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Heterosis in Relation to Development in Upland Cotton, *Gossypium hirsutum* L.[1]

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ABSTRACT

The primary objective was to examine hybrids and their parents throughout their life span to detect the onset of heterotic behavior and its cumulative effect. Four single-cross hybrids of Upland cotton, *Gossypium hirsutum* L., and their parents were studied. No heterotic effects were detected during the embryonic stage of development. Neither did hybrids appear to be any more vigorous than their parents during the first 7 days of seedling growth following germination. The first significant divergence of hybrids from their parents was recorded at approximately 6 weeks after planting. Relative rates of dry matter production continued to diverge for a period of about 3 weeks and then proceeded at comparable rates in both parents and hybrids to maturity. Hybrids thus maintained their vegetative superiority throughout the period of fruition with the result that hybrid plants were larger than parental plants and produced a greater yield of fruit.

HETEROSIS as measured by comparison of the F_1 with the average performance of the parental lines has been amply demonstrated for yield and general vigor in Upland cotton, *Gossypium hirsutum* L. (2, 5, 6, 8). The majority of these studies, however, were primarily concerned with heterotic effects as measured on mature plant characteristics such as lint yield and boll and fiber traits.

Only limited information is available concerning heterotic effects on rate of vegetative and fruiting development. Kime and Tilley (2) reported that F_1 hybrids had a significantly higher rate of blooming and also produced open bolls earlier than their parents. Turner (9) reported heterosis for early flowering. Marani (4) observed that interspecific crosses between strains of *G. hirsutum* and *Gossypium barbadense* L. started to flower earlier than their parents and exhibited a large heterotic effect for number of flowers. Intraspecific crosses of *G. hirsutum,* however, exhibited heterosis for earlier flowering in only 1 of the 2 years in which the material was grown. Miller and Marani (6) reported that the percentage of total lint production harvested at the first picking was significantly higher for hybrids than for their parents. Although results are variable it appears that hybrids generally initiate flowering sooner and mature their fruits earlier than their parents.

Since considerable effort is now being given to developing methods for producing hybrid cotton seed economically, a better understanding of heterosis in cotton would be helpful. The primary objective of this study was to examine hybrids and their parents throughout their life span for evaluating the onset and cumulative effect of heterotic behavior.

MATERIALS AND METHODS

Four hybrids and their respective parents were studied. Designation of the hybrids and description of the parental lines follows:

Cross 1, Glandless Empire (P_1) $\times$ Sealand I (P_2) — Glandless Empire was derived from the commercial variety 'Empire'. Sealand I is characterized as "long staple Upland" developed by recurrent backcrossing to *G. hirsutum* of an interspecific cross between *G. hirsutum* and *G. barbadense.*

Cross 2, North Carolina Margin (P_1) $\times$ Empire 10 (P_2) — The former parent is an Upland cotton introgressed with germplasm from *Gossypium arboreum* L. and the latter an inbred line derived from the Empire variety.

Cross 3, A-1 (P_1) $\times$ F-3 (P_2) — Both parents were extracted from commercial varieties by pedigreed selfing with visual selection for vigor and adaptation; the former from 'Deltapine 15', the latter from 'Stoneville 2B'.

Cross 4, M-8 (P_1) $\times$ Z106 (P_2) — Both parents are doubled polyhaploids derived from the varieties 'Deltapine 14' and 'Stoneville 2B' respectively.

All parental strains were highly inbred and generally adapted to the Southeastern cotton belt, although their level of agronomic performance is below that of commercial varieties. Reciprocal F_1's from each cross were evaluated separately.

Four types of experiments were conducted to evaluate heterotic effects during the diffreent stages of growth and development of the cotton plant.

Experiment 1 — Yield at the Mature Plant Stage. The primary objective of this investigation was to confirm preliminary information on heterosis for lint yield displayed by these four F_1 hybrids and to measure the effects of heterosis on various components of yield. In 1963 the experiment was planted at the Central Crops Research Station, Clayton, N.C. and in 1964 at the Upper Coastal Plains Research Station, Rocky Mount, N.C. A split-plot design with 4 replications was used with the 4 crosses (hybrid families) making up the whole plots. A hybrid family consisted of 4 entries (2 parental strains and their reciprocal F_1's). Each subplot contained a single entry planted in 3 rows of 25 hills each. The hills were spaced 12 inches apart with 3 plants per hill. Fifty-boll samples were picked at random from each plot to provide data on weight per boll, lint percentage, seed index (weight of 100 seed in grams), and number of seed per boll. Lint yield values were calculated by multiplying the total weight of seed cotton per plot by the lint percentage value for that plot.

Experiment 2 — Embryonic Stage. The two parental lines of each of the four crosses were planted in paired rows in the field for self pollination and reciprocal crossing. Selfed and crossed bolls borne on the same female parent were harvested separately and their seed and boll characteristics compared. Two samples, each consisting of 30 bolls, were picked at random from each of the four entries within a cross. Traits measured were weight per boll, lint percentage, seed index (weight of 100 seed in grams), and number of seeds per boll.

Experiment 3 — Early Seedling Growth. Early seedling growth was measured in terms of the rate of transfer of cotyledonary dry weight to the axial parts of the seedling following the method described by Christiansen (1). Seed were hulled in order to increase uniformity in water uptake. A split plot design with 4 replications was used to evaluate the same entries described for Experiment 1. All entries in a replicate were placed on a single shelf in a growth chamber. Twenty-five seeds were used for each entry. The pads were kept in the chamber for 7 days at 70° F. At the end of the week, cotyledons were separated from the axial portion (hypocotyl and radical) and the material was oven dried. Ratio of cotyledon dry weight to total seedling dry weight was computed.

Experiment 4 — Vegetative Growth and Fruiting Development.

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Comparison of the parental lines and their respective hybrids were made on the basis of vegetative growth and fruiting development curves covering the seedling to mature plant stages. This experiment was conducted for two growing seasons at Clayton, N.C. The experimental design was similar to that used in Experiment 1 except that each row consisted of 10 single-plant hills with plants spaced 18 inches apart. The following traits were measured:

Seedling dry weight. Five seeds were planted per hill. Beginning one month after planting three successive harvests of seedlings were made at 12-day intervals leaving a final stand of one plant per hill. Harvested seedlings were cut off at soil level, oven dried, weighed, and average weight per seedling in grams was determined for each entry.

Vegetative and fruiting dry weight. Six plants were periodically harvested from each subplot border. An individual plant was cut off at soil level each 2-week interval beginning at the 4-leaf stage. Flower buds and bolls were separated from vegetative growth and each was oven dried. Vegetative and fruiting parts were weighed after drying and weight per plant was recorded in grams. Growth curves obtained on vegetative dry weight in this portion of the experiment are a continuation of those obtained during the earlier seedling stage of growth.

Number of flowers produced. Number of flowers opening each day was recorded for the four bordered plants in the central row in each plot. Daily flowering data for each plot were pooled over 6-day periods.

Number of matured bolls. Tags recording date of blooming were fastened on each flower. At the end of the season the tags on the opened bolls were collected. These represented the actual number of bolls which reached maturity. Data for this trait were also pooled over 6-day periods.

Generations, crosses and periods studied were analysed on a fixed model basis, while locations in Experiment 1 and years in Experiment 4 were assumed to be random. Differences between reciprocal F_1's were very small in magnitude in Experiments 1, 3, and 4 and generally not statistically significant. Thus the average value was used for the comparison of generation means.

EXPERIMENTAL RESULTS

Experiment 1 — Yield at Mature Plant Stage

Comparative data on yield and certain yield components of parental lines and their F_1 hybrids are presented in Table 1. Yields of F_1 hybrids exceeded midparent values in all four crosses with the increases ranging from 15.5 to 24.5%. In three of the crosses the hybrids also outyielded their higher yielding parent. The F_1 hybrids also exceeded midparent values for weight per boll, lint percentage, and number of seeds per boll. No consistent differences between hybrids and parents were observed for seed size. Results of this experiment are in general agreement with similar data reported in the literature (2, 5, 6, 8), and indicate heterotic effects for yield in each of the four crosses examined in this study.

Table 1. Average performance of parental lines and F_1 hybrids for yield and several components of yield at the mature plant stage, combined data 1963 and 1964.

Cross	Generation	Lint yield, g/plot	Weight /boll, g	Lint, %	Seed index	Seed /boll
1	P_1	2648	8.05	38.5	12.9	38.6
	P_2	1777	7.20	34.0	13.9	34.5
	F_1	2555	8.12	36.6	13.3	38.7
	$(F_1$-MP)/MP, %	15.5	6.5*	1.0	-0.7	5.9
2	P_1	2225	7.03	40.7	11.5	36.1
	P_2	2766	8.22	39.5	13.5	37.3
	F_1	3093	8.23	41.6	12.7	38.2
	$(F_1$-MP)/MP, %	23.9*	7.9**	3.7**	1.6	4.1
3	P_1	2548	6.86	39.3	11.0	37.9
	P_2	2112	7.49	35.9	13.2	36.5
	F_1	2695	7.65	38.6	12.0	39.2
	$(F_1$-MP)/MP, %	15.7	6.6*	2.7*	-0.8	5.4
4	P_1	2494	6.19	41.5	9.9	36.6
	P_2	2325	7.64	38.5	12.3	38.6
	F_1	3001	7.40	41.2	11.3	38.6
	$(F_1$-MP)/MP, %	24.5*	7.0*	3.0*	1.8	2.7

*, ** F_1 significantly different from midparent at 5% and 1% levels, respectively.

Experiment 2 — Embryonic Stage

Embryonic tissues account for the major portion of the weight of acid-delinted, mature cotton seed. Comparisons were made between selfed and crossed seed produced on the same female parent to evaluate possible heterotic effects during embryonic development. Data are presented in Table 2. As measured by the weight of the mature seeds produced, no consistent or significant differences were observed between inbred and hybrid seed produced on the same female parent. Characteristics of the bolls bearing the selfed or crossed seed were likewise very similar. Thus heterotic effects were not detected during the embryonic stage in any of the four hybrids of this study.

Experiment 3 — Early Seedling Growth

Early seedling growth of the parental lines and their F_1 hybrids was measured in terms of the rate of transfer of cotyledonary dry weight to the axial parts of the seedling (1). Comparisons are presented in Table 3. No significant differences were detected between the generations within a cross, although differences between crosses were significant. Thus the hybrids do not appear to be any more vigorous than their parents during the first 7 days of seedling growth following germination.

Experiment 4 — Vegetative Growth and Fruiting Development

Data comparing vegetative growth and fruiting development rates for inbreds and their hybrids are presented graphically in Figures 1 to 4. Interactions of "midparent versus $F_1 \times$ years" were generally small

Table 2. Comparison of seed and boll characteristics of selfed and crossed seed produced on the same female parent.

Cross	Generation	Dry weight /seedling, g	Vegetative dry wt. /plant, g	Flowers /plant	Mature bolls /plant	Flowers setting mature bolls, %	Fruit dry wt. /plant, g
1	P_1	9.99	303	38.3	12.8	33.5	232
	P_2	9.99	258	54.9	21.8	39.8	256
	F_1	13.33	386	52.3	22.6	43.0	323
	$(F_1$-MP)/MP, %	33.4*	37.6*	12.2**	30.6**	17.3*	32.4*
2	P_1	9.12	228	46.6	21.5	46.0	250
	P_2	10.00	248	42.0	15.3	36.8	229
	F_1	12.78	288	44.3	17.8	40.0	339
	$(F_1$-MP)/MP, %	33.7**	21.0**	0.0	-3.3	-3.4*	41.5*
3	P_1	9.85	351	50.2	20.2	40.5	268
	P_2	10.57	291	44.5	18.9	42.5	334
	F_1	12.11	366	47.7	20.9	44.0	324
	$(F_1$-MP)/MP, %	18.6	14.0	0.7	6.9**	6.0*	7.6
4	P_1	10.03	262	53.0	25.2	48.0	283
	P_2	8.11	298	48.7	19.8	40.8	308
	F_1	11.12	361	48.9	22.3	45.3	363
	$(F_1$-MP)/MP, %	22.6	28.9**	-3.8	-0.9	2.0	22.8

*, ** F_1 significantly different from midparent at 5% and 1% levels, respectively.

Table 3. Comparison of F_1 hybrids and mid-parent values for the ratio of cotyledon dry weight to total seedling dry weight 7 days after germination.

Cross	Generation	Cotyledon dry weight/ total seedling dry weight
1	Midparent	.5708
	F_1	.5563
2	Midparent	.6492
	F_1	.5968
3	Midparent	.5742
	F_1	.5956
4	Midparent	.5831
	F_1	.5812

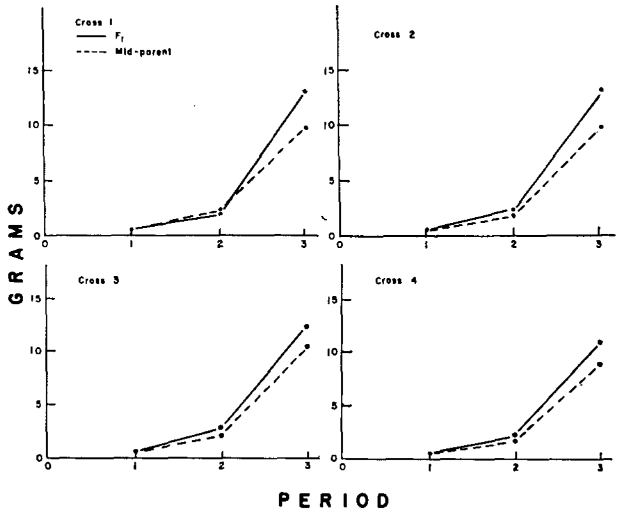

Figure 1. Cumulative growth curves for dry weight per seedling for F_1 hybrids and their respective mid-parent values. Periods 1, 2, and 3 correspond to data obtained at 30, 42, and 54 days after planting, respectively.

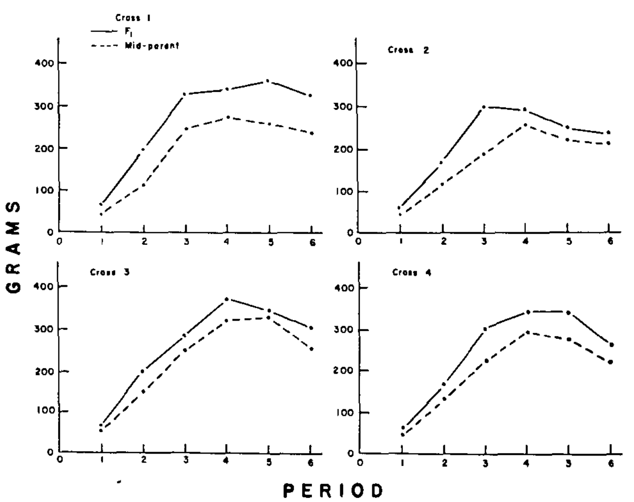

Figure 2. Cumulative growth curves for vegetative dry weight per plant during the post seedling growth stage for F_1 hybrids and their respective mid-parent values. Initial measurements were made at the 4-leaf stage of growth (period 1) with successive measurements at 14-day intervals.

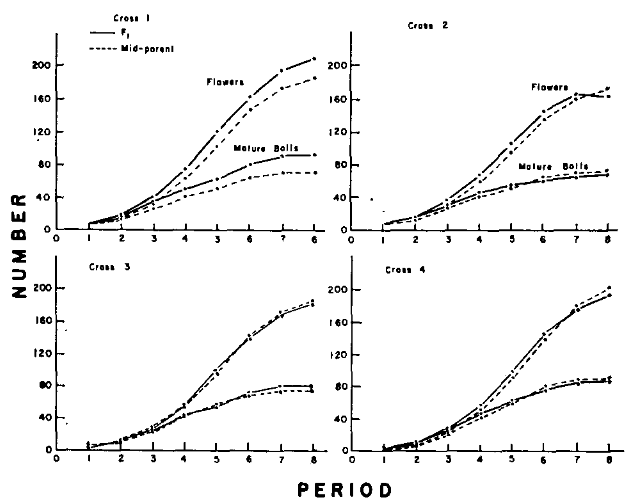

Figure 3. Cumulative curves of number of flowers and number of bolls retained to maturity per plot (4 plants) for F_1 hybrids and their respective mid-parent values. Data were pooled over 6-day intervals beginning with initiation of blooming (period 1) and continuing to maturity (period 8).

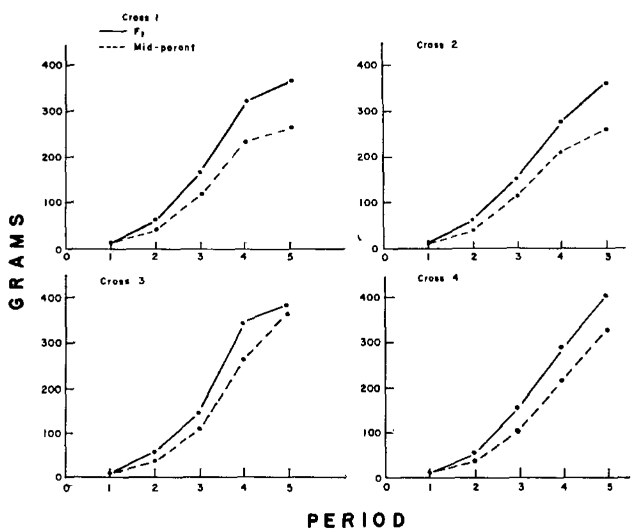

Table 4. Maximum points on vegetative growth and fruiting development curves of parental lines and F_1 hybrids, combined data 1963 and 1964.

and non significant. Data from the two years were thus combined for presentation. Mean values of the maximum points on the respective growth curves are presented in Table 4.

Seedling growth. Cumulative curves of seedling dry weight for the F_1 hybrids and their midparent values are presented in Figure 1. Hybrids did not differ from their midparent values at the time of the first harvest (period 1). They began to diverge at the second thinning, however, and the hybrids were clearly superior to midparent values at the third harvest. Magnitudes of the hybrid superiority ranged from 18.6 to 33.7% of respective midparent values at this last date. Results of the analysis of variance indicated that this observed divergence of hybrids from their midparent values was statistically significant for seedling weight in three of the four crosses. Thus the first significant superiority of hybrids over their midparent values appeared to take place at this stage of plant development.

Vegetative growth. Cumulative curves of the vegetative dry weights following the seedling stage of growth are presented in Figure 2. The decrease in cumulative dry weight observed for the 5th and 6th periods reflect the shedding of leaves after the plants reached maturity and vegetative growth ceased. The F_1 hybrids clearly and consistently showed superiority to their midparent values. Thus the differences observed between hybrids and their parents by the third period of measurement in the seedling stage of growth were maintained throughout the remainder of the vegetative growth phase. No significant or consistent trend was observed, however, for the hybrids either to increase or decrease their superiority as the growing season progressed. The interaction of "F_1 vs midparent $\times$ periods" was not significant in any of the four crosses. Thus it appears that the initial advantage in vegetative growth of the hybrids was established during the seedling growth stage and was maintained at approximately the same level thereafter.

Figure 4. Cumulative curves for dry weight of fruit per plant for F_1 hybrids and their respective mid-parent values. Harvests were made at 14-day intervals beginning with the onset of fruiting (period 1) and continuing to maturity (period 5).

Cross	Treatment	Seed index	Weight /boll, g	Seed /boll	Lint, %
1	P_1 - selfed	12.9	7.34	37.0	36.6
	P_1 (♀) × P_2	13.0	7.24	35.8	38.0
	P_2 - selfed	13.6	6.15	35.0	33.4
	P_2 (♀) × P_1	13.8	6.55	34.1	32.6
2	P_1 - selfed	11.7	5.95	31.9	41.2
	P_1 (♀) × P_2	11.7	5.85	31.3	42.7
	P_2 - selfed	13.3	7.02	33.3	39.4
	P_2 (♀) × P_1	13.9	7.20	33.4	38.7
3	P_1 - selfed	11.5	6.10	35.1	38.8
	P_1 (♀) × P_2	11.3	6.59	38.7	38.5
	P_2 - selfed	13.1	7.70	39.3	34.7
	P_2 (♀) × P_1	14.0	7.32	35.7	34.5
4	P_1 - selfed	10.0	5.52	33.9	41.1
	P_1 (♀) × P_2	10.1	4.95	31.0	40.4
	P_2 - selfed	12.1	6.90	37.3	38.4
	P_2 (♀) × P_1	12.6	7.47	36.8	41.3

Although weekly plant height measurement were recorded as another index of vegetative growth, no sizeable or consistent differences between parents and hybrids were observed for this trait.

Flowering and fruiting. Cumulative curves for number of flowers and number of bolls per plot (four plants) retained to maturity are presented in Figure 3. Small differences in the curves during the first part of the season indicated that the hybrids tended to initiate flowering and fruit-set earlier than their parents. Except for Cross 1, however, differences between F_1 hybrids and their midparent values were generally small and inconsistent during the remainder of the season. Total number of flowers produced and bolls matured for the different generations are shown in Table 4. The F_1 hybrids of Cross 1 produced 12.2% more flowers and 30.6% more bolls than the average of its parents. The only other significant value for the remaining three crosses was the 6.9% increase in number of mature bolls for the F_1 of Cross 3 as compared to its parents. A significantly higher percentage of the flowers developed mature bolls on the F_1 hybrids of Crosses 1 and 3 than on their respective parents (Table 4). Thus the increased number of bolls on the F_1 hybrids of Cross 1 was due both to an increase in number of flowers produced as well as to a higher percentage of the flowers developing into mature fruits. For Cross 3 on the other hand the smaller increase in number of bolls was due almost entirely to the increased percentage of flowers which set fruit.

Cumulative curves for fruiting dry weight are presented in Figure 4. Shortly after initiation of fruiting the hybrids began to diverge from their midparent values and generally maintained or increased their superiority as the season progressed. These differences as averaged across periods were statistically significant for all four crosses.

DISCUSSION

Comparisons of accumulated dry matter in the current experiments showed that the hybrids began to diverge sharply from their parents when all plants were about 6 weeks of age. The relative rates of dry matter accumulation continued to diverge for a period of about 3 weeks and then proceeded at comparable rates in both parents and offspring to the end of the season. The hybrids thus maintained their vegetative superiority throughout the period of fruition with the result that hybrid plants were larger than parental plants and bore more fruits per individual. White and Richmond (10) showed that the hybrid combinations in their experiments which displayed heterosis for lint yield were not more efficient than their parents in terms of the amount of lint produced per unit of plant matter. Rather, some hybrids grew to a larger size within the same time-span and consequently produced more fruit. The present data are consistent with this finding. White and Richmond conducted their experiments at College Station, Texas, a region where climatic and edaphic conditions are quite different from those of central North Carolina. Moreover, they used a much broader array of germ plasm than was used in the current experiments. When these facts are taken into account the similarity of the results lends the aspect of generality to the proposition that cotton hybrids are not more efficient in fruit production than parental lines, but merely grow larger under at least some conditions, and, as a result, produce more fruits.

There is, at present, no explanation as to why certain cotton hybrids are able to produce significantly more vegetative matter within an allotted time span than the best of the parents. Any hypothetical explanation must involve physiological considerations such as are elaborated in the work of Muramoto et al. (7) on the relative rates of photosynthesis among a number of cotton strains. These workers were able to show that under optimum conditions of light and temperature the strains employed did not differ in net photosynthetic rate. However, small differences in net photosynthetic rate were difficult to measure under optimum natural conditions. They did find marked differences in the rate of increase in leaf area and total dry matter. A hybrid between *Gossypium hirsutum* and *Gossypium barbadense* was particularly striking in this respect. At the age of 9 weeks this entry had 3.9 times the leaf area of the best parent and 3.1 times the total dry matter, whereas at 1 week of age it did not exceed its best parent in leaf area. The problem is how the hybrid could gain superiority in leaf area and dry matter in a period of only 2 or 3 weeks and continue to diverge from the parents in these two categories until at least the ninth week, if it did not differ in net photosynthetic rate.

Heterotic hybrids must be more efficient in the use of their environmental resources than their parents. Under sub-optimal conditions they might have a higher net photosynthetic rate than the parental strains. Thus over a given segment of time the net assimilation rate could be, on the average, higher for the hybrid. Heterotic hybrids could be those which withstand better the environmental shocks inherant in most experimental situations, particularly those conducted under field conditions. Better buffering of physiological processes would lead to better canalization of development in the sense of Lerner (3). The use of pre-fruition dry matter assays as a tool for identifying potentially useful hybrid combination merits further study.

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