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Seasonal and Fertility-Related Changes in Cottonseed Protein Quantity and Quality[1]

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ABSTRACT

Utilization of seed protein from cotton (*Gossypium hirsutum* L.) as an ingredient of food products for non-ruminant animals may provide producers with a salable seed commodity in addition to oil and meal. It was therefore important to establish the effects that environmental conditions might have on cottonseed protein quantity and quality (amino acid composition).

Cotton was grown at two levels of N fertility at each of two locations in Mississippi. At each location, N concentration was higher in cottonseed produced in high-N plots than in seed produced in low-N plots. For the single harvest at Starkville, enhancement of seed N by fertilization was 1.18%. For the first five harvests at Stoneville, enhancement of seed N ranged from 0.85 to 1.10%. A stratified harvest at Stoneville enabled the identification of seasonal influences on seed size and chemical composition. Both seed size and seed N concentration decreased with later developmental periods. Seed oil concentration, however, increased with later developmental periods.

Both N fertility and seasonal patterns caused slight, but statistically significant, changes in the amino acid composition of cottonseed protein. Generally, as seed N increased, the concentrations of lysine, threonine, glycine, and alanine in the seed protein decreased; concentrations of arginine and glutamic acid increased. Because both N fertilization and seasonal factors can influence cottonseed protein quantity and amino acid composition, comparisons of these factors should be made only among environmentally similar samples.

***Additional index words:* *Gossypium hirsutum* L., Seed development, Amino acid composition, Storage protein.**

SEED characteristics of cotton (*Gossypium hirsutum* L.) have increasingly become subjects of interest. Much early work on cottonseed concentrated on the oil fraction and the identification of the inverse relationship between oil and protein concentrations (Garner, et al., 1914; Pope and Ware, 1945).

Environmental and cultural factors have been demonstrated to affect the level of seed protein in several experiments (Wadleigh, 1944; Sturkie, 1934, 1947; Ham et al., 1975; Bhangoo and Albritton, 1976). The level of N nutrition of the parent plant is especially important in determining the concentration of protein in cottonseed (Wadleigh, 1944; Sturkie, 1947). A seasonal pattern affects development so that later-forming seeds are smaller and lower in N than their earlier-forming counterparts (Leffler and Tubertini, 1976).

Significant changes in the amino acid composition of developing cottonseed were observed during the period of N accumulation (Elmore and Leffler, 1976). Some of the variability within cotton for both seed N and amino acid composition has been identified as genetic (Meredith and Elmore, 1976). Because of these developmentally-related and genetically-associated changes in cottonseed amino acid composition we wanted to determine if environmental factors might also significantly alter the quality (amino acid composition) of cottonseed protein. We therefore examined the effects of N fertility and developmental period on the size and chemical composition of cottonseed.

MATERIALS AND METHODS

Cottonseed produced in fields at Starkville and Stoneville, Miss. were analyzed in this study. At Starkville, the cultivar 'Deltapine 16' was planted in field plots which differed in N fertility: no N fertilization and 200 kg N/ha, applied before planting. At Stoneville, a glandless derivative of 'Stoneville 7A' was provided by Dr. W. R. Meredith, Jr. Each plot at Stoneville was side-dressed with 112 kg N/ha 3 weeks after planting. The high-N plots were then top-dressed with an additional 112 kg N/ha when the cotton was in the early bloom stage.

At Starkville, tags were placed on 1st-day flowers on 1 August. Thereafter, at intervals during development, bolls at tagged nodes were harvested and processed as described by Leffler (1976).

The Stoneville experiment was a stratified harvest of cotton produced under the two N regimes. The first harvest was made on 19 September and consisted of a sample of all bolls that were open on that date. Open bolls that were not harvested were removed from the plants and discarded. Each subsequent harvest consisted of samples of bolls that had opened since the preceding harvest. Each of the seven harvests was then ginned and seed analyses were conducted on the separate samples.

Seeds were dehulled and the kernels were ground in a knife mill and analyzed. Chemical analyses were conducted in duplicate or triplicate on each sample. Nitrogen was analyzed as described by Leffler and Tubertini (1976). Oil analyses were made by the AOAC method (1965). Amino acids of both the hydrolysates and the nonprotein nitrogen (NPN) fractions of cottonseed were analyzed as indicated by Elmore and Leffler (1976).

The Stoneville experiment was a split plot with two replications; harvest dates were the main plots. Analyses of variance were conducted on the amino acid data. For those data in which significant differences were detected, least significant differences were calculated using the pooled errors from the analysis of variance. Parameters of seed produced at the two levels of N fertility were compared with the *t* test. The experiment at Starkville was not replicated; the within-plot errors were used to compare values from the two N fertility levels. Tests of significance were made using the *t* test.

RESULTS AND DISCUSSION

Throughout development at Starkville, cottonseed produced in the high-N plots contained a higher concentration of N than did seed produced in the low-N plots (Fig. 1). At maturity (bolls were open on 6 October), seed N concentrations were 6.15 and 4.96% for the high-N and low-N plots, respectively. The pattern of N accumulation by cottonseed was similar to that described by Leffler and Tubertini (1976); N fertilization appeared to affect only the level, not the duration, of N accumulation.

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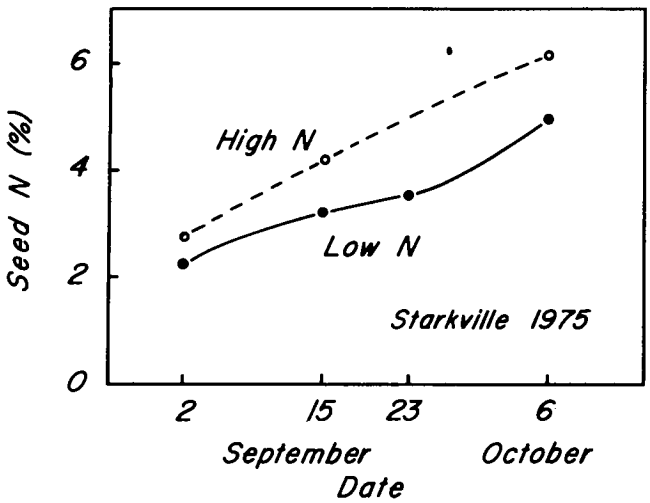

Fig. 1. Concentrations of N in cottonseed developing under conditions of low- and high-N fertility.

That cottonseed from the high-N plots were higher in N than those from the low-N plots would be expected (Wadleigh, 1944; Sturkie, 1947). Our data however, do not agree with those reported by Wanjura and Sunderman (1976), who found the level of cottonseed N to be unaffected by N fertilization. Differences between their study and ours may be seasonal, varietal, or geographic; the causes are not, however, readily apparent.

Recently matured, unweathered bolls were harvested periodically between mid-September and mid-November at Stoneville. Selected characteristics of cottonseed from these bolls are presented in Table 1. Within each level of N fertility, seed from early-formed bolls were larger than those from later-formed bolls. Similarly, within each developmental-harvest period, seed from the high-N plots were larger than those from the low-N plots. The relative enhancement of seed size by N fertilization became significant at the later harvests. The beneficial effect of N fertilization on seed size was additionally demonstrated by the fact that the seasonal seed size decrease was 21.6% for the low N plots, but only 8.4% for the high N plots.

Supplemental N fertilization also increased the concentration of N in cottonseed at each harvest at Stoneville. As has been described for seed index, the seed N concentration declined as the season advanced. In contrast to the seed size response, however, the relative enhancement of seed N concentration by N fertilization was more pronounced at the earlier harvests than at the later harvests. Responses of seed size and seed N concentration to both seasonal and N fertility differences coupled to produce considerably more protein in the earlier-formed seed, especially when high levels of fertilizer N were available. These results are consistent with those of Wadleigh (1944).

The oil concentration in cottonseed increased as the season progressed; supplemental N fertilization reduced the oil concentration. These results were expected because of the well-documented inverse relationship between oil and protein percentages in cottonseed (Garner, et al., 1914; Wadleigh, 1944; Sturkie, 1947), and in other oilseeds (Cartter, 1940; Lyons and Earley, 1952; Ham et al., 1975). Although kernel weights were not recorded, approximate comparisons of oil content per seed can be drawn from the product of seed index and seed oil concentration. From these comparisons, it appears that conditions which result in high protein content may also result in high oil content per seed, although the oil concentration was reduced.

Table 1. Characteristics of cottonseed developed during intervals of the 1975 season at Stoneville.

Date of harvest	Seed parameter					
	Seed index		N		Oil	
	Low N	High N	Low N	High N	Low N	High N
	g·100 seed^{-1}		% dry weight			
19 Sept.	12.19	12.49	6.14**	7.01	44.07*	39.55
26 Sept.	11.45	11.62	5.87**	6.72	44.97**	38.86
3 Oct.	10.80	11.32	5.64**	6.50	45.24†	41.86
10 Oct.	10.66*	11.48	5.46**	6.56	45.79†	42.93
20 Oct.	10.82**	12.03	5.49**	6.44	46.90	43.36
31 Oct.	9.98**	12.27	5.41*	5.79	47.08	44.50
13 Nov.	9.56†	11.44	5.42	5.53	45.76	44.31

*,**,† Significant (at the 5. 1, and 10% levels, respectively; *t* test) difference between measured parameters of seed produced at the two levels of N.

At both locations and with both cultivars, high N fertility increased both the concentration and the content of cottonseed N. This elevated level of seed N could reflect an increase in protein synthesis in the seed, an elevation of the free amino acid pool, or a combination of these phenomena. To resolve among these alternatives, we analyzed the NPN fractions of cottonseed produced under the different environmental conditions (Table 2).

There was generally more NPN in cottonseed from the high-N plots, but the differences were not always significant. The additional N available to developing seed did, however, appear to cause a qualitative change in the NPN fraction. In each of the harvests examined, soluble arginine was higher in cottonseed from high-N plots than it was in seed from low-N plots. When averaged over the four harvests, the increase in arginine-N accounted for about 72% of the total increase in NPN due to N fertilization.

Elmore and Leffler (1976) reported that NPN of mature cottonseed accounted for only about 1% of the total seed N. In this study, the NPN fraction of low-N cottonseed accounted for about 1.0% of the seed N while that fraction of high-N cottonseed accounted for about 1.4% of the total seed N. We conclude that the high level of N fertility did not result in severe perturbations of seed protein synthesis, and that nearly all of the increased seed N could be accounted for by the protein fraction. The apparent relationship between N fertility level and soluble arginine-N in cottonseed will require additional investigation to identify its importance to regulatory systems operating in developing seed.

Since most of the increased seed N due to N fertilization was apparently in the protein fraction, amino acid analyses were conducted to determine if this quality characteristic of cottonseed protein had been affected by N fertilization. These analyses are summarized in Table 3.

Table 2. Concentrations of factors in the nonprotein N fractions of cottonseed produced under different environmental conditions.

	Stoneville							Starkville	
	19 Sept.		10 Oct.		13 Nov.			6 Oct.	
Factor	Low N	High N	Low N	High N	Low N	High N	L.S.D.†	Low N	High N
	μmoles·gdw⁻¹								
Lysine	(0.22)‡	0.42	0.20	(0.40)	0.35	0.37	–	0.81	0.97
Histidine	0.18	0.22	0.18	0.24	0.19	0.18	ns	0.38	0.43
Ammonia	(4.11)	(6.18)	1.56	3.96	(1.07)	(1.26)	–	12.74	14.45
Arginine	3.30	6.94	3.74	11.11	2.50	3.58	1.45	3.07*	6.99
Aspartic acid	3.44	4.10	2.86	3.64	4.00	4.14	ns	0.34	0.40
Asparagine	1.90	5.87	1.83	3.72	2.83	3.54	ns	1.98	1.69
Threonine	0.31	0.43	0.23	0.29	0.30	0.28	0.07	0.90	1.11
Serine	0.38	0.34	0.68	1.44	0.66	0.81	0.21	1.30	1.31
Glutamic acid-glutamine	3.24	4.54	2.98	4.05	5.26	4.38	ns	0.34	0.31
Proline	TR	TR	TR	TR	TR	TR	ns	TR	TR
Glycine	1.18	0.98	0.58	0.76	0.42	0.46	0.49	2.26	2.47
Alanine	0.72	1.00	0.48	0.65	0.74	0.58	ns	0.55	0.78
1/2 Cystine	TR	TR	TR	TR	TR	TR	ns	0.65	0.73
Valine	TR	TR	TR	TR	TR	TR	ns	0.38	0.52
Methionine	TR	0.13	TR	TR	TR	TR	ns	0.47	0.46
Isoleucine	TR	0.16	TR	TR	TR	TR	ns	0.43	0.51
Leucine	TR	0.14	TR	TR	TR	TR	ns	0.55	0.69
Tyrosine	TR	0.15	TR	TR	TR	TR	ns	0.38	0.47
Phenylalanine	0.20	q0.28	0.17	0.19	0.24	0.26	ns	0.25	0.32
γ-Amino butyric acid	0.16	0.25	TR	TR	0.18	0.07	ns	7.22	6.01
Ethanolamine	1.51	0.76	0.50	0.58	1.83	1.80	ns	1.00	1.00
Nonprotein N	33.23	60.44	29.60	68.96	31.63	36.72	–	48.76	66.11

* Difference between values for the two Starkville samples was significant (5% level, *t* test).
† Least significant difference (5% level) for those factors indicated by the analysis of variance to be significant. ns, not significant; –, not analyzed.
‡ Data enclosed in parentheses are for a single replication only.

Table 3. Concentrations of amino acids and ammonia in seed protein and of nitrogen in cottonseed produced under different environmental conditions.

	Stoneville							Starkville	
	19 Sept.		10 Oct.		13 Nov.			6 Oct.	
Factor	Low N	High N	Low N	High N	Low N	High N	L.S.D.†	Low N	High N
	g·16 g N⁻¹								
Lysine	4.6	4.3	4.6	4.4	4.7	4.5	0.1	4.8*	4.2
Histidine	2.6	2.6	2.7	2.5	2.6	2.5	ns	2.7	2.6
Ammonia	2.7	2.6	2.7	2.6	2.6	2.6	ns	2.4	2.2
Arginine	12.1	12.3	11.7	12.2	11.4	11.7	0.3	11.4*	12.2
Aspartic acid	9.9	10.0	9.9	10.0	10.0	10.0	ns	10.1	10.2
Threonine	3.0	2.8	3.0	2.9	3.1	3.0	<0.1	3.4*	3.2
Serine	3.7	3.7	3.8	3.7	3.8	3.8	ns	4.9	4.8
Glutamic acid	23.3	24.7	23.6	24.5	23.6	24.4	0.6	23.6	24.5
Proline	3.7	3.7	3.8	3.8	3.9	3.8	ns	4.2	3.9
Glycine	4.8	4.5	4.7	4.6	4.8	4.6	0.1	4.6	4.4
Alanine	4.2	4.0	4.2	4.1	4.3	4.2	0.1	4.3*	4.1
1/2 Cystine	1.0	1.2	0.8	1.0	0.7	1.1	ns	1.1	1.1
Valine	5.0	5.0	5.1	4.9	5.1	5.0	ns	4.1	4.1
Methionine	1.7	1.5	1.7	1.5	1.6	1.3	0.2	1.4	1.4
Isoleucine	3.2	3.2	3.2	3.2	3.3	3.2	<0.1	2.8	2.7
Leucine	6.2	6.1	6.3	6.1	6.3	6.2	0.1	6.5	6.4
Tyrosine	2.8	2.6	2.8	2.6	2.8	2.7	<0.1	2.8	2.8
Phenylalanine	5.3	5.4	5.3	5.3	5.3	5.3	ns	4.9*	5.2
N, %	6.1	7.0	5.5	6.6	5.4	5.5		5.0	6.1

* Difference between values for the two Starkville samples was significant (5% level, *t* test).
† Least significant difference (5% level) for those factors indicated by the analysis of variance to be significant. ns, not significant.

Significant differences in amino acid composition were found between N fertility levels at each location. Several of the differences in amino acid composition were also significant among the developmental periods within N fertility levels at Stoneville. These data demontrate that cottonseed samples must be chronologically, developmentally, and environmentally similar in order for valid comparisons of amino acid compositions to be made. These similarities would be particularly important, for example, if an effort were made to survey *Gossypium* germplasm for specific amino acid variants.

Much of the additional protein accumulated by cottonseed in response to N fertilization can be assumed to be storage proteins. This assumption is supported by reports describing storage protein accumulation in other crops (Hansen et al., 1946; Frey, 1951; Schneider et al., 1952; Wiggans and Frey, 1958;

Choe et al., 1974). Based upon this assumption, some inferences can be drawn concerning the probable amino acid composition of cottonseed storage proteins.

The data in Table 3 show lower concentrations of alanine, glycine, leucine, lysine, methionine, threonine, and tyrosine in cottonseed from high-N plots. These results suggest that these amino acids are probably present in low concentrations in cottonseed storage proteins. Conversely, the concentrations of arginine and glutamic acid were higher in samples from high-N plots, indicating relatively high concentrations of these amino acids in cottonseed storage proteins. These interpretations are supported by preliminary evaluation of the amino acid composition of an isolated cottonseed storage protein fraction (E. E. King and C. D. Elmore, unpublished results). If arginine is present in high concentration in a cottonseed storage protein, the increase in soluble arginine may be simply the result of the system's capacity to produce the amino acids required by protein synthesis.

We have shown that cottonseed may respond to elevated N fertility by synthesizing additional storage proteins. The additional storage proteins, in turn, cause significant shifts in the total amino acid profile of the cottonseed meal. Furthermore, we have found that a seasonal pattern of seed N accumulation exists which also affects the seed amino acid profile. Although the absolute magnitudes of these changes were generally slight, their statistical significance emphasizes the importance of adequate sampling procedures. Increases in seed N due to N fertilization did not appear to be associated with massive increases in the soluble amino acid pool.

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