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Effects of Genotype and Heterozygosity of Pollen Source and Method of Application of Pollen on Seed Set and Seed and Fiber Development in Cotton[1]

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ABSTRACT

This paper reports results of a factorial experiment involving four methods of pollen application, four genotypes, and five levels of heterozygosity of pollen source from Upland cotton, *Gossypium hirsutum* L. Pollen was applied to a common female parent. Data obtained were seed number per boll, seed number per locule, seed index, lint index, grams of lint per boll, and staple length. Different methods of pollination caused varying degrees of physical damage to the young flowers, and this was reflected in all characters measured except staple length. Heterozygosity of the pollen source provided interesting results. Larger seeds developed when flowers of the female parent (F_0^1) were pollinated with pollen of the same source than when pollinated with pollen from F_1 or from genetically different parental sources (F_0^2). Seed development (seed index) was less variable with F_0^1 than F_1 or F_0^2 pollen; this indicated greater developmental stability and adaptation of self-fertilization.

Additional index words: **Upland cotton, *Gossypium hirsutum* L., and homeostasis.**

CONTROLLED pollinations form an integral part of the routine procedures associated with a cotton genetics and improvment program. The researcher frequently observes variation in boll set and seed formation resulting from controlled pollinations. Experimental control, however, is usually not adequate to separate the causes of this variation. Loden, Lewis, and Richmond (1950) showed that boll-set and number of seeds per boll were not significantly different from hand-crossed, selfed, or open-pollinated flowers. Earlier observations by Harrison (1931) indicated that fiber development was influenced by the pollen parent of the seed embryo. Pressley (1937) attempted unsuccessfully to duplicate these results in a later experiment.

The purpose of this experiment was to study the effects on the seed set and subsequent seed and fiber devolpment of methods of pollination, differing genotypes, and levels of heterozygosity of source of pollen applied to flowers of Upland cotton, *Gossypium hirsutum* L.

MATERIALS AND METHODS

A long term inbred line (TM 1) was used as a common female parent. Four pollen parent genotypes were selected to represent varying degrees of divergence from the female genotype. *Genotype 1* was a Brown linted (Lc_1) stock derived from four generations of backcrossing to TM 1. *Genotype 2* was a Naked seeded (N) stock derived from four generations of backcrossing to TM 1. *Genotype 3* was a multiple marker stock (T 586) that combines the following genetic markers Naked seed (N), Brown lint (Lc_1), Yellow pollen (P), Yellow petals (Y), Red plant body (R_1), Red petal spot (R_2), Hirsute (H_2), and Okra leaf (L^o). *Genotype 4* (T 34) was *G. hirsutum* race *latifolium* collected in Central America, and thus represented the greatest degree of divergence from TM 1. The first three genotypes represented variation primarily associated with qualitative genetic characters. *Genotype 4*, while of the same race as the other genotypes, presumably differed quantitatively from TM 1, because it was collected recently and has not been included in current programs of agronomic selection.

Five levels of heterozygosity were employed. *Heterozygosity 1* was TM 1 pollen (F_0^1), and *heterozygosity 5* was pollen from each of the contrasting genotypes (F_0^2). *Heterozygosity 3* was pollen from the F_1 plants ($F_0^1 \times F_0^2$), and *heterozygosity 2* and *4* was pollen from BC_1^1 ($F_1 \times F_0^1$) and BC_1^2 ($F_1 \times F_0^2$) plants, respectively.

The four methods of pollination were: *Method 1* flowers were hand-emasculated with the thumbnail-soda-straw technique then hand-pollinated; *Method 2* glasine bags were placed over floral buds the day prior to anthesis and on the day of anthesis the bag was removed, the flower was hand pollinated, and the bag was replaced; *Method 3* the flower was allowed to open normally and then it was hand pollinated; and *Method 4* the flower was allowed to undergo normal anthesis and open pollination. Outcrossing generally averaged about 30% in the experimental nursery.

The experiment was a factorial arranged in split-plot design with two replications. Genotype-heterozygosity treatment combinations made up the main plots, and mode-of-pollination treatments made up the subplots. In the field, two female parents were surrounded by six plants of each genotype-heterozygosity treatment combination. All four methods of pollination were applied to each female plant with a pooled pollen sample from no fewer than three plants, and an attempt was made to set two bolls for each pollination treatment. Two bolls were not obtained in all cases, so that a single boll average for each female plant was used as the basic experimental unit.

The following characters were evaluated; seed number per boll, seed number per locule, seed index (the weight in grams of 100 seeds), lint index (the weight in grams of lint from 100 seeds), grams of lint per boll, and staple length.[3]

RESULTS

Differences among methods of pollination were statistically significant for all characters measured except staple length (Table 1). The F ratios for testing the effects of mode of pollination on seeds per locule, lint per boll, seeds per boll, seed index, lint index, and staple length were 88.86, 80.08, 48.44, 12.01, 10.92, and 2.25, respectively. This change in F ratio reflects the anticipated effect that physical damage to the bolls might have on the characters studied. The observed differences associated with mode of pollination must have been caused mainly by physical damage to

Table 1. Analysis-of-variance significance test of treatments for each character measured.

		Mean squares of character measured					
Treatment	D. F.	Lint index	Seed index	Staple length	Lint/ boll	Seeds/ boll	Seeds per locule
Genotype	3	20.17*	35.31	11	2.59	28	1.42
Heterozygosity	4	26.81**	50.66**	67	3.56*	94	2.58
G × H	12	7.24	7.83	16	.88	78	.80
Pollination	3	28.16*	24.69*	9	10.41**	3,778**	79.09**
G × P	9	.77	2.14	6	.10	38	1.56
H × P	12	1.54	3.83	3	.33	56	1.34
G × H × P	36	1.87	2.68	3	.32	74**	.97

*, ** Statistically significant at the 5 and 1% levels, respectively.

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the flower. Loden, Lewis, and Richmond (1950) did not detect significant differences in seed per boll caused by method of pollination, although hand crossing did cause some reduction in seeds per boll. The care taken by the individual performing the pollinations can influence the results. All the pollinations in the experiment reported herein were carried out by one individual in a normal, routine manner without any special techniques.

Mode-of-pollination means for each character are presented in Table 2. The increased floral manipulation, and consequently greater physical damage, resulted in the reduction of seeds set per boll. This reduction in seed number resulted in larger seeds (seed index), although the differences were not great enough to show significant subgroupings among the means. The slight reduction in lint index values from flowers hand-emasculated and pollinated, compared to values from bagged and hand-emasculated flowers, are ascribed to physical damage. Otherwise, lint index followed a response similar to seed index. Lint per boll is a product of lint and seed number, and its response followed this relationship.

The only character which showed a significant response to the genotype of the pollen source was lint index (Table 3). Lint index values were highest when the genotype of the pollen parent differed the least from the female parent, and decreased as the genotype of the pollen parent increased in genetic diversity from the ovule parent.

Lint index, seed index, and lint per boll responded significantly to the differences in heterozygosity of the pollen source. The characters were consistently low in *heterozygosity 2* (Table 4), the backcrosses to the ovule parent (BC_1^1). Because the plants in the backcross generations represented genetic recombinants, the distribution of genotypes could have been non-random. Among the homogeneous genotypes, *heterozygosity 1*, TM 1 pollen applied to TM 1 plants, consistently yielded the highest value. *Heterozygosity 3* and *5*, the F_1 and F_0^2 pollen sources, gave lower values and belonged to the same significance subgroup. The genotype $\times$ heterozygosity interaction was not significant, and when *heterozygosity 1* and *5*, F_0^1 and F_0^2 pollen, for lint index, seed index, and lint per boll were plotted against genotypes, *heterozygosity 1* was consistently higher than 5.

Table 2. Mode-of-pollination mean values for characters measured.

Method of pollination	Character measured*				
	Seeds per locule	Lint index	Seed index	Seeds per boll	Lint per boll (gm.)
(1) Hand emasculated and pollinated	7.15 c	6.07 a	11.14 a	26.36 b	1.64 b
(2) Bagged, hand pollinated	7.90 b	6.32 a	10.94 a	32.65 a	2.06 a
(3) Unbagged, hand pollinated	8.15 a	5.85 ab	10.70 a	32.43 a	1.96 a
(4) Open-pollinated	8.25 a	5.80 b	10.50 a	32.49 a	1.86 a

* Means followed by a common letter indicate nonsignificant subgroups (Duncan's test, 5% level of significance).

Table 3. Genotype mean values for lint index.

Genotype of pollen parent	Lint index*
(1) Brown lint	6.32 a
(2) Naked seed	6.08 ab
(3) Multiple marker	5.80 b
(4) race latifolium	5.79 b

* Means followed by a common letter indicate nonsignificant subgroups (Duncan's test, 5% level of significance).

Table 4. Level of heterozygosity mean values for characters measured.

Heterozygosity of pollen source	Character measured*		
	Lint index	Seed index	Lint/boll
(1) F_0^1 (TM 1)	6.48 a	11.42 a	2.06 a
(2) BC_1^1	5.62 c	10.28 c	1.74 b
(3) F_1	5.90 bc	10.63 be	1.86 b
(4) BC_1^2	6.14 ab	11.12 ab	1.88 ab
(5) F_0^2	5.87 bc	10.66 be	1.88 ab

* Means followed by a common letter indicate nonsignificant subgroups (Duncan's test, 5% level of significance).

DISCUSSION

One of the questions asked in designing the experiment was: "What is the effect of mode of pollination on seed set and on seed and fiber development?" The results showed that hand emasculation and pollination can cause reduced seed set, which in turn increased seed size (seed index) and lint index. When hand cross-pollinating, one is concerned mainly with the number and the development of seed. The present study, as contrasted to the report of Loden et al. (1950), indicates that the individual performing the operations can influence the results.

Of basic interest is the effect of genotype and level of heterozygosity of the pollen on seed set and on seed and fiber development. Lint index was the only character that responded significantly to differences in the genotype of the pollen; the response was such that a decrease in lint index value (less desirable) was associated with an increase in genetic diversity of the pollen parent in relation to the ovule parent. Fiber is maternal tissue, and it is difficult to understand how the genotype of the embryo could directly influence fiber development. Seed index could have been influenced by the genotype of the pollen, but this character was not significantly affected in this experiment. Seed index did follow a trend similar to that of lint index. Seed and fiber follow parallel development patterns (Kerr, 1966), and the general inverse relation of seed index and lint index to seed number can be accounted for by the additional substrate available for individual boll development when total seed number has been reduced by physical damage. Pressley (1937) observed that heavier seeds had longer lint; and in the present study, seed index and lint were significantly correlated, $r = .64$ with 594 d.f. The significant lint index response to genotype may have been a result of this relationship.

The level of heterozygosity of the pollen parent significantly influenced seed index, lint index, and weight of line per boll. When pollen was genetically identical to the ovule parent, seed and fiber development was greater than when pollen was genetically different. Of special interest was the fact that pollen from the F_1 and from the F_0^2 parents affected seed and fiber development at about the same level. The pollen from the backcross generations, BC_1^1 and BC_1^2, were erratic in their performance and were not too informative.

Kohel and White (1963) analyzed the variability of five parents and their F_1 progeny in *Gossypium hirsutum*. In 7 out of 10 characters measured, the parents were less variable. However, differences were significant in only two cases, in one, the F_0 was least variable and in the other the F_1 was least variable.

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The results indicated that the F_0 exhibited a greater degree of homeostasis than the F_1.

Homeostasis could be manifested in early embryo development as well as in plant development. The lack of influence of level of heterozygosity on seed number indicates that a gross reaction such as pollen incompatibility is not involved, but reaction must take place in zygote development. Differences in zygote development would be reflected primarily in seed index. A larger seed would provide more surface area for fiber development and thus result in a larger lint index. The calculated correlation between seed index and lint index was $r = .64$ with 594 d.f. Since weight of lint per boll is a product of seed index and lint index, it would be expected to follow this same relationship.

The within mean squares for the three homogeneous heterozygosity levels (1, 3, and 5) were computed for seed index and lint index to obtain estimates of environmental variability, and conversely of developmental stability. Mean squares for seed index were 107, 440, and 262 and lint index 74, 206, and 203 for levels 1, 3, and 5, respectively. In both cases F_0^1 pollen on F_0^1 ovule parents caused less variable development of the character measured than F_0^2 pollen on F_0^1 ovule parents. The treatment with F_1 pollen was most variable. This result could have been caused by the segregation of the F_1 gametes. Thus, seed development, as measured by seed index, was most favorable and least variable when TM 1 pollen was applied to TM 1 flowers.

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