Frequency of Cross-Pollination of Soybeans After Seed Irradiation[1]

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ABSTRACT

Soybean [*Glycine max* (L.) Merrill] seeds of white-flowered varieties were irradiated with 0, 5, 10, or 20 k rads of gamma rays and planted in rows bordered by purple-flowered varieties of the same maturity. Honeybees were kept in the vicinity of the plots during the flowering period. Seedlings grown from seeds harvested from the white-flowered plants were classified for hypocotyl color. The frequency of outcrossing varied with location, with varieties, and with treatment level of irradiation. Outcrossing in the nonirradiated populations varied from 0.30% to 3.62%. An increase of outcrossing, to as much as 11 to 14% in some cases, was observed in the irradiated soybeans. A mixture of the white- and purple-flowered varieties in the same row increased outcrossing slightly over similar treatments with the varieties in separate rows. Seed treatment with ethyl methanesulphonate (EMS) increased outcrossing about the same as the irradiation. In this study a combination of mutagen-treated seed plus large honeybee populations did not increase outcrossing over earlier reported frequencies from each used separately.

Additional index words: **Ethyl methanesulphonate, Natural hybridization, Outcrossing, Honeybees, [*Glycine max* (L.) Merrill].**

THIS study evaluates the combined effects of seed irradiation and honeybees in promoting outcrossing in varieties of soybeans [*Glycine max* (L.) Merrill] in different maturity groups at different locations in California. A preliminary evaluation of the effect of ethyl methanesulphonate (EMS) for one variety at one location is included for comparison.

Estimates of natural crossing in soybeans vary from 0.03% (3) to 3.35% (8). Estimates by Casas (2), Cutler (4), Garber and Odland (5), Piper and Morse (7), Starnes and Hadley (9, 10), Takaji (11), Weber and Hanson (12), and Woodworth (13) fall between these extremes. The frequency estimates have varied owing to locations (12), season (3), date of sowing (8), and distance from the pollen donor (3, 12). Hybrid plants were determined from contrasting characteristics such as flower color (3, 12, 13), hilum color (8), seed coat color (5, 12), and pubescence (4).

Johnson and Bernard (6) reviewed the literature dealing with outcrossing in soybeans. Weber and Hanson (12) found that treatment of seed with x-rays and thermal neutrons increased outcrossing slightly (average 1% for control, 4.4% irradiated). Using alpha, betadichloroisobutyrate on plants, Starnes and Hadley (9, 10) found that 250 to 500 ppm increased outcrossing slightly (0.35%) but 750 to 1,000 ppm decreased outcrossing (0.08%) compared to nontreated plants (0.21%), because the flowers were progressively more cleistogamous with increasing concentration of the chemical. Casas (2) found the frequency of natural crossing was increased from 0.6% to 5% by enclosing soybean plants in cages with a population of honeybees.

Casas (2) found that bees did transfer pollen. Rosas (8) and Woodworth (13) suggested that thrips may transfer pollen. Weber and Hanson (12) speculated that bees, thrips, other insects, and wind are possible agents for pollen transfer.

MATERIALS AND METHODS

This study consisted of two main experiments: First, an attempt to determine the optimum mutagenic treatment of the seed for maximum outcrossing (sections 1A, 1B, and 2 below), and second, the use of seed irradiation to intercross the germ plasm (predominantly within maturity groups) in the USDA Soybean World Collection (section 3). In all experiments honeybees in hives were placed near the plots during the flowering period.

Purple petals and purple hypocotyl are due to the same gene (1, 14), and there is complete dominance for both characteristics. Consequently, in our study seedlings with purple hypocotyls were considered due to outcrossing because the seeds were taken from white-flowered plants. These are minimal estimates of outcrossing since white × white crosses are scored as selfs.

Estimates of the frequency of natural-hybridization were obtained from three sources as outlined below.

(*1*) White-flowered plants grown from irradiated or ethyl methanesulphonate (EMS) treated seed in a center row bordered by rows of purple-flowered plants:

A. White- and purple-flowered varieties were selected from each of maturity groups 00, 0, I, II, III, and IV. Seeds of the white-flowered variety from each pair were exposed to various treatments of gamma rays in the ^{60}Co facility at the University of California, Davis. Different seed lots of each variety received 0, 5, 10, or 20 k rads of gamma rays. The varieties and locations where each pair were grown in the field are shown in Table 1.

B. Different seed lots of the white-flowered variety A-100 were soaked for 4 hours in 0, 0.5, 1.25, or 2.0% EMS at 20 C. These seeds were then rinsed in running tap water for 18 hours and dried in a forced draft oven at 30 C. This test was grown in the field at Five Points only.

(*2*) White- and purple-flowered plants grown in the same row, bordered by purple-flowered plants:

Seeds of the white-flowered variety A-100 irradiated as described in 1A above were mixed with an equal amount of nonirradiated seed from the purple flowered variety Hark before planting. Only tagged white-flowered plants were harvested. This test was located at Five Points only.

At each location approximately 100 seeds from each irradiated and control lot in 1A, 1B, and 2 above were sown in 6 m rows spaced at 80 cm. There were three replications at each location.

Outcrossing was determined (1A, 1B, and 2) from 10 to 15 white-flowered plants tagged during the flowering period. The seeds from each plant were sown in vermiculite; and hybrid seedlings were determined by the purple color of the hypocotyl about one month after sowing. Rows of Lee (purple flowers) and Bragg (white flowers) seedlings were also grown with each planting, as checks for the development of hypocotyl color.

In most cases 1,500 to 2,000 seedlings were classified for each value shown in Table 1 except for the plants that were grown at Shafter. Emergence was poor at this location and in some plots less than 10 plants reached maturity. The varieties in

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Table 1. Percentage outcrossing on control and M_1 plants after seed irradiation or treatment with ethyl methanesulphonate (EMS).

Variety treated	Pollen source	Control	Irradiation 5 k rads	10 k rads	20 k rads
		Plants grown at Davis			
M54-12-2	Portage	0.31	0.34	11.97	1.96
Merit	M391-1	2.28	2.56	6.79	3.24
A-100	Hark	0.50	1.22	2.39	1.59
Mukden-Seneca	Harosoy 63	3.49	4.00	4.40	2.25
Avg.		1.65	2.03	6.39	2.26
		Plants grown at Shafter			
M54-12-2	Portage	0.56	0.02	0.40	0.41
Merit	M391-1	1.00	0.71	3.38	0.00
A-100	Hark	0.30	0.36	0.95	1.23
Mukden-Seneca	Harosoy 63	0.47	0.00	3.92	0.00
Avg.		0.58	0.27	2.16	0.41
		Plants grown at Five Points			
M54-12-2	Portage	3.46	6.41	7.01	7.52
Merit	M391-1	1.54	5.40	14.94	6.32
A-100	Hark	3.62	2.16	5.84	4.54
Mukden-Seneca	Harosoy 63	0.83	2.13	1.59	14.64
Avg		2.36	4.03	7.35	8.26
Wayne	Shelby	0.44	2.22	5.80	2.91
Delmar	Clark 63	0.67	1.21	0.78	1.22
Avg all varieties		1.76	3.26	5.99	6.19
A-100 & Hark mixed		2.12	4.47	6.70	7.75
		At Five Points			
			Aqueous EMS		
		Control	0.5%	1.25%	2.0%
A-100	Hark	2.47	1.25	2.12	7.45

groups 00, 0, I, and II were also sown at Brawley, but these varieties are not adapted and flowering occurred during a period of extremely hot dry weather. There was very little seed set and no outcrossing with the few seeds that matured.

(*3*) White-flowered plants selected from a mixed population: Approximately 100 seeds from each entry in each maturity group 00 through VII in the USDA Soybean World Collection were bulked. Half of these seeds were then exposed to 5 k rads of gamma rays from the ^{60}Co facility at the University of California, Davis, in 1967. The other half served as nonirradiated controls. The maturity groups that were grown in each location are shown in Table 2. Honeybees in hives were placed near the plots during the flowering period. During flowering 20 white-flowered plants were tagged in each maturity group population at each location. The seeds from the tagged plants were sown in vermiculite, and hypocotyl color was used to distinguish hybrids.

After harvesting the tagged plants, the seeds from the remaining plants were bulked within maturity-treatment populations. A 1-kg sample of the bulked seeds from irradiated populations was irradiated with 10 k rads and sown at the same location. Seeds from control populations were sown without irradiation at the same location. These experiments were continued through 1968 and 1969.

RESULTS

Table 1 gives the results obtained from studies of hypocotyl color of seedlings from single white-flowered plants from sources 1A, 1B, and 2. At Davis there were no significant increases in outcrossing as a result of irradiation. At Shafter varieties were not significantly different, but irradiation treatment differences were highly significant. The variety × irradiation interaction was also highly significant, probably due to a lack of outcrossing in Merit with a treatment of 20 k rads. At Five Points, where outcrossing was higher than at Davis or Shafter, there were significant differences between varieties and significantly increased outcrossing was observed with all irradiation treatments. The variety × irradiation interaction was not significant.

The level of outcrossing in A-100 was significantly higher when it was mixed and bordered by Hark than when it was grown in a solid row and bordered by rows of Hark (Table 1, Five Points).

Table 2. Percentage outcrossing after seed irradiation on white-flowered plants selected from the USDA World Collection Bulks for each maturity group.

Maturity group	1967 5 k rads	1968 Control	1968 10 k rads	1969 Control	1969 10 k rads
	M_1 plants grown at Five Points				
Group I	5.21	2.44	6.40	0.80	3.60
II	3.10	1.73	0.50	0.46	0.43
III	2.34	0.59	0.70	0.54	0.50
IV	1.90	0.22	0.44	0.50	0.33
	M_1 plants grown at Shafter				
II	2.02	0.19	0.00		0.55
III	0.42	0.17	0.00		0.59
IV	0.14	0.12	0.30		0.37
V	0.54				
	M_1 plants grown at Brawley				
V			3.14		1.53
VI		0.15	0.00	0.00	0.24
VII		0.31	0.00	0.88	0.81

Significantly increased outcrossing was observed on plants from EMS treated seed (Table 1, Five Points), and levels of outcrossing were roughly comparable to those obtained from irradiation treatments on the same variety.

Table 2 gives average values obtained from seedlings grown from single white-flowered plants from World Collection Bulks (Source 3). Results were extremely erratic. There was no replication, so the data cannot be analyzed. White-flowered plants of group I maturity grown at Five Points had the highest frequency of seedlings with purple hypocotyl. Although the average for 10 to 20 plants is shown in Table 2, the progeny of each plant was checked and none approached a 3:1 ratio. Thus, the purple hypocotyls must have been due to outcrossing.

DISCUSSION

Two main factors are involved in outcrossing. One is the failure of self-pollination or fertilization; the other is the transfer of foreign pollen. If all self-pollinations are effective, the pollen transfer process is not important, but, if the pollen available for selfing is sterile or otherwise ineffective, the pollen transfer process determines the number of seeds, and the frequency of outcrossing. In soybeans the normal situation is a complex interaction effect of these two extremes. It is not known whether the low level of outcrossing is due to a highly efficient self-fertilization process or a lack of pollen transfer.

Speculation regarding the importance of each factor can be enlightening, although supporting data are scanty in this experiment. Calculations of the frequency of outcrossing, using various frequencies of self-pollination-failure can be plotted against seed set, assuming various frequencies of effective pollen transfer. By plotting the actual observed outcrossing frequencies and seed set, some interesting relationships can be shown (Fig. 1). All data must be converted to percentage of the control for comparison with the theoretical relationships.

It appears that the frequency of pollen transfer is the limiting factor in soybean outcrossing, judging from the shape and position of the plotted actual data line (Fig. 1). Data from individual locations (not shown) were more erratic, but indicated effective pollen-transfer-frequency was about 10% at Five Points,

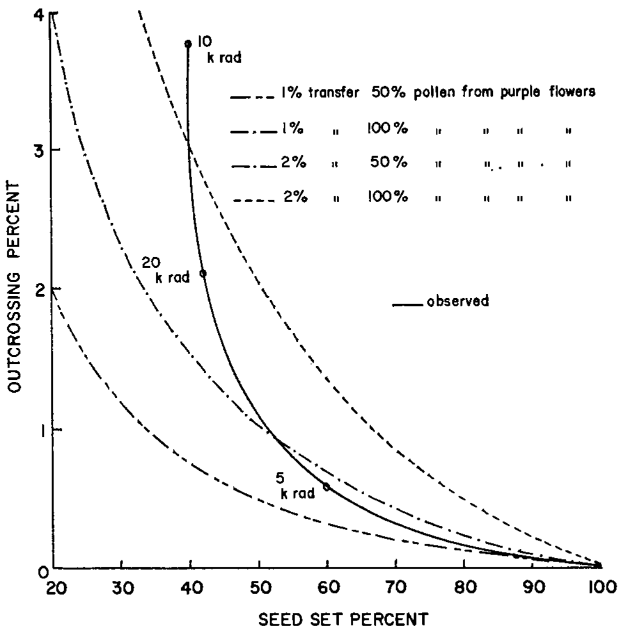

Fig. 1. Theoretical relationship between frequency of outcrossing and percent seed set for 1% and 5% effective pollen transfer, and the relationship between actual outcrossing and seed set of soybeans.

from 1 to 5% at Davis, and between 0 and 1% at Shafter.

In this and similar studies, outcrossing was determined if the foreign pollen came from a purple flower. However, pollen from a white flower—even from another flower on the same plant—should be considered an outcross from a purely academic or mechanical standpoint.

If pollen transfer occurs at random, the frequency of pollen transfer from purple flowers to white flowers and from white flowers to white flowers is a function of the ratio of white to purple flowers. The values for this ratio may vary for each location and variety pairs involved, but a base level of outcrossing will be established for each situation. If seed irradiation reduced the number of plants, or the number of flowers per plant of the white-flowered variety, the ratio of white to purple flowers would change, but the number of pollen transfers should remain the same. Because we score only the purple to white flower pollen transfers, the detected outcrossing frequency would increase even with no change in the pollen sterility of the white-flowered plants.

At Five Points and Shafter there was a highly significant association between irradiation treatment level and frequency of outcrossing. This total effect could consist of two parts: (*1*) a direct effect by producing chromosomal aberrations or mutations that make the pollen ineffective, and (*2*) an indirect effect by decreasing the number of plants, or the number of flowers per plant of the white-flowered variety.

One measure of the indirect effect of the irradiation is the correlation between the frequency of outcrossing and the number of viable seeds per white-flowered plant. This correlation for the experiment at Five Points was negative but nonsignificant, and at Shafter was negative but nonsignificant. For the total study, including Davis, the correlation was negative and significant, but the r^2 value was only 0.04%, not large enough to be useful. The r values showing the association between outcrossing and number of seeds were also calculated for each variety and each irradiation level. In each case the correlation was negative but nonsignificant. This indicates there is some indirect effect of seed irradiation.

The use of mutagens plus honeybees did not increase outcrossing to a useful level. Furthermore, there seems to be a poor association between the results obtained in the experiment to determine optimum irradiation levels and the attempt to mix the germ plasm.

In comparing the single variety at only one location, EMS and irradiation appear to be equally effective in promoting outcrossing. Our method of treatment with EMS was extremely damaging to the seeds, but some modification of the treatment might be found that would cause less damage.

There was more outcrossing at Five Points than at Davis, and more outcrossing at Davis than at Shafter. The thrip population was about the same at all locations, or perhaps slightly higher at Davis. Spider mites, present at all locations, would not be expected to affect outcrossing. There were fewer honeybees at Shafter than at Five Points or Davis. Leaf cutter bees are used extensively around Five Points to pollinate alfalfa fields, and these may have strayed into the soybean plots, but no data are available concerning this hypothesis.

In order to effectively mix the germ plasm in the USDA Soybean World Collection with low levels of outcrossing, hybrid identification is necessary. A variety with three or four recessive characteristics could be developed for each maturity group. The seed from this varitey would be treated with the mutagen causing the highest level of pollen sterility with the least damage to the rest of the physiological and reproductive systems of the plant. Plants from the irradiated seeds would be grown between border rows of the bulked World Collection of the same maturity. In the next generation most of the hybrids could be identified in the progeny of the variety with the recessive characteristics. The seeds from the hybrid plants could be grown either in plant rows or as a bulk for a generation or two to increase the seed supply and to build up the frequency of homozygosity. Then a portion of the new bulk could be used as the pollen donor to start another cycle. Decisions regarding: (*1*) method of generation advance of the bulk, (*2*) method of selection within the bulk for material to start the next cycle, and (*3*) use of the original or a reselected line with the recessive characteristics for the mutagen-treated variety would depend on the objectives of the experiment. If outcrossing is genetically controlled this new bulk should have a higher frequency of genes that favor outcrossing. This procedure is similar to recurrent selection programs and could be continued through any number of additional cycles. It would tend to select for gene combinations that increase the frequency of outcrossing in addition to mixing the germ plasm. Selections from any individual hybrid or the bulk population could be made and tested with standard varieties to determine progress.

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