossing. Seed irradiation induced pollen sterility which in turn increased the amount of natural field hybridization in *Avena* and *Glycine* (2, 12, 19, 21). Weber and Hanson (21) reported a 4- to 6-fold increase in outcrossing in soybeans following a 20 kr gamma- or X-ray seed treatment. This increase in outcrossing was attributed to radiation-induced male sterility which permitted ovules to remain unfertilized for longer periods of time during which they were more vulnerable to foreign pollen. Konzak (12) found that radiation-induced pollen sterility caused an increase in outcrossing which was related to radiation dose. The worth of seed irradiation to produce hybrid seeds of self-fertilized plants in which hand-crossing is difficult and tedious was discussed by Weber and Hanson (21).

In view of this, a field experiment was conducted at Oak Ridge, Tennessee, during 1962 to ascertain the feasibility of using gamma-radiation seed treatment as a means of producing commercial quantities of F_1 hybrid cotton seed through increased natural outcrossing. In this respect, data are presented concerning M_1 seedling survival, M_1 yield performance, M_1 percent outcrossing, and M_2 seedling survival.

MATERIALS AND METHODS

The tester varieties were *G. hirsutum* L., 'Deltapine Smooth Leaf', 'Empire W. R. 61', and 'Pope'; 'Red Empire' (homozygous for an incompletely dominant gene governing red plant color) was used as the marker stock. Acid-delinted, fungicide-treated seeds of tester varieties were stored 3 weeks at 70 ± 10% relative humidity and 50 ± 2° F. which resulted in an average seed moisture content of 11%. These seeds were then sealed in 4-mil polyethylene bags and exposed at room temperature to 5, 10, 20, or 40 kr of gamma-radiation from Co^{60} (405 r/min.). Control seeds were handled similarly except that they were not exposed to radiation. All seeds of a treatment were sown in the field within 50 minutes post-irradiation.

The alternate row procedure of testing for degree of outcrossing was utilized and all border rows were planted to marker stock. Seeds were sown in hills (5 seeds/hill) 12 inches apart on 15-foot rows, and plants were thinned at 6 weeks post-planting to 2 per hill. Growing plants were sprinkler irrigated when necessary and insecticides were used until plants were past the seedling stage. Use of insecticides was discontinued to maintain an undisturbed population of pollinator insects. Insect damage was trivial.

A split plot design was utilized in which 2 rows per treatment of each variety were planted in each of 3 main blocks. Varieties were planted randomly in separate subblocks of each main block. Thus, there was a total of 90 rows planted to seeds of the 3 tester varieties. In the analysis of variance, the between-treatment-row variation was removed to provide a better estimate of treatment effects.

The effect of seed irradiation on the extent of outcrossing was determined by scoring M_2 populations from open-pollinated seeds of tester varieties. The M_2 populations were grown in a greenhouse and under these conditions the seedlings from outcross seeds, heterozygous for red plant color, were unquestionably recognizable. Percent outcrossing represents the frequency of these heterozygous red seedlings among survivors of 720-seed plantings from each treatment of each variety. The number of survivors at 3 weeks post-planting expressed as percentage values is reflected in the M_2 seedling survival data. M_1 seedling survival was determined by an actual count in the field at 5 weeks post-planting and expressed as percentage values. Yield performance of M_1 plants is shown as the total seed-cotton harvested expressed in grams per row. These data were used to evaluate seed irradiation as a tool in producing large quantities of F_1 hybrid cotton seed by means of increased outcrossing due to induced pollen sterility.

RESULTS AND DISCUSSION

Plant growth and development of the tester varieties and the marker variety were comparable. Plants from seeds that received 20 and 40 kr of gamma-radiation were stunted, and delayed in respect to blooming. However, there was sufficient overlap in blooming dates to permit unbiased outcrossing between the irradiated tester and non-irradiated marker plants. There were no differences among Deltapine Smooth Leaf, Empire W. R. 61, and Pope (three commercial southeastern cotton varieties) in respect to radiation sensitivity of the dormant seeds. The 50% growth-reduction dose for all 3 varieties was approximately 38 kr, whereas the 50% yield-reduction dose was 23 kr, administered to dormant seeds containing 11% seed moisture (Figure 1). The array of radiation doses administered to the dormant seeds of the three varieties had no effect on M_1 survival, and had a highly significant effect on M_1 percent outcrossing, M_1 yield, and M_2 seedling survival (Table 1). No significant differences were observed due to varieties, blocks, or varieties × blocks interaction.

The mean outcrossing for control plants of all varieties was approximately 28%, which is considerably less than the 46% natural outcrossing found on the USDA Cotton Field Station near Knoxville, Tennessee (16). The distance between the Oak Ridge experimental field and the USDA Cotton Feld Station is less than 30 miles, but there are considerable differences in environmental conditions. This particular experiment was a small isolated planting nestled between the Clinch River and rather high wooded ridges which may have had a profound influence on the population density of pollinator insects. However, the magnitude of the difference observed in this case is not unique. Kohel and Richmond (10) reported 26% and 7% outcrossing in test

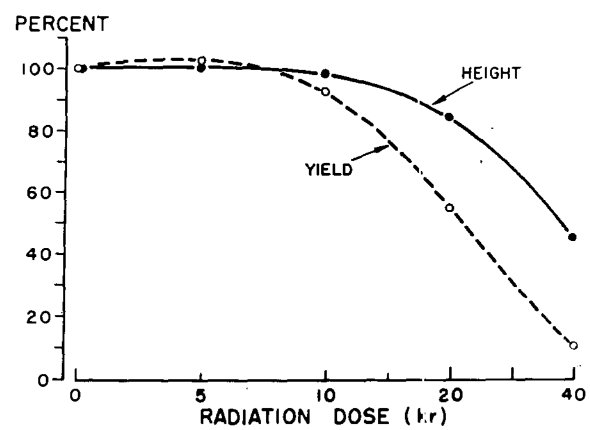

Figure 1. Effects of gamma radiation on plant height at 11 weeks post-planting and total seed-cotton yield relative to unirradiated control.

Table 1. Analysis of radiation effects for observed traits on the basis of Duncan's Multiple Range Test (4).

% M_1 seedling survival		Row yield, g./row		% M_1 outerossing		% M_2 seedling survival	
Dose (kr)	Mean	Dose (kr)	Mean	Dose (kr)	Mean	Dose (kr)	Mean
0	59.57*	5	686.3	40	48.64	5	60.97
10	57.59	0	667.6	20	42.12	0	55.97
20	56.48	10	618.8	10	30.78	10	53.33
5	55.86	20	367.4	5	29.09	20	50.69
40	52.28	40	69.3	0	27.88	40	36.03

* All means included within the same solid vertical line are similar statistically; all means outside are different statistically at $P < 0.01$.

plantings on the Agronomy Farm at College Station, Texas, and the A. and M. College Plantation, respectively, and these 2 areas are only 8 miles apart.

It is interesting to note that seed irradiation at levels of 20 and 40 kr increased the percent outcrossing from 28% in the controls to 42% and 48%, respectively—almost a 2-fold (and highly significant) increase. On this basis only, one could conclude that 100% outcrossing might be attained by growing plants from seeds irradiated at 20 to 40 kr in areas of 50% or more natural outcrossing. However, this is highly misleading as we shall see later.

Economics force the hybrid seed producer to pay particular attention to crop yield; hybrid cotton seed production would be no exception. As seen in Table 1, a radiation dose (administered to dormant seeds) of 20 kr reduced seed-cotton yield by almost 50% while a 40 kr dose reduced yield to approximately 10% of the controls. This reduction in yield was highly significant. These were the same radiation doses necessary to increase the incidence of outcrossing above that of the control populations. There were no differences among plants of the controls, 5 kr, and 10 kr doses of radiation.

Another economic facet of this problem is germination of M_2 seeds and survival of M_2 seedlings. Farmers cannot afford to plant, nor do the various state governments permit the sale of, cotton seeds with substandard germination percentage. Data were not collected on seed germination; however, when open-pollinated seeds of tester varieties were planted in greenhouse beds, a reduction in M_2 seedling survival at 3 weeks post-planting was observed at the 40 kr radiation dose. At this level of seed irradiation seedling survival was 36% as compared to 56% for the controls, a difference which was highly significant (Table 1). There were no differences in this respect among the controls, 5, 10, or 20 kr seed treatments.

Results of computations to ascertain the value of seed irradiation as a tool in producing large quantities of F_1 hybrid cotton seed are presented in Table 2. Relative seed yield, "A", multiplied by percent M_2 survival (Table 1) gives the M_2 potential survivors, "B". The potential F_1 hybrids, "C", is "B" multiplied by percent M_1 outcrossing (Table 1). Without seed irradiation one could expect approximately 16 potential F_1 hybrid units; the potential F_1 hybrids increased to 18 units when seeds were exposed to 5 kr, but dropped to only 2 when exposure was 40 kr (Table 2). The severe reduction is a direct reflection of the decrease in seed-cotton yield and M_2 seedling survival caused by the 40 kr gamma-radiation seed treatment. Thus the slightly less than 2-fold increase in percent outcrossing induced by 40 kr radiation treatment is more than offset by the drastic decrease in seed-cotton yield of M_1 plants and reduced survival of M_2 seedlings from open-pollinated seeds. It is evident from these results that seed irradiation at present is impractical as a tool in the production of F_1 hybrid cotton seeds for commercial planting.

Table 2. Relative potential F_1 hybrid seed production as influenced by radiation treatment.

Production component	Radiation treatment (kr)				
	0	5	10	20	40
A. M_1 seed yield (relative)	100	103	93	54	10
B. (A) × % M_2 survival	56	63	49	28	4
C. (B) × % M_1 outcrossing	16	18	15	12	2

SUMMARY

Dormant seeds (11% moisture) of 3 American Upland Cotton varieties—Deltapine Smooth Leaf, Empire W. R. 61, and Pope—were exposed to 0, 5, 10, 20, or 40 kr of gamma-radiation. Response of all varieties to seed irradiation was comparable. The following responses were observed: *(1)* M_1 seedling survival was not affected, *(2)* yield of seed-cotton was reduced severely by the two highest radiation treatments, *(3)* outcrossing was increased from 28% in the controls to 42% and 48% in the 20 kr and 40 kr populations, respectively, and *(4)* M_2 seedling survival decreased from 56% in the controls to 36% at the 40 kr dose.

The increase in percent outcrossing, due probably to radiation-induced pollen sterility, was more than offset by the severe decrease in seed-cotton yield and M_2 seedling survival. Therefore, on the basis of this experiment, it was considered impractical to recommend seed irradiation as a means of producing large quantities of F_1 hybrid cotton seed.

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