

## Variation of Cottonseed Quality With Stratified Harvests<sup>1</sup>

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### ABSTRACT

Selected constituents of mature cottonseed harvested at weekly intervals (up to 6 weeks) from opened bolls of seven entries were measured during 2 crop years (1976, 1979). The seed were analyzed for weight, oil, protein, gossypol, and selected fatty acids and amino acids. Major weather differences between years during the periods of flowering and seed maturity caused variations in seed composition. Part of these variations was probably due to weather conditions that affected cottonseed development and differentiation, and energy demands within the cotton plants as the growing seasons progressed. Seed weights decreased, oil and free gossypol percentages declined and proteins increased with progressively later harvest dates during the 2 crop years. In oil, palmitic acid percentages decreased with later harvest dates during both crop years, and oleic and linoleic acid values were variable between seasons and over harvest dates, but these fatty acids followed a consistent reciprocal relationship. The percentages of amino acids in proteins including lysine, threonine, isoleucine, methionine, and half-cystine increased during later harvest dates in 1976. Positive correlations for all amino acids and harvest dates were noted, but these values were significant only for total amino acids (similar to total protein), lysine, and methionine. The amount of oil and protein in seed decreased with harvest date, but the greatest change was in the amount of oil. These data provide information on the variation of the composition within and between crop years, and they indicate the need for additional research on factors contributing to variation in cottonseed composition.

*Additional index words:* *Gossypium hirsutum* L., Seed oil, Fatty acids, Proteins, Amino acids, Gossypol, Intra- and interseasonal variation.

PROGRAMS to improve seed quality begin with the identification of the variability caused by genetic and environmental factors. Most investigations on the effects of environment on cotton (*Gossypium hirsutum* L.) seed quality used year or location effects as their sources of variation (Cherry et al., 1981). The traits measured most often were oil, protein, and gossypol; oil and gossypol were shown to exhibit a great amount of environmental variation (Stansbury et al., 1954, 1956; Turner et al., 1976; Cherry et al., 1978, 1981).

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Only one investigator found greater environmental variation for protein than for oil (Hancock, 1942). In most cases, the environmental variation exceeded that attributable to genetic sources. Of major concern was the effect on cottonseed composition of adverse environmental conditions, or prolonged field weathering, after boll opening. The effects of intra-seasonal environmental variation has received little attention.

Since cotton is an indeterminate flowering species, the extended periods of flowering and boll development during the crop season produce mature bolls that are exposed to a progression of different environmental factors. These factors can influence the final crop quality and thus be important considerations in sampling to measure potential performance characteristics of the seeds (Leffler, 1976; Meredith and Bridge, 1973; Turner et al., 1979; Verhalen et al., 1975). For these reasons, we investigated seed quality variation during two crop seasons by harvesting open bolls with mature cottonseeds from seven entries at weekly intervals for up to 6 weeks.

### MATERIALS AND METHODS

Cotton plants were grown at the Texas A&M University's Brazos River Research Farm during the crop seasons of 1976 and 1979. The crops were managed with conventional cultural practices (weed and insect control, fertilization and supplemental irrigation) used in typical cotton performance tests at this field location. Each entry was grown in two row (each 1.0 × 7.6 m) plots in a randomized complete block; the tests were replicated five times. As bolls opened, seed cotton was harvested weekly. A total of six and five weekly harvests were made in 1976 and 1979, respectively; rain prevented the second week's harvest in 1979, thus it was combined with that of the third week.

Test entries included: 1) ORS, from L. S. Bird; 2) 'Tamcot SP-37', L. S. Bird; 3) 'Stoneville 213', commercial seed; 4) Lubbock Dwarf, J. E. Quisenberry; 5) Stoneville Okra Leaf, C. W. Manning; 6) 6M10, G. A. Niles; and 7) Lyman, L. S. Bird; entries 1) to 6) were glanded, and 7) was glandless.

Seed index was the weight, in g, of 100 acid-delinted and dried seeds. Percentages of oil in acid delinted and seeds dried to moisture equilibrium at 38°C (10 g) were determined by nuclear magnetic resonance (NMR) (Kohel, 1980).

Assays for percentages of kernel oil, protein ( $N \times 6.25$ ), and free gossypol, and percentages of fatty acids in hexane-extracted oil were made in accordance with the official and tentative methods of the American Oil Chemists' Society (Anon., 1976). The method of Kaiser et al. (1974) was used to determine quantities of amino acids and their totals (g/hg of sample) in flours from seed ground and defatted with hexane. Most analyses were completed as two replications. The seed index and NMR studies were done as five replications. Amino acids and fatty acids were conducted as single replications in 1979.

## RESULTS AND DISCUSSION

Mean squares and F-test results for the effects of entry, harvest date, and date by entry are presented in Table 1. Means of seed traits for the entries and harvest dates are presented in Table 2. Table 3 presents correlations of selected traits with harvest dates and with each other.

Interpretations of responses to growing conditions within the 2 years were influenced by the greater amount of intraseasonal variation in 1976 than 1979. In 1976, very high temperatures occurred during the flowering period in July and declined through the remainder of the growing season. Supplemental irrigation was required in 1976 to minimize the moisture stress on the plants. In 1979, moderate temperatures and high rainfall occurred throughout the growing season, therefore, the plants were not severely stressed by heat or moisture.

### Oil and Fatty Acids

Percentages of oil in acid delinted and dried whole seeds determined by NMR differed among entries and harvest dates during both years (Table 1). Entry by harvest date interactions existed for both years (Table 1). The glandless entry 7 had the highest oil percentage both years, 30.9%, while entry 4 had the lowest, 29.0 and 23.0% in 1976 and 1979, respectively. The other entries were not consistently high or low in their percentages of oil; they varied both within and between years (Table 2). The range among the entry means of all harvest dates within a crop year was 1.9% in 1976 and 7.9% in 1979. When all

entries were averaged, the overall trend of oil percentages was a decrease from the first to last harvest dates (Tables 2 and 3). In 1976, however, the oil percentages of the last two harvest dates increased. The percentages of each individual entry followed a similar pattern. The only apparent reason for the interaction was that, although the entries performed similarly over harvest dates, they varied differently.

As with NMR estimates, oil percentages of dehulled kernels determined by AOCS methods were different among entries in both years (Table 1). However, kernel oil percentages from the different harvest dates did not vary in either year. Chemical analysis confirmed that glandless entry 7 had the highest oil percentages in both years. Excluding this glandless entry, little variation among entries for percentages of kernel oil was noted. An entry by harvest date interaction was detected only in the 1976 data. There was a trend for percentage of kernel oil to decrease from the first to last harvest dates as was observed for percentages of seed oil. The correlations between kernel oil percentages and harvest dates,  $r = -0.74$  and  $r = -0.82$  for 1976 and 1979, respectively, were significant (Table 3). The trends were similar for all entries except 4 and 6, which showed little response to harvest dates. These two entries are early maturing entries and, because of this feature, they may not be as greatly influenced by environmental factors as the longer growing cottons.

Significant positive correlations were obtained between seed oil and kernel oil percentages in 1976 and 1979. This result indicates that these values changed similarly and that both methods of analysis produced comparable results (Table 3). The difference in the correlations for oil percentages of the 2 years may be technique related; however variation in seed hull percentages among entries is also a possibility.

Since palmitic, oleic, and linoleic acids account for approximately 95% of the fatty acids in cottonseed oil (Cherry et al., 1981), only these fatty acids were analyzed statistically (Table 2). In 1976, palmitic and linoleic acids differed significantly, both among entries and harvest dates; the entry by harvest date in-

Table 1. Mean squares and results of significant tests for cottonseed properties.

Property	Mean squares					
	Entries (E)		Harvest dates (D)		D × E	
	1976	1979	1976	1979	1976	1979
Seed oil (%)	15.35**	23.07**	35.27**	9.52**	1.44**	1.36**
Kernel oil (%)	23.57**	20.78**	10.00	12.25	2.74*	1.46
Kernel protein (%)	20.51	14.21**	17.14**	19.97	3.38**	1.47
Seed index (g/100 seed)	16.91**	15.74**	9.57**	16.72**	0.49**	0.19**
Seed oil index (g/100 seeds)	1.37*	1.96**	0.67**	1.72**	6.65	0.02
Seed protein index (g/100 seeds)	0.987*	0.850**	0.182*	0.449*	0.077	0.027
Palmitic acid (%)	60.34*	11.43**	7.65**	2.94**	0.71	-
Oleic acid (%)	30.08	18.21**	5.32	4.54	1.00	-
Linoleic acid (%)	45.58**	28.82**	27.78**	2.20	0.69	-
Total amino acids (g/hg sample)	16.91*	4.03	15.32	9.78	2.80	-
Lysine (g/hg sample)	0.017	0.015	0.018*	0.028	0.004	-
Threonine (g/hg sample)	0.025	0.004	0.011	0.018	0.004	-
Isoleucine (g/hg sample)	0.025	0.005	0.019*	0.013	0.055	-
Methionine (g/hg sample)	0.012	0.005**	0.007	0.011**	0.002	-
1/2 Cystine (g/hg sample)	0.094*	0.003	0.009	0.009**	0.002	-
Free gossypol (%)	0.334	0.2708**	0.0202	0.0105**	0.0193	0.0038

\*, \*\* Significant at the 0.05 and the 0.01 levels, respectively.

teractions were not significant. There were no significant differences for oleic acid. In 1979, fatty acid composition was determined for only one replication, therefore, only entries and harvest dates could be tested statistically. All three fatty acids varied significantly among entries, but only palmitic acid, which decreased from the first to last harvest dates in both

years, showed significant variability among harvest dates. The correlations of palmitic, oleic, and linoleic acid percentages between the 2 years were 0.99, 0.66, and 0.79, respectively. The correlations of these fatty acids with harvest dates in 1976 were -0.83, -0.68, and 0.91, and in 1979 they were -0.95, 0.77, and -0.11, respectively (Table 3).

Table 2. Means of seed traits for entries and harvest dates in 1976 and 1979.

Year		Seed oil	Kernel oil	Kernel protein	Seed index	Seed oil index	Seed protein index	Palmitic acid	Oleic acid	Linoleic acid
		%			g/100 seeds			%		
<b>1976</b>										
Entry	1	30.4	37.1	39.1	8.3	3.0	3.1	25.4	18.5	51.5
	2	29.5	37.0	38.3	9.4	3.7	3.8	23.3	17.4	55.0
	3	29.2	36.1	35.9	8.6	3.2	3.2	22.1	17.3	56.6
	4	29.0	36.3	39.9	9.0	3.3	3.6	19.2	19.5	57.2
	5	29.1	36.2	39.1	8.5	3.1	3.3	20.8	19.3	55.4
	6	29.4	36.0	37.5	10.3	3.7	3.8	21.9	20.5	53.2
	7	30.9	40.0	37.8	9.9	3.8	3.6	25.0	15.9	54.6
LSD <sub>05</sub>		0.6	1.6	-	0.5	0.4	0.4	3.4	-	1.0
Harvest date	1	30.9	37.8	36.8	9.9	3.7	3.6	23.3	19.3	53.0
	2	30.8	37.9	37.0	9.4	3.5	3.4	23.0	18.4	54.3
	3	29.4	37.2	38.2	9.2	3.5	3.5	22.8	18.2	54.6
	4	28.4	36.4	38.6	9.0	3.3	3.5	22.8	18.6	54.2
	5	28.9	36.7	39.4	9.0	3.4	3.6	22.0	18.0	55.6
	6	29.3	35.7	39.4	8.3	3.0	3.3	21.3	17.5	57.1
LSD <sub>05</sub>		0.4	-	1.0	0.3	0.2	0.6	-	-	0.5
<b>1979</b>										
Entry	1	28.5	38.9	33.4	7.4	2.8	2.4	23.7	22.2	50.0
	2	27.9	38.7	33.8	8.3	3.2	2.7	22.2	18.0	54.8
	3	27.9	39.1	33.1	8.4	3.3	2.8	21.0	17.4	58.0
	4	23.0	38.4	35.3	8.9	3.4	3.1	18.8	21.3	55.1
	5	28.2	40.3	32.3	8.4	3.4	2.7	20.8	17.8	58.0
	6	28.8	39.9	32.6	10.2	4.2	3.4	21.2	20.8	54.3
	7	30.9	43.1	31.1	9.4	4.0	2.9	23.4	17.0	54.8
LSD <sub>05</sub>		0.6	1.1	0.8	0.4	0.2	0.1	0.8	2.6	2.6
Harvest date	1	29.2	40.4	32.2	9.7	3.9	3.1	22.2	18.3	55.4
	2	28.9	40.7	32.0	8.6	3.5	2.8	21.9	19.6	54.2
	3	28.1	39.4	33.7	8.4	3.3	2.8	21.6	18.9	55.4
	4	28.2	38.7	34.5	8.2	3.2	2.8	20.7	20.1	54.9
LSD <sub>05</sub>		0.4	-	-	0.1	0.1	0.2	0.6	-	-
<b>1976</b>										
Year		Total amino acid	Lysine	Threonine	Isoleucine	Methionine	Half cystine	Free gossypol		
		g/hg of sample								
<b>1976</b>										
Entry	1	46.8	2.20	1.58	1.65	0.64	0.77	0.86		
	2	44.9	2.18	1.53	1.57	0.68	0.74	0.96		
	3	46.0	2.24	1.54	1.64	0.72	0.78	0.88		
	4	48.7	2.30	1.66	1.71	0.68	0.98	0.93		
	5	47.5	2.24	1.58	1.68	0.68	0.74	0.94		
	6	46.5	2.22	1.61	1.62	0.67	0.74	0.82		
	7	47.1	2.19	1.56	1.66	0.74	0.78	0.00		
LSD <sub>05</sub>		1.9	-	-	-	-	0.14	-		
Harvest date	1	45.3	2.17	1.56	1.60	0.65	0.76	0.96		
	2	45.7	2.19	1.54	1.61	0.68	0.76	0.90		
	3	47.0	2.22	1.60	1.68	0.69	0.81	0.86		
	4	47.5	2.25	1.60	1.68	0.71	0.80	0.93		
	5	47.8	2.25	1.61	1.67	0.71	0.81	0.88		
	6	47.4	2.26	1.53	1.65	0.69	0.81	0.86		
LSD <sub>05</sub>		-	0.06	-	0.06	-	-	-		
<b>1979</b>										
Entry	1	39.8	1.98	1.46	1.43	0.74	0.68	0.84		
	2	39.5	1.96	1.42	1.42	0.70	0.67	0.76		
	3	40.8	2.04	1.50	1.48	0.75	0.71	1.12		
	4	42.0	2.10	1.50	1.48	0.81	0.76	0.82		
	5	40.2	2.01	1.46	1.46	0.75	0.69	1.15		
	6	40.0	1.96	1.45	1.46	0.76	0.69	0.73		
	7	38.9	1.91	1.42	1.38	0.72	0.69	0.00		
LSD <sub>05</sub>		-	-	-	-	0.05	-	0.05		
Harvest date	1	39.4	1.97	1.44	1.40	0.72	0.65	0.92		
	2	41.2	2.06	1.50	1.48	0.80	0.70	0.93		
	3	41.2	2.04	1.50	1.48	0.74	0.71	0.90		
	4	38.9	1.92	1.40	1.40	0.71	0.73	0.86		
LSD <sub>05</sub>		-	-	0.08	-	0.04	0.04	0.18		

dates was a negative, but nonsignificant, value for both years (Table 3).

### Seed Index

In both years, the values for seed index (seed weight) were significantly different for entries, harvest dates, and the interactions of entry with harvest dates (Table 1). Entries 6 and 7 had the largest seeds, and those of entry 1 were the smallest (Table 2). These relationships were consistent between years, but the intermediate seed entries 2 and 5 varied. Of all cottonseed properties studied, seed indexes of the entries was most consistent between years.

An interaction of entry with harvest date for seed index was detected each year. The values of each individual entry were plotted over harvest date to determine the source of this interaction. In 1976, changes in seed indexes of the entries were similar in their response to harvest dates, except that entry 7 and entry 5 had a more rapid decline in seed index at each successively later harvest date than the other entries, and entries 1 and 3 declined sharply in seed index at the last date. These different rates of change could account for the interactions of entries with harvest dates in 1976. A comparison of the seed index of individual entries over harvest dates in 1979 appeared to show a more consistent separation of entries over harvest dates than in 1976; no meaningful interpretation of the statistically significant interaction could be made from these data.

The observation that seed index decreased from first to last harvest dates (Table 2) was consistent with previous reports (Nelson, 1949; Meredith and Bridge, 1973; Leffler et al., 1977; Turner et al., 1979). The correlations of seed indexes with harvest dates were  $-0.75$  and  $-0.88$  in 1976 and 1979, respectively (Table 3). The greatest changes were associated with the first and last harvest dates (Table 2). This effect could be influenced by differences in seed maturities of entries combined with variations in seed index, but the comparison of individual entries over harvest dates indicated that the response was common to all entries.

The earliest harvest date should be associated with favorable environmental conditions and because there are few bolls, the plants are capable of providing abundant substrate for seed growth. In contrast, seed index may decrease with harvest dates and decrease most dramatically at the last date because seeds are developing at a time when there is highest competition for available substrates with previously set seeds. In addition, the low temperatures at the end of the season are not favorable to seed development (Gipson and Joham, 1969).

### Seed Oil and Protein Indexes

Consistent with most reports, the percentages of oil and protein in cottonseed kernels are negatively correlated (Table 3). Significantly positive correlations were observed between seed indexes and percentages of oil in seeds and kernels, and nonsignificantly negative correlations were observed between seed indexes and percentages of protein in kernels (Table 3).

Most reports stress the negative correlation between percentages of oil and protein, which give the impression that these constituents are synthesized competitively. However, Stansbury et al. (1956), and a review by Cherry et al. (1981), pointed out that positive correlations between the amounts of oil and protein do occur in cottonseeds. The weights of oil and protein in cottonseed kernels were estimated by assuming that seed hulls were constant among entries and over harvest dates, and then multiplying percentages by seed indexes (Table 2). The results were expressed as indexes (weight per 100 seeds).

In both years, seed oil and protein indexes were different among entries and over harvest dates, but the interactions of entries and harvest dates were not detected (Table 1). The correlations between seed oil index and seed protein index were positive and significant (Table 3). As with seed index, the indexes of oil and protein decreased from the first to last harvest dates (Table 2). The correlation of seed protein index versus harvest date was not significant. However, inspection of the data revealed that in 1976 the first harvest date was significantly high (Table 2). Recalculation of the correlations after excluding the data of the final harvest date in 1976, and the first harvest in 1979, showed that the seed oil index versus harvest date correlations remained significantly negative,  $r = -0.74$  and  $-0.79$  in 1976 and 1979, respectively, but those of the protein index became positive. Excluding these two extreme harvest dates also changed the correlations between seed oil and protein indexes from a significant to a nonsignificant positive correlation in 1976 ( $r = 0.35$ ), and a nonsignificant negative correlation in 1979 ( $r = -0.44$ ). Inspection of the mean seed protein indexes reveals that they changed little. These data suggest a relatively constant amount of protein among seeds of the normally mature bolls with most of the variation occurring in oil content.

### IMPLICATIONS

Intraseasonal variation influences cottonseed composition when evaluated in stratified harvests of a crop year. Concentrations of any constituent measured on a whole seed basis can be influenced by the ratio of the embryo to the seed coat (Cherry et al., 1981). However, this variable did not appear important in our experiments because the percentages of seed oil and kernel oil responded similarly and were highly correlated. In both seasons, the percentages of oil decreased during later harvest dates. The composition of the oil as measured by the percentages of three fatty acids representing 95% of the oil also varied. Palmitic acid consistently decreased with later harvest dates. Oleic and linoleic acids followed no consistent pattern with respect to harvest dates, but maintained a consistent reciprocal relation with each other. These responses and the significant entry by harvest date interactions point out the need to exercise care in sampling cottonseeds when evaluating genotypic and environmental influences on oil and fatty acid compositions.

The percentages of kernel protein increased with

**Table 3. Correlations of selected seed properties and harvest date.**

Characters compared	Correlation	
	1976	1979
Seed oil vs. harvest date	-0.69**	-0.81**
Kernel oil vs. harvest date	-0.74**	-0.82*
Seed oil vs. kernel oil	0.80**	0.90**
Kernel protein vs. harvest date	0.93**	0.82*
Kernel protein vs. kernel oil	-0.83**	-0.96**
Seed index vs. harvest date	-0.75**	-0.88**
Seed index vs. seed oil	0.53**	0.78*
Seed index vs. kernel oil	0.76**	0.60
Seed index vs. kernel protein	-0.38	-0.66
Seed oil index vs. harvest date	-0.83**	-0.96**
Seed protein index vs. harvest date	-0.34	-0.70
Seed protein index vs. seed oil index	0.72**	0.82*
Palmitic acid of oil vs. harvest date	-0.83**	-0.95**
Oleic acid of oil vs. harvest date	-0.68*	0.77
Linoleic acid of oil vs. harvest date	0.91**	0.11
Palmitic acid vs. oleic acid	0.40	-0.79
Palmitic acid vs. linoleic acid	-0.98**	0.09
Oleic acid vs. linoleic acid	-0.97**	-0.68*
Total amino acids vs. harvest date	0.75**	-0.15
Lysine vs. harvest date	0.86**	-0.34
Threonine vs. harvest date	0.52	-0.32
Isoleucine vs. harvest date	0.54	0.00
Methionine vs. harvest date	0.65*	-0.26
Half-cystine vs. harvest date	0.56	0.95*
Lysine vs. isoleucine	0.37	0.94**
Free gossypol vs. harvest date	-0.32	-0.56
Free gossypol vs. kernel oil	0.25	0.34
Free gossypol vs. kernel protein	-0.29	-0.29

\*,\*\* Significant at the 0.05 and the 0.01 levels, respectively.

Linoleic acid has been reported in other oilseeds to have a negative association with temperature and a reciprocal relation with oleic acid (Robertson et al., 1979). We calculated correlations of linoleic acid with temperature changes in 1976. The mean daily maximum and minimum temperatures were determined for the 1-, 2-, and 3-week periods prior to each harvest date. During the 3-week period before boll opening, major fatty acid synthesis and oil production occurs (Ali and Ullah, 1963; Benedict et al., 1976; Grindley, 1950). Temperatures were significantly and negatively correlated with linoleic acid percentages. The highest correlations were with mean daily minimum temperatures; the correlation was the same ( $r = -0.96$ ) for each period of 1, 2, or 3 weeks prior to harvest. Gipson and Joham (1969) showed that night temperatures (minimum temperatures) had the greatest effect on seed quality. However, our correlations were about the same for heat units and only slightly lower for maximum temperature. These results may not reflect a real causal relation because the temperature changed uniformly. That is, a steady decrease in temperature occurred simultaneously with seed development so that changes in linoleic acid followed a decline in temperature and increase in developmental period.

Similar calculations for mean daily maximum and minimum temperatures and total heat units were completed for the 1979 study. No weekly period or combination of weekly intervals were significantly correlated to linoleic acid percentages at the various harvest dates. The temperatures were moderate for the entire boll development period, and cyclic fluctuations lasted for about a week. Perhaps the temperature range was too narrow to trigger a change.

Although there were no major temperature-induced changes in linoleic acid, a significant reciprocal relation of linoleic and oleic acids was noted in both years that probably reflected the interrelated biosynthetic pathways of these fatty acids (Table 3).

### Protein and Amino Acid Percentages

Concentrations of proteins in kernels were significantly different for harvest dates and the harvest date by entry interaction in 1976; they varied only among entries in 1979 (Table 1). The correlation of entries between years was 0.43, and there was little consistency in entry ranking between years to attribute biological significance in the statistically significant differences among entries in 1979. A plot of entries vs. 1976 harvest dates revealed obvious differences in their entry responses to account for the entry by harvest date interaction. Specifically, the two early maturing entries, 4 and 6, exhibited little change in kernel protein over harvest dates, while entry 7 increased from the lowest to highest values between the first and last 1976 harvests. No increase in protein for this latter entry was noted in 1979. Overall, there was a trend in both years for protein percentages to increase from first to last harvests (Table 2). The correlations of protein versus harvest dates were positive and significant (Table 3). Total amino-acid percentages had no significant source of variation except among entries in 1976 (Table 1). In 1976, the seeds harvested the last 4 weeks had higher total amino acid values than those from the first 2 weeks (Table 2). The correlation between total amino acid percentages and harvest dates was positive and significant in 1976, but was a nonsignificant negative value in 1979; as expected, these changes were similar to those of total proteins.

Essential amino acids needing improvement in cottonseed are lysine, threonine, isoleucine, methionine, and half-cystine; for simplicity, only the data for these amino acids are reported. Differences among entries were detected for half-cystine in 1976, and methionine in 1979 (Table 1). The differences among entries associated with half-cystine were apparently due to its high level in seeds of entry 4 in both years (Table 2). Only in 1979, entry 4 had a high level of methionine. Significant differences over harvest dates were detected for lysine and isoleucine in 1976, and were similarly shown for threonine, half-cystine, and methionine in 1979 (Table 2). Each of these amino acids had a positive correlation with harvest dates in 1976, but these values are significant only for lysine and methionine (Table 3). In 1979, the amino acid to harvest date relations were nonsignificantly negative, except for a 0.0 correlation with isoleucine and a significantly positive correlation with cystine, which was positive in both years.

### Free Gossypol Percentage

The glandless entry 7 was excluded from the statistical analysis of free gossypol percentages. Differences were detected for free gossypol percentages among entries and among harvest dates in 1979 (Tables 1 and 2); no other source of variation was detected. The correlation of gossypol versus harvest

progressively later harvest dates. The amino acid composition of the protein, measured as total, or the selected amino acids lysine, threonine, isoleucine, methionine, and half-cystine, demonstrated no consistent patterns with harvest dates. The change in percentages of kernel protein over harvest dates and the significant interaction between entries and harvest dates in 1976 further indicate that sampling of cottonseed during a crop year must be carefully exercised. The percentages of oil and protein were negatively correlated in these data, which is consistent with other results (Cherry et al., 1981). However, the effects of high and normal nitrogen fertilization on the composition of cottonseed in a single cultivar over stratified harvests were shown to increase percentages of oil and decrease those of nitrogen in successive harvest dates (Leffler et al., 1977). These results and those of Elmore and Leffler (1976) are in contrast to those observed in our experiments.

Leffler et al. (1977) measured seed weight (seed index) and found that normal nitrogen level resulted in decreased seed weight with progressively later harvest dates as was observed in our experiments. The high nitrogen treatment appeared to overcome most of the variation due to harvest dates (Leffler et al., 1977). Their low nitrogen level is similar to the amount usually applied to the College Station, Texas, cotton production area.

The use of percentages to characterize cottonseed composition can be misleading, especially when seed weights change. For this reason, the seed oil and protein indexes were computed to express compositions as weights rather than percentages. Similar calculations were made in the normal nitrogen treatment study of Leffler et al. (1977). In both experiments, the seed weights and protein and oil quantities decreased with later harvest dates. The responses in the Mississippi and Texas experiments were similar when the compositions were based on seed weight. However, the percentages of change from first to last harvest dates differed between the two locations, but were similar between the 2 years in Texas. These results demonstrate not only the variation associated with sampling seed during a crop year, but also the need for additional research to identify the environmental and genetic factors contributing to variation in cottonseed composition.

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