The *Journal of Cotton Science 18:410–419 (2014)*
http://journal.cotton.org, © The Cotton Foundation 2014

MOLECULAR BIOLOGY AND PHYSIOLOGY

Nitrogen Fertility and Irrigation Effects on Cottonseed Composition

William T. Pettigrew* and Michael K. Dowd

ABSTRACT

Cottonseed products are a valuable secondary revenue source for cotton (*Gossypium hirsutum* L.) producers, but how production practices impact cottonseed composition is unclear. This research evaluated the effect on cottonseed composition by varying irrigation and nitrogen (N) fertilization regimes. Four cotton cultivars were grown from 2010 through 2012 under irrigated or dryland conditions and given a fertilizer application of 0 kg N ha^{-1}, 56 kg N ha^{-1}, or 112 kg N ha^{-1}. Ginned seed from the plots were dehulled, and the kernels were analyzed for protein, crude oil, gossypol, soluble carbohydrates, and the oil's fatty acid distribution. Few cultivar interactions with either irrigation or fertilization were detected. Irrigation increased kernel gossypol (18%) and oil (8%) levels but decreased kernel protein levels (13%). In contrast, the highest rate of N fertilization increased kernel protein concentration (18%) but decreased gossypol (14%), oil (9%), and soluble carbohydrate (3%) levels compared to seed kernels grown without fertilizer. In addition, N fertilization shifted the distribution of the oil's unsaturated fatty acids toward more oleic acid and less linoleic acid. Practices growers implement to optimize lint production alter some desirable and some less desirable seed compositional traits.

Harvesting and ginning a cotton (*Gossypium hirsutum* L.) crop generates two marketable products, lint and seed. Although lint contributes the majority of income produced with a cotton crop, the seed also produces a consistent secondary revenue stream. Increased utilization of whole cottonseed and cottonseed meal by the dairy industry (Arieli, 1998) and cottonseed oil by the restaurant and food processing industries (O'Brien and Wakelyn, 2005) has helped to elevate and stabilize the price received by producers and ginners for the seed. As these and other industries further recognize the inherent value of cottonseed products, further appreciation in the price for cottonseed might be expected.

Both environmental influences and the genetic background are involved in determining cottonseed composition. The genetic contribution has been well established over the years by National Cotton Variety Trial reports (USDA, 2012), book reviews (Cherry and Leffler, 1984; Tharp, 1948) and research articles (Cherry, 1983; Dowd et al., 2010; Kohel and Cherry, 1983; Lawhon et al., 1977; Lukonge et al., 2007; Pandey and Thejappa, 1975; Pettigrew and Dowd, 2012; Pons et al., 1953; Stansbury et al., 1953, 1954; Turner et al., 1976). Despite this genetic variability among cotton cultivars, little effort or resources have been devoted to breeding seed for improved composition.

While genetics accounts for considerable variation in the composition of cottonseed, the growth environment also influences the phenotypic expression of compositional traits (Dowd et al., 2010). Although there have not been as many studies on the environmental aspects of seed composition, some studies have correlated seed composition with precipitation and temperature levels across years and locations (Dowd et al., 2010; Pons et al., 1953; Stansbury et al., 1953, 1956). Recently, we demonstrated a direct environmental effect on seed composition by varying planting dates and water regimes (Pettigrew and Dowd, 2011). The response to irrigation was also found to vary depending upon the cultivar being grown (Pettigrew and Dowd, 2012).

Most environmental studies of cottonseed composition have involved climatic variables, such as seasonal temperature and precipitation, with only a few studies addressing production aspect contributions to seed composition. As previously mentioned, planting dates impacted seed composition (Pettigrew and Dowd, 2011). The effects that nitrogen (N) and phosphorus (P) fertilizers, and growth regulators had on cottonseed protein and oil contents were investigated by Sawan et al. (1988). Hunt et al. (1998) examined a soil fertilization effect on seed N content, which would be analogous to the seed protein, but

W.T. Pettigrew*, USDA-ARS Crop Production Systems Res. Unit, Stoneville, MS 38776 and M.K. Dowd, USDA-ARS Commodity Utilization Res. Unit, New Orleans, LA
*Corresponding author: bill.pettigrew@ars.usda.gov

did not address how seed gossypol, carbohydrate, oil, and fatty acid compositions were impacted. Main et al. (2013) studied how cotton fiber traits were affected by fertilizer applications and included analysis of whole seed oil and protein levels. He et al. (2013) looked at how the mineral composition of cottonseed was affected by the application of poultry litter as a fertilizer but they did not report how the traditional traits associated with composition (protein, carbohydrates, oil, fatty acids and gossypol) were impacted.

It is important to understand the factors that affect cottonseed composition, because the composition impacts the usefulness and value of seed products. Soil moisture status, N fertility, and cultivars have all been shown to influence some aspects of cottonseed composition. What is not known is what level of interplay exists among these variables for seed composition. The irrigation response has been documented to vary depending upon the cultivar grown (Pettigrew and Dowd, 2012). However, it is not known if the irrigation response changes when the level of N fertilization is altered. Similarly, it is less well known if cultivars respond differently to varying levels of N fertilization. The primary objective behind this research was to determine how varying N fertilization affects seed composition. Secondary objectives were to determine if the N fertilization response was altered in response to irrigation or by growing different cultivars.

MATERIALS AND METHODS

Research was conducted in the field over a three-year period from 2010 to 2012 at two locations near Stoneville, MS. During 2010, cotton was planted on a Dubbs silt loam soil (fine-silty, mixed, thermic Typic Hapludalfs). In 2011 and 2012, the site was a Dundee silty clay loam soil (fine-silty, mixed, active, thermic Typic Endoaqualfs). At each location, half of the plots were irrigated and the other half grown under dryland conditions. Four cotton cultivars were grown each year of the study. The cultivars were 'DP 0935B2RF', 'FM 840B2RF', 'PHY 485WRF', and 'ST 4554B2RF' representing a range of maturities and breeding programs. Delta and Pine Land Co., Scott, MS provided the seed of DP 0935B2RF. Seed of FM-840B2RF and ST 4554B2RF were provided by Bayer CropScience, Research Triangle Park, NC, and Dow AgroSciences-Phytogen Seed Company, Indianapolis, IN provided seeds of PHY 485WRF. Each cultivar was grown under three rates of N fertilization (0 kg N ha^{-1}, 56 kg N ha^{-1}, and 112 kg N ha^{-1}), which were applied pre-plant as a urea-ammonium nitrate solution. The plots were planted in four rows spaced 1 m apart with plot lengths of 18.3 m in 2010 and 15.2 m in 2011-2012. Planting dates were 31 March 2010, 7 April 2011, and 28 March 2012. Recommended insect and weed control measures were employed throughout each growing season as needed.

A randomized complete block with a modified split-plot treatment arrangement was the experimental design utilized for this study. Irrigation regimes were the main plots and the sub plots were the cultivars and N application rates, which were arranged factorially. Irrigation regimes were randomly assigned and replicated in three blocks. In addition, within each of these blocks there were two replications containing each of the cultivars by N rate combinations, resulting in a total of six replications. The cultivar by N rate combination subplots were randomly assigned within each replication for the first year of the study (2010). In 2011, the subplots were re-randomized within each replication due to the experiment being moved to a new location. To minimize N treatment carryover effects from one year to the next, the subplots remained in their initial location the following year (2012). Two furrow irrigation applications occurred in both 2010 and 2011, and three applications occurred in 2012. Approximately 2.54 cm of water was applied during each irrigation event.

Each year, the plots were defoliated when approximately 60% of the bolls had opened in the latest maturing treatment (usually early-to-mid September). The initial defoliation step involved the application of a mixture of 0.035 kg thidiazuron ha^{-1} and 0.0175 kg diuron ha^{-1} to the canopy. One week later a mixture of 0.035 kg thidiazuron ha^{-1}, 0.0175 kg diuron ha^{-1}, and 1.68 kg ethephon ha^{-1} was applied to complete defoliation and open most of the remaining unopened bolls. After defoliation but prior to mechanical harvest, a 50-boll sample was hand-harvested from each plot. These samples were subsequently ginned on a 10-saw laboratory gin, the lint and seed were weighed and saved, and the seed were utilized for subsequent chemical analyses.

As seed kernels contain almost all of the valuable oil and protein components, analysis was conducted on kernels after separation of the seed hull. To prepare kernels for analyses, approximately 75 g of fuzzy seed from each plot was cracked by milling for several seconds in a blender. The resulting material was sifted through a series of #4 (4.75 mm opening) and # 12

(1.70 mm opening) sieves. Material collected on the # 4 sieve was re-milled, increasing the duration and intensity of the grinding process and then resifted. Dehulled kernels and large kernel pieces that collected on the #12 sieve were ground in a food chopper to pass through a # 20 (0.85 mm opening) sieve. These ground seed samples were then freeze-dried and stored at –20°C in the dark until analyzed.

An in depth description of the specific details of each chemical assay procedure to quantify the various kernel composition traits has been described earlier (Pettigrew and Dowd, 2011). Therefore, we will only provide a brief synopsis of the techniques here. Petroleum ether (CAS #8032-32-4) was used to extract crude oil from the ground tissue, and this crude oil was subsequently quantified gravimetrically after evaporation of the ether. Oil was extracted from the kernels with hexane (CAS #110-54-3) and was converted to fatty acid methyl esters by heating with the addition of 0.5 N Methanolic base (Sigma-Aldrich-Supelco, Bellefonte, PA). A gas chromatograph with a polar capillary column was then used to separate and quantify the fatty acid methyl esters. High pressure liquid chromatography was used to detect (+)- and (-)-gossypol after the compounds were extracted and transformed into Schiff's base derivatives with *R*-(–)-2-amino-1-propanol (CAS #35320-23-1). The assay was based upon AOCS Recommended Practice Ba 8a-99 (AOCS, 1998). Kernel N was determined by combustion, and the N value was multiplied by 6.0 to give protein concentration (Dowd and Wakelyn, 2010). Soluble sugars were extracted from the tissue and silylated by heating at 70°C in a solution containing pyridine (CAS #110-86-1), hexamethyldisilazane (CAS #999-97-3), and trifluoroacetic acid (CAS #76-05-1). After derivatization, the sugars were separated and quantified by gas chromatography on a nonpolar capillary column.

Statistical analyses were performed by analysis of variance (PROC MIXED, SAS Institute, 1996). Because all the irrigation, N rate, and cultivar treatments remained in the same place each year for a given location, years were treated as a repeated measurement when conducting a combined analysis across years for a given location. Random effects were block; block X water; rep (block water); block X fertilizer X cultivar(water); rep X fertilizer X cultivar(block water); and block X rep X year. Irrigation, N rate, and cultivar means were averaged across years and each other when statistically important interactions were not detected. Means were separated by a protected LSD test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Year-to-year variability generated contrasting climatic conditions across the three years of this study (Table 1). The years 2010 and 2011experienced relatively dry periods from June through August (encompassing the early squaring through boll setting periods) with an accompanying high accumulation of thermal units. In contrast, 2012 had over 10 cm of precipitation each month from June through August. Consequently, 2010 and 2011 were extremely good years for testing the irrigation regime effects, while 2012 was not.

Table 1. Monthly weather summary for 2010 to 2012 at Stoneville, MS.[z]

Month	2010	2011	2012
	Precipitation (cm)		
April	6.0	16.0	10.6
May	13.4	7.0	5.2
June	3.1	4.0	16.2
July	4.8	5.0	11.6
August	0.6	6.1	10.9
September	5.4	10.1	8.3
October	4.5	2.7	14.7
	Thermal Units[y]		
April	124	159	137
May	273	224	293
June	401	404	316
July	412	436	409
August	458	425	370
September	315	228	264
October	129	101	68
	Solar Radiation (MJ m^{-2})		
April	-	626	638
May	681	748	688
June	743	743	751
July	710	723	700
August	667	689	634
September	609	530	528
October	566	523	462

[z] All observations made by NOAA, Mid-South Agric. Weather Service, and Delta Research and Extension Center Weather, Stoneville, MS.

[y] [(Max. temp + Min. temp.)/2] – 15

Data for both locations were analyzed separately because of the year and soil type differences between locations. At the second location (2011-12), the year effect was significant (Table 2) due to the contrasting weather conditions. Although year interacted with water and fertilizer for several of the traits measured at the second location, the F-values for those interactions were small relative to those of the main effects. In addition, the dramatic difference in precipitation totals between the two years (Table 1) explains why in 2011 there was a significant irrigation response and in 2012 there was not a significant response. The irrigation-dryland differences were in the same direction for both years, in one year the comparison was significant and in the other it was not significant. Therefore, irrigation and fertility treatment means were averaged across years. Variety consistently produced the largest F-value relative to the other sources of variation. The variety effect on seed composition has been well documented in earlier publications (Dowd et. al., 2010; Pettigrew and Dowd, 2012; USDA, 2012) and therefore will not be dealt with in this report. When the occasional variety X water or variety X fertility interaction was significant, the F-value for the interaction was small relative to that of the main effects. Therefore, irrigation and fertility means were also averaged across varieties.

The irrigation response on gossypol, crude oil and protein was similar to that previously reported (Pettigrew and Dowd, 2011). Total gossypol (18%) and crude oil (8%) concentration were increased when irrigation was applied, while the protein (13%)

Table 2. Analysis of variance table containing sources of variation, degrees of freedom, and f values for seed gossypol, oil, protein, carbohydrate, and fatty acid concentrations.

Location	Source of [z] Variation	df	Total Gossypol	% (+) Gossypol	Crude Oil	Protein	Total Soluble Carbohydrates	Saturated Fatty Acids	Unsaturated Fatty Acids
2010	Water	1	99.95 (0.01) [y]	80.81 (0.01)	233.91 (0.01)	339.71 (0.01)	6.48 (0.04)	137.57 (0.01)	103.36 (0.01)
	Fertilizer.	2	68.31 (0.01)	10.87 (0.01)	155.42 (0.01)	225.31 (0.01)	18.10 (0.01)	2.47 (0.09)	3.40 (0.04)
	Water*Fertilizer	2	3.20 (0.04)	1.06 (0.35)	5.36 (0.01)	10.79 (0.01)	1.41 (0.25)	0.21 (0.81)	0.53 (0.59)
	Variety	3	189.82 (0.01)	803.97 (0.01)	38.69 (0.01)	32.57 (0.01)	36.53 (0.01)	1260.26 (0.01)	1258.68 (0.01)
	Water*Variety	3	1.65 (0.18)	2.46 (0.07)	2.26 (0.09)	0.13 (0.94)	0.96 (0.42)	8.60 (0.01)	6.31 (0.01)
	Fertilizer*Variety	6	1.86 (0.24)	0.76 (0.60)	2.13 (0.05)	1.18 (0.32)	1.41 (0.23)	6.64 (0.01)	6.60 (0.01)
	Water*Fertilizer*Variety	6	0.45 (0.85)	0.56 (0.76)	0.87 (0.52)	0.88 (0.53)	1.88 (0.10)	1.20 (0.31)	1.14 (0.34)
2011-12	Water	1	39.94 (0.01)	77.53 (0.01)	19.03 (0.01)	29.83 (0.01)	8.66 (0.07)	43.46 (0.02)	36.62 (0.02)
	Fertilizer.	2	17.11 (0.01)	1.38 (0.26)	46.30 (0.01)	53.83 (0.01)	9.18 (0.01)	5.86 (0.01)	5.19 (0.01)
	Water*Fertilizer	2	0.45 (0.64)	3.16 (0.05)	0.27 (0.76)	0.12 (0.89)	1.29 (0.28)	1.16 (0.32)	1.35 (0.27)
	Variety	3	208.20 (0.01)	1026.44 (0.01)	19.72 (0.01)	13.96 (0.01)	82.58 (0.01)	1146.40 (0.01)	1088.76 (0.01)
	Water*Variety	3	2.00 (0.13)	0.34 (0.79)	0.59 (0.62)	0.87 (0.47)	0.92 (0.43)	5.68 (0.01)	4.96 (0.01)
	Fertilizer*Variety	6	0.24 (0.96)	1.75 (0.14)	0.18 (0.98)	0.56 (0.76)	1.74 (0.12)	1.10 (0.37)	0.99 (0.45)
	Water*Fertilizer*Variety	6	0.73 (0.63)	0.61 (0.72)	0.45 (0.84)	0.51 (0.80)	1.05 (0.40)	0.37 (0.90)	0.32 (0.92)
	Year	1	35.64 (0.01)	15.22 (0.01)	82.21 (0.01)	2.96 (0.14)	8.38 (0.02)	65.29 (0.01)	46.63 (0.01)
	Year*Water	1	6.64 (0.01)	41.75 (0.01)	2.65 (0.11)	2.30 (0.13)	0.68 (0.41)	17.28 (0.01)	10.80 (0.01)
	Year*Fertilizer	2	2.36 (0.10)	0.60 (0.55)	7.23 (0.01)	14.03 (0.01)	6.38 (0.01)	1.18 (0.31)	0.99 (0.38)
	Year*Water*Fertilizer	2	0.90 (0.41)	2.41 (0.10)	1.30 (0.28)	1.12 (0.33)	3.30 (0.04)	0.36 (0.70)	0.40 (0.67)
	Year*Variety	3	3.20 (0.03)	4.15 (0.01)	0.59 (0.62)	0.36 (0.78)	0.74 (0.53)	2.55 (0.06)	2.58 (0.06)
	Year*Water*Variety	3	0.13 (0.94)	4.73 (0.01)	0.28 (0.84)	0.31 (0.82)	0.03 (0.99)	1.76 (0.16)	1.89 (0.14)
	Year *Fertilizer*Variety	6	0.59 (0.73)	1.71 (0.13)	0.57 (0.76)	0.55 (0.77)	1.27 (0.28)	2.09 (0.06)	2.13 (0.06)
	Year*Water*Fertilizer*Variety	6	1.52 (0.18)	2.12 (0.06)	0.68 (0.67)	0.89 (0.51)	1.40 (0.22)	0.20 (0.98)	0.30 (0.94)

[z] **Random effects used in this model were block, block*water, rep(block water), block*fertilizer*variety(water), rep*fertilizer*variety(block water), and block*rep*year.**

Nested effects denoted with parentheses (i.e. rep(block) denotes rep within block).

[y] **Values with parentheses represent P > F. Values < 0.01 were rounded up.**

concentration and the percentage of the (+)-gossypol isomer (3%) were decreased (Table 3). Alternatively, increasing the rate of N fertilization produced the opposite effect of an irrigation application. The highest rate of N fertilization (112 kg N ha^{-1}) decreased total gossypol (14%) and crude oil (9%) levels but increased the seed kernel protein (18%) concentration compared with kernels from plants not receiving any fertilizer. This N effect on seed protein is not surprising considering the direct role that N has in forming the peptide bonds that are integral to all proteins.

Table 3. Effects of varying water regimes and N fertilization rates on seed gossypol, crude oil, and protein concentrations for cotton grown at Stoneville, MS during two periods (2010) and (2011-2012).

Years	Water Regime	N Fertility	Total Gossypol	% (+) Gossypol	Crude Oil	Protein
			g kg^{-1}	% [z]	g kg^{-1}	g kg^{-1}
2010	Dryland		10.0	59.8	355	342
	Irrigated		12.0	57.9	388	290
	LSD 0.05		1.0	0.4	4	6
		0 kg N ha^{-1}	12.0	59.4	389	287
		56 kg N ha^{-1}	11.4	58.8	380	303
		112 kg N ha^{-1}	9.7	58.3	345	357
		LSD 0.05	0.4	0.5	5	7
2011-12	Dryland		10.0	59.8	335	382
	Irrigated		11.6	58.4	357	345
	LSD 0.05		0.8	0.3	16	22
		0 kg N ha^{-1}	11.3	58.9	359	344
		56 kg N ha^{-1}	10.8	59.2	345	364
		112 kg N ha^{-1}	10.3	59.2	334	381
		LSD 0.05	0.4	0.4 (ns) [y]	5	7

[z] Percentage of the total gossypol.

[y] ns = not significantly different at the $P \leq 0.05$ level

Kernel carbohydrates were also impacted by both irrigation and N fertilization (Table 4). The predominate seed kernel carbohydrate, raffinose, was increased 3% with irrigation while the second most prominent seed kernel carbohydrate, stachyose, was decreased 7% by irrigation. Total soluble carbohydrates and sucrose concentrations were inconsistently impacted by irrigation. The irrigation effects on raffinose and stachyose concentrations found in this study were similar to results that we reported earlier (Pettigrew and Dowd, 2011). For the most part N fertilization decreased kernel carbohydrate levels. Fertilization at the highest rate (112 kg N ha^{-1}) decreased total soluble carbohydrates (3%), sucrose (9%), and stachyose (7%) compared with levels measured in the kernels of unfertilized plants. Seed raffinose concentrations were not consistently impacted by N fertilization.

The proportion of saturated fatty acids in the seed oil was decreased by both irrigation (2%) and N fertilization (0.5%), although individual fatty acids responded differently to these two treatments (Table 5). The proportion of myristic (19%), palmitic (2%), behenic (11%), and lignoceric (13%) acids were decreased by irrigation. However, stearic acid levels were increased (2%) by irrigation and arachidic acid levels were not consistently impacted by irrigation. The 112 kg N ha^{-1} rate of fertilization increased the proportion of myristic (14%), behenic (10%), and lignoceric (11%) acids compared to these acids in seed from plants not fertilized. In contrast, palmitic (1%) and stearic (2%) acids levels were decreased by N fertilization. Palmitic and stearic acids are the most prominent saturated fatty acids in the seed; hence, their relative change parallels the overall change in the saturated fatty acid levels. Arachidic acid levels were not affected by N application, similar to the lack of response observed from irrigation.

Corresponding to the changes seen in the saturated fatty acids, both irrigation and N fertilization increased the percentage of unsaturated fatty acids by 0.9 and 0.4%, respectively (Table 6). Irrigation increased the amount of the poly-unsaturated linoleic acid in the oil by 6%. At one location, irrigation increased the relative level of the other poly-unsaturated fatty acid, α-linolenic acid by 3% but at the second location irrigation decreased its relative level by 2%. The relative levels of the mono-unsaturated fatty acids palmitoleic acid, vaccenic acid, and oleic acid in the oil were all decreased with irrigation by 9, 10, and 10%, respectively. In contrast to the increased the level of the predominant unsaturated fatty acid seen with irrigation treatments, linoleic acid, applying N fertilization had the opposite effect and decreased the level of linoleic acid in the oil by 3%. Fertilization also increased the oil's palmitoleic acid level by 7% and oleic acid level by 10%, which contrasts with the way irrigation decreased the levels of these acids. The levels of α-linolenic acid were not altered by N fertilization, while there was an inconsistent response with *cis*-vaccenic acid levels as N fertilization increased levels at the first location but not at the second location.

Table 4. Effects of varying water regimes and N fertilization rates on various seed carbohydrate concentrations for cotton grown at Stoneville, MS during two periods (2010) and (2011-2012).

Years	Water Regime	N Fertility	Total Soluble Carbohydrates	Sucrose	Raffinose	Stachyose
			g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
2010	Dryland		68.9	9.2	48.0	11.7
	Irrigated		69.9	9.8	49.2	10.9
	LSD 0.05		0.9	0.2	0.8	0.2
		0 kg N ha^{-1}	70.3	9.9	48.6	11.8
		56 kg N ha^{-1}	70.2	9.6	49.1	11.5
		112 kg N ha^{-1}	67.8	9.0	48.2	10.6
		LSD 0.05	0.9	0.2	0.7	0.2
2011-12	Dryland		71.2	11.2	48.8	11.3
	Irrigated		72.5	11.6	50.3	10.6
	LSD 0.05		1.4 (ns) [z]	0.8 (ns)	1.4	0.6
		0 kg N ha^{-1}	72.8	11.9	49.8	11.1
		56 kg N ha^{-1}	71.6	11.2	49.4	11.0
		112 kg N ha^{-1}	71.2	11.0	49.5	10.7
		LSD 0.05	0.7	0.3	0.7 (ns)	0.2

[z] ns = not significantly different at the $P \leq 0.05$ level

Table 5. Effects of varying water regimes and N fertilization rates on various seed saturated fatty acid distributions for cotton grown at Stoneville, MS during two periods (2010) and (2011-2012).

Years	Water Regime	N Fertility	Myristic Acid 14:0	Palmitic Acid 16:0	Stearic Acid 18:0	Arachidic Acid 20:0	Behenic Acid 22:0	Lignoceric Acid 24:0	Total Saturated Fatty Acids
			% [z]	%	%	%	%	%	%
2010	Dryland		1.029	26.61	2.79	0.319	0.128	0.099	30.98
	Irrigated		0.817	26.12	2.88	0.298	0.111	0.085	30.30
	LSD 0.05		0.030	0.10	0.08	0.005	0.003	0.003	0.11
		0 kg N ha^{-1}	0.853	26.50	2.87	0.306	0.114	0.087	30.73
		56 kg N ha^{-1}	0.897	26.33	2.86	0.308	0.117	0.090	30.60
		112 kg N ha^{-1}	1.020	26.25	2.78	0.312	0.127	0.099	30.59
		LSD 0.05	0.021	0.12	0.03	0.006 (ns) [y]	0.003	0.003	0.14
2011-12	Dryland		1.048	26.18	2.75	0.219	0.141	0.107	30.44
	Irrigated		0.870	25.64	2.79	0.210	0.129	0.095	29.73
	LSD 0.05		0.025	0.42	0.02	0.018 (ns)	0.007	0.006	0.40
		0 kg N ha^{-1}	0.922	26.04	2.80	0.214	0.130	0.098	30.21
		56 kg N ha^{-1}	0.965	25.92	2.76	0.214	0.135	0.100	30.10
		112 kg N ha^{-1}	0.989	25.77	2.74	0.216	0.140	0.105	29.95
		LSD 0.05	0.025	0.13	0.03	0.004 (ns)	0.003	0.002	0.15

[z] Percentage of the total fatty acid fraction.

[y] ns = not significantly different at the $P \leq 0.05$ level

Table 6. Effects of varying water regimes and N fertilization rates on various seed unsaturated fatty acid distributions for cotton grown at Stoneville, MS during two periods (2010) and (2011-2012).

Years	Water Regime	N Fertility	Palmitoleic Acid 16:1	Vaccenic Acid 18:1 (n-7)	Oleic Acid 18:1 (n-9)	Linoleic Acid 18:2	α-Linolenic Acid 18:3	Total Unsaturated Fatty Acids
			% [z]	%	%	%	%	%
2010	Dryland		0.641	1.067	17.62	49.02	0.104	68.4
	Irrigated		0.584	0.950	15.96	51.43	0.107	69.0
	LSD 0.05		0.014	0.026	0.31	0.44	0.002	0.1
		0 kg N ha^{-1}	0.589	0.978	15.99	50.97	0.106	68.6
		56 kg N ha^{-1}	0.604	0.994	16.53	50.52	0.106	68.8
		112 kg N ha^{-1}	0.645	1.053	17.84	49.17	0.105	68.8
		LSD 0.05	0.012	0.032	0.31	0.35	0.003 (ns) [y]	0.1
2011-12	Dryland		0.687	0.967	19.25	47.79	0.216	68.9
	Irrigated		0.633	0.882	17.21	50.60	0.212	69.5
	LSD 0.05		0.019	0.071	0.82	0.94	0.002	0.3
		0 kg N ha^{-1}	0.649	0.915	17.67	49.66	0.213	69.1
		56 kg N ha^{-1}	0.659	0.925	18.15	49.26	0.214	69.2
		112 kg N ha^{-1}	0.673	0.933	18.86	48.67	0.216	69.4
		LSD 0.05	0.009	0.020 (ns)	0.32	0.36	0.003 (ns)	0.2

[z] Percentage of the total fatty acid fraction.

[y] ns = not significantly different at the $P \leq 0.05$ level

An unfortunate consequence from irrigation is an increase in the level of cyclopropenoid fatty acids in the oil, which are undesirable minor components (Table 7). Irrigation increased the total cyclopropenoid level by 14% compared with their level in the seed oil of dryland plants. Of the individual cyclopropenoid acids, both malvalic and sterculic acids were increased by irrigation. This irrigation response is similar to the response reported earlier (Pettigrew and Dowd, 2011). Varying N fertilization had no effect on the cyclopropenoid acid levels.

Based upon the effect N fertilization and irrigation had on some of the individual fatty acid levels, it was not surprising to find that both treatments impacted calculated fatty acid parameters (Table 8). The oleic desaturation ratio (ODR), which describes the efficiency of the desaturation reaction to convert oleic acid to linoleic acid, was increased 5% by irrigation. In contrast, N fertilization decreased the ODR by 3%. The linoleic acid desaturation ratio (LDR) estimates the conversion of linoleic acid into linolenic acid and was inconsistently impacted by irrigation and N fertilization depending upon the location. At the first location (year 2010), neither irrigation nor N affected the LDR. However, at the second location (years 2011-12) irrigation decrease the LDR by 7% but N fertilization increased LDR by 4%. Irrigation decreased the ratio of 16-carbon to 18-carbon fatty acids (C16:C18) an average of 3% across both locations. Fertilization did not alter the C16:C18 ratio at the first location and only minimally decreased it (1%) at the second location. Irrigation decreased the percentage of longer chain fatty acids (>C18) in the oil. In contrast, N fertilization increased the level of fatty acids in the oil with more than 18-carbon atoms by 6%. These irrigation responses were similar to those reported earlier (Pettigrew and Dowd, 2011).

Both irrigation and N fertilization impacted cottonseed composition. For the most part, the irrigation effects on cottonseed composition described in this research were very similar to those described earlier (Pettigrew and Dowd, 2011). With the exception of one year, a lack of difference in seed size between the water regimes (Pettigrew and Zeng, 2014) indicates that there should not be any dilution effect for the various composition traits due to volume constraints placed on assimilated deposition that might be associated with smaller seeds. However, the reduction in the total reproductive sink size because of reduced yields under dryland conditions for most years (Pettigrew and Zeng, 2014), could explain the increased kernel protein levels seen for dryland seed (Pettigrew and Dowd, 2011).

Table 7. Effects of varying water regimes and N fertilization rates on various seed cyclopropenoid fatty acid distributions for cotton grown at Stoneville, MS during two periods (2010) and (2011-2012).

Years	Water Regime	N Fertility	Cyclopropenoid Fatty Acids	Malvalic Acid cpe 18:1	Sterculic Acid cpe 19:1
			% [z]	%	%
2010	Dryland		0.583	0.323	0.260
	Irrigated		0.670	0.382	0.288
	LSD 0.05		0.064	0.046	0.018
		0 kg N ha^{-1}	0.638	0.360	0.278
		56 kg N ha^{-1}	0.640	0.362	0.278
		112 kg N ha^{-1}	0.601	0.335	0.266
		LSD 0.05	0.024	0.017	0.008
2011-12	Dryland		0.651	0.361	0.290
	Irrigated		0.727	0.410	0.317
	LSD 0.05		0.062	0.043	0.012
		0 kg N ha^{-1}	0.684	0.383	0.301
		56 kg N ha^{-1}	0.692	0.388	0.304
		112 kg N ha^{-1}	0.691	0.385	0.306
		LSD 0.05	0.024 (ns) [y]	0.016 (ns)	0.009 (ns)

[z] Percentage of the total fatty acid fraction.

[y] ns = not significantly different at the $P \leq 0.05$ level

Table 8. Effects of varying water regimes and N fertilization rates on various calculated seed fatty acid components for cotton grown at Stoneville, MS during two periods (2010) and (2011-2012).

Years	Water Regime	N Fertility	Oleic Acid Desaturation Ratio (ODR)	Linoleic Acid Desaturation Ratio (LDR)	C16:C18 Fatty Acid Ratio	Total Fatty Acids w > C18
						% [z]
2010	Dryland		0.736	0.00211	0.393	0.545
	Irrigated		0.764	0.00207	0.380	0.494
	LSD 0.05		0.005	0.00005 (ns) [y]	0.002	0.010
		0 kg N ha^{-1}	0.762	0.00206	0.388	0.507
		56 kg N ha^{-1}	0.754	0.00209	0.386	0.514
		112 kg N ha^{-1}	0.734	0.00212	0.386	0.537
		LSD 0.05	0.005	0.00006 (ns)	0.003 (ns)	0.012
2011-12	Dryland		0.714	0.00449	0.385	0.467
	Irrigated		0.747	0.00419	0.372	0.435
	LSD 0.05		0.012	0.00005	0.009	0.018
		0 kg N ha^{-1}	0.738	0.00426	0.380	0.442
		56 kg N ha^{-1}	0.732	0.00434	0.379	0.449
		112 kg N ha^{-1}	0.721	0.00442	0.376	0.462
		LSD 0.05	0.005	0.00007	0.003	0.008

[z] Percentage of the total fatty acid fraction.

[y] ns = not significantly different at the $P \leq 0.05$ level.

The increased seed protein concentration produced at the highest rate of N fertilization is comparable to the increased seed N concentrations seen in response to fertilization as reported by Sawan et al. (1988), Hunt et al. (1998) and Main et al. (2013). This response of seed protein to fertilization was expected because N is a component of the peptide bond that forms the backbone of all proteins. In contrast to the lack of an irrigation effect on seed size, N fertilization increased both seed mass and yield in most instances (Pettigrew and Zeng, 2014). Sawan et al. (1988) and Main et al. (2013) also reported similar N fertilization effects on seed mass and seed yield. The decreased oil content caused by N fertilization is similar to that reported by Sawan et al. (1988) and Main et al. (2013). One explanation for the decreased seed gossypol, crude oil, and total soluble carbohydrate levels is that these components may have been diluted in the seed from the highest N fertility rate because of the larger seed size and yield found in the higher N rate plants compared with the seed size and yield of the non-fertilized plants. The effect of N fertilization on the fatty acid distribution within the oil fraction is not as easily explained by a dilution effect. Although seed total crude oil levels were decreased by applying N fertilization, the distribution of fatty acids in that oil was shifted away from linoleic acid production and toward more oleic acid production. Although the fatty acid changes due to agronomic practices are too small to be important commercially, they are in the direction that would produce oil that would have higher oxidative stability in fryers and would require less hydrogen during hydrogenation processes while maintaining the health benefits of high unsaturated fatty acid levels (Lukonge et al., 2007).

Producers will always be predominately interested the amount and quality of the lint because that provides the majority of their income. However, the choice of production practices impact not only lint production but also alters seed composition. Irrigation increased both seed oil concentration (the most valuable seed component) and gossypol levels (a negative seed trait that affects utilization of the defatted meal). Although N fertilization decreased crude oil concentration in individual seed, the amount of oil produced per unit land area is greater than produced without fertilization due to the increased yields associated with fertilization (Pettigrew and Zeng, 2014). Sawan et al. (1988) also reported increased oil yields with N fertilization. Furthermore, the shift of fatty acid profiles away from linoleic acid by the N fertilization could contribute, albeit modestly, to the need for less post harvest processing of the oil.

In general, choosing production practices for optimal yield performance also increases the quality of the seed. The most prominent exception is that irrigation significantly increases seed gossypol levels. In addition, more of the gossypol is in the (-) isomeric form, which is considered to be a more toxic isomer. In contrast, fertilization reduces gossypol levels potentially increasing the value of the meal. Although genetics still accounts for the majority of variation in cottonseed composition, environmental influences and production practices, such as irrigation and fertilization, also impact seed composition.

REFERENCES

AOCS. 1998. Various methods. *In* D. Firestone (ed.) Official Methods and Recommended Practices of the American Oil Chemists' Society. AOCS Press, Champaign, IL.

Arieli, A. 1998. Whole cottonseed in dairy cattle feeding: A review. Anim. Feed Sci. Technol. 72:97-110. doi:10.1016/S0377-8401(97)00169-7.

Cherry, J.P. 1983. Cottonseed oil. J. Am. Oil Chem. Soc. 60:362-367.

Cherry J.P., and H.R. Leffler. 1984. Seed p. 511-569. *In* R.J. Kohel and C.F. Lewis (eds.) Cotton. ASA, CSSA, and SSSA, Madison, WI.

Dowd, M.K., D.L. Boykin, W.R. Meredith, Jr., B.T. Campbell, F.M. Bourland, J.R. Gannaway, K.M. Glass, and J.Zhang. 2010. Fatty acid profiles of cottonseed genotypes from the National Cotton Variety Trials. J. Cotton Sci. 14:64-73.

Dowd, M.K., and P.J. Wakelyn. 2010. Cottonseed current and future utilization. p. 437-460. *In* P.J. Wakelyn and R. Chaudhry (eds.) Cotton: Technology for the 21st Century. ICAC Press, Washington, DC.

He, Z., M. Shankle, H. Zhang, T.R. Way, H. Tewolde, and M. Uchimiya. 2013. Mineral composition of cottonseed is affected by fertilization management practices. Agron. J. 105:341-350.

Hunt, P.G., P.J. Bauer, C.R. Camp, and T.A. Matheyn. 1998. Nitrogen accumulation in cotton grown continuously or in rotation with peanut using subsurface microirrigation and GOSSYM/COMAX management. Crop Sci. 38:410-415.

Kohel, R.J., and J.P. Cherry. 1983. Variation of cottonseed quality with stratified harvests. Crop Sci. 23:1119-1124.

Lawhon, J.T., C.M. Cater, and K.F. Mattil. 1977. Evaluation of the food potential of sixteen varieties of cottonseed. J. Am. Oil Chem. Soc. 54:75-80.

Lukonge, E., M.T. Labuschagne, and A. Hugo. 2007. The evaluation of oil and fatty acid composition in seed of cotton accessions from various countries. J. Sci. Food Agric. 87:340-347.

Main, C.L., L.T. Barber, R.K. Boman, K. Chapma, D.M. Dodds, S. Duncan, K.L. Edmisten, P. Horn, M.A. Jones, G.D. Morgan, E.R. Norton, S. Osborne, J.R. Whitaker, R.L. Nichols, and K.F. Bronson. 2013. Effects of nitrogen and planting seed size on cotton growth, development, and yield. Agron. J. 105:1853-1859.

O'Brien, R.D., and P.J. Wakelyn. 2005. Cottonseed oil: An oil for *trans*-free options. INFORM 16:677-679.

Pandey, S.N., and N. Thejappa. 1975. Study on relationaship between oil, protein, and gossypol in cottonseed kernels. J. Am. Oil Chem. Soc. 52:312-315.

Pettigrew, W.T., and M.K. Dowd. 2011. Varying planting date or irrigation regimes alters cottonseed composition. Crop Sci. 51:2155-2164.

Pettigrew, W.T., and M.K. Dowd. 2012. Interactions between irrigation regimes and varieties result in altered cottonseed composition. J. Cotton Sci. 16:42-52.

Pettigrew, W.T., and L. Zeng. 2014. Interactions among irrigation and nitrogen fertility regimes on Mid-South cotton production. Agron. J. 106:1614-1622.

Pons, W.A., Jr., C.L. Hoffpauir, and T.H. Hopper. 1953. Gossypol in cottonseed: influence of variety of cottonseed and environment. J. Agric. Food Chem. 1:1115-1118.

SAS Institute. 1996. SAS systems for mixed models. SAS Inst. Cary, NC.

Sawan, Z.M, A.A. El-Farra, and S.A. El-Latif. 1988. Cottonseed, protein and oil yields, and oil properties as affected by nitrogen and phosphorus fertilization and growth regulators. J. Agron. and Crop Sci. 161:50-56.

Stansbury, M.F., C.L. Hoffpauir, and T.H. Hopper. 1953. Influence of variety and environment on the iodine value of cottonseed oil. J. Am. Oil Chem. Soc. 30:120-123.

Stansbury, M.F., A.F. Cucullu, and G.T. Den Hartog. 1954. Cottonseed contents variation: Influence of variety and environment on oil content of cottonseed kernels. J. Agric. Food Chem. 2:692-696.

Tharp W.H. 1948. Cottonseed composition—relation to variety, maturity, and environment of the plant. P. 117-156. *In* A.E. Bailey (ed.) Cottonseed and Cottonseed Products. Interscience, New York, NY.

Turner, J.H., H.H. Ramey, Jr., and S. Worley, Jr. 1976. Influence of environment on seed quality of four cotton cultivars. Crop Sci. 16:407-409.

USDA, National Cotton Variety Trials. 2012. Available at http://www.ars.usda.gov/main/docs.htm?docid=23813. (verified 18 November 2014)