

Varying Planting Dates or Irrigation Regimes Alters Cottonseed Composition

William T. Pettigrew^{*} and Michael K. Dowd

ABSTRACT

Despite continued utilization of cottonseed (*Gossypium hirsutum* L.), little information exists regarding the effect of production factors on cottonseed composition. The objective of this study was to determine how irrigation regime and planting date affect cottonseed composition. Six cotton cultivars were planted early (late April) and at a normal time (late May) in a field near Stoneville, MS, from 2005 to 2008. Half of the plots were irrigated and half were cultivated dry. Seed from the 2006 through 2008 seasons were analyzed for protein, crude oil, gossypol, soluble carbohydrates, and the oil's fatty acid composition. Irrigation increased oil and total soluble carbohydrate levels by 7 and 4%, respectively, and reduced protein levels by 10%. Irrigation also increased the level of total gossypol by 21%, modestly decreased the percentage of the gossypol (+) isomer, and slightly decreased the level of saturated fatty acids in the oil. Early planting decreased gossypol in the seed kernel by 8% under dryland conditions but not under irrigated conditions. The early planting effect on the fatty acid distribution was similar to the effect observed under dryland conditions, but the differences were more modest. Early planting and irrigation offer potential for improved fiber and seed yield but with altered seed composition. Given the proper economic incentives, producers could alter some production strategies to produce seed with more valuable composition traits.

W.T. Pettigrew, USDA-ARS, Crop Production Systems Research Unit, P.O. Box 350, Stoneville, MS 38776; M.K. Dowd, USDA-ARS SRRC, Commodity Utilization Research Unit, New Orleans, LA 70124. Trade names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product or service, and the use of the name by USDA implies no approval of the product or service to the exclusion of others that may also be suitable. Received 17 Feb. 2011. ^{*}Corresponding author (bill.pettigrew@ars.usda.gov).

Abbreviations: AOCS, American Oil Chemists' Society; FID, flame ionization detector; HPLC, high performance liquid chromatography; LDR, linoleic acid desaturation ratio; ODR, oleic acid desaturation ratio; PE, petroleum ether.

COTTON (*Gossypium hirsutum* L.) is grown primarily for its fiber, but there is also inherent value in the seed due to its high levels of protein and oil. The processing of cottonseed yields four products: linters, hulls, oil, and meal (i.e., protein) (Cherry and Leffler, 1982). While the former two products have commercial uses, it is the oil and protein that account for most of the value of the seed. Whole cottonseed and cottonseed meal are widely used as a protein ingredient in ruminant diets. Whole seed is particularly valued by the dairy industry, which has increased its use in recent years (Arieli, 1998). This is due in part to the seed's protein component and in part to the oil component, the latter of which is associated with increased butterfat levels in milk (Smith et al., 1981). Gossypol (a polyphenolic terpene) is an anti-nutritive component of the seed that limits the amount of seed or meal that can be fed to ruminant animals and completely prevents the feeding of cottonseed products to nonruminant animals

Published in Crop Sci. 51:2155–2164 (2011).

doi: 10.2135/cropsci2011.02.0085

Published online 6 July 2011.

© Crop Science Society of America | 5585 Guilford Rd., Madison, WI 53711 USA

All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Permission for printing and for reprinting the material contained herein has been obtained by the publisher.

(Bernardi and Goldblatt, 1980). Oil extracted from cottonseed is an important ingredient for the food processing and restaurant industries (O'Brien and Wakelyn, 2005). It is valued for the functionality it contributes to processed foods and for the nutty aroma that it imparts to food when it is used as a frying oil. The oil has enough saturated fatty acids (~30%) to allow it to be relatively stable frying oil that reduces the need for hydrogenation (a process that produces undesirable trans-fatty acids) and enough unsaturated fatty acids (~70%) to provide health benefits (O'Brien and Wakelyn, 2005). Nevertheless, a redistribution of the oil's fatty acids away from linoleic acid, the predominant unsaturated fatty acid, and toward oleic acid would yield an oil requiring even less processing while maintaining the benefits of high levels of unsaturated fatty acids (Lukonge et al., 2007). Understanding the factors that influence cottonseed composition, both genetic and environmental, could lead to more useful products.

It has been demonstrated that both the oil and protein components of cottonseed exhibit genetic variation (Cherry et al., 1986). It is also known that seed gossypol levels can vary dramatically among *G. hirsutum* cultivars (Pons et al., 1953; Stansbury et al., 1956; Stipanovic et al., 2005; Romano and Scheffler, 2008), and surveys of seed fatty acid composition indicate that some genetic variability exists among cotton genotypes and cultivars (Yunusova et al., 1991; Lukonge et al., 2007; Dowd et al., 2010). Sufficient variation appears to exist for some traits to allow breeding for altered or improved seed composition. Nevertheless, the principal goal of cotton breeding has historically been the improvement of fiber yield and lint quality, with the seed composition garnering little attention.

Environmental influences on cottonseed composition have not been studied in any significant detail. This reality most likely exists due to the fact that genetics accounts for more than half of the variation in many seed composition traits, with environment and environment \times variety interactions accounting for less than half (Dowd et al., 2010). The few existing studies that address the environment generally involve variety trials across multiple years or locations where the location or environment component of variation had been partitioned out (Turner et al., 1976; Cherry and Leffler, 1982; and Cherry et al., 1986, and references therein). Correlations have been noted for gossypol, protein, oil, and the oil's iodine value (an indication of unsaturated fatty acids) with precipitation and temperature patterns from data recorded across several locations and years (Pons et al., 1953; Stansbury et al., 1953, 1956). Data from recent variety trials hint at associations between cottonseed fatty acid composition and weather conditions (Dowd et al., 2010). While these studies suggest that environment affects seed composition and properties, it is impossible to assign direct "cause and effect" from these experiments, as the trials

were not designed to test for environmental effects and the associations are confounded by any other differences that might exist among the diverse environments used in these studies. In the single study designed to test for differences in seed composition with field conditions, Hunt et al. (1998) demonstrated that cottonseed N content is altered by varying irrigation and N fertilization regimes.

Alteration or optimization of cottonseed composition could enhance the value of the seed and possibly expand the use of cottonseed products. Although genetic variation in seed traits offers potential and promise for future compositional improvements, a void remains in understanding how environmental factors alter seed composition. Hence, the objective of this study was to determine how two controllable factors, that is, planting date (early and normal) and irrigation regime (irrigated and dryland), affect seed composition for a diverse group of cotton varieties.

MATERIALS AND METHODS

Field studies were conducted on a Dubbs silt loam (fine-silty, mixed, active, thermic Typic Hapludalfs) near Stoneville, MS, during the years 2005 through 2008. Six cotton cultivars were grown in this study ('DP 445BR', 'DP 555BR', 'FM 800BR', 'FM 960BR', 'ST 4892BR', and 'ST 5599BR'). Delta and Pine Land Co., Scott, MS, provided the DP 445BR and DP 555BR seed, while Bayer CropScience, Research Triangle Park, NC, provided the FM 800BR, FM 960BR, ST 4892BR, and ST 5599BR seed. Each year, half the plots were planted around the beginning of April (early) and the other half were planted around the beginning of May (normal). Early planting dates were 4 Apr. 2005, 30 Mar. 2006, 2 Apr. 2007, and 31 Mar. 2008. Normal planting dates were 2 May 2005, 2 May 2006, 27 Apr. 2007, and 6 May 2008. In addition to planting date, two different irrigation regimes were considered. Half the experimental area was furrow irrigated (irrigated) and the other half of the area was left nonirrigated (dryland). One irrigation treatment was applied in 2005 (seed was not collected for this year), three were applied in 2006, two were applied in 2007, and four were applied in 2008, with approximately 2.54 cm of water being applied during each irrigation event. All other cultural practices were as described earlier (Pettigrew, 2010).

Individual plots consisted of four rows spaced 1-m apart and 18.3-m long. These were planted with a seeding rate that resulted in a final plant density of approximately 97,000 plants ha⁻¹. The overall experimental design was a randomized complete block with a modified split-split treatment arrangement. Irrigation regimes were the main plots, planting dates were the split plots, and cultivars were the split-split plots. The irrigation regimes were replicated in three blocks. Within each block, there were two replications of planting date for each block \times irrigation combination. Cultivars were randomly assigned within each irrigation \times block \times planting date combination. All treatments and cultivars were randomly assigned the first year of the study and remained in their initial location for subsequent years.

After defoliation but before mechanical harvest, a 50-boll sample was hand harvested from one of the two inner plot rows of each plot and was then ginned on a 10-saw laboratory gin.

The percentage of lint was calculated and other yield components were determined from these 50-boll samples (Pettigrew, 2010). For the final 3 yr of the study (2006–2008), seeds recovered from the ginning process were saved and used for compositional analysis.

Seed Preparation

To separate the hulls, whole seed from each sample was cracked in a Model 7011 1-L Waring blender (New Hartford, CT). Ten-to-fifteen gram portions were blended for several seconds and then the partially cracked seed was sifted through a series of #4 (4.75 mm opening) and #12 sieves (1.70 mm opening) with a bottom pan. Material retained on the #4 sieve was re-run through the blending and sieving operation, progressively increasing the speed and duration of the blender. The dehulled kernels and larger kernel pieces were collected from the surface of the #12 sieve. The procedure yielded ~25 g of dehulled kernels from ~75 g of whole seed. Recovered kernels were ground with a Braun food chopper (The Gillette Co., Woburn, MA) to pass a #20 mesh sieve (0.85 mm opening). The ground kernels were then freeze-dried and stored in the dark at -20°C until used. All analyses were made on dehulled dry ground kernels in duplicate.

Crude Oil

Extractable oil was determined with a Soxtec extractor (Foss North America, Eden Prairie, MN). Approximately 3 g of ground seed was weighed into a cellulose thimble. The thimble was fitted into the extractor with 40 mL of petroleum ether (PE) (CAS #8032-32-4, Mallinckrodt Baker, Inc., Philipburg, NJ), and the sample was boiled for 15 min. The thimble was then raised and PE was refluxed through the thimbles for 2 h to extract the oil. Petroleum ether was stripped from the oil by diverting the reflux condensate away from the sample cup for a period of 20 min. The mostly solvent-free sample was then dried in an oven for 30 min at 130°C before being transferred to a dessicator to cool to room temperature. Samples were weighed to determine the amount of crude oil.

Fatty Acid Distribution

Approximately 100 mg of ground seed sample was added to a 2-mL microcentrifuge tube with 1.5 mL hexane (CAS #110-54-3, Mallinckrodt Baker, Inc.), and the contents were shaken on a Vibrex shaker (IKA Works, Inc., Wilmington, NC) overnight. Samples were then centrifuged, and the miscella (crude oil in hexane) was poured into a 10-mL screw-cap test tube. To recover the oil in the hold-up volume, an additional 1.5 mL of hexane was added to the tube, and the tube was vortex mixed to break up the pellet and was recentrifuged. The recovered hexane wash was added to the miscella.

To convert glycerides to fatty acid methyl esters, 200 µL of 0.5 N methanolic base (Supelco Analytical, Sigma-Aldrich Co., Bellefonte, PA) were added to the tube with combined miscella and hexane wash, and the tube was capped and heated at 70°C for 10 min with periodic mixing. After cooling, 1 mL of hexane and 1 mL of brine (salt-saturated water) were added and the tube was vortex mixed again. Upon standing, the solution separated into organic and aqueous phases. The organic phase was transferred into a second tube and mixed with ~20 mg of

anhydrous magnesium sulfate. After allowing the drying agent to settle, the organic phase was then transferred to a sample vial for chromatography.

A Model 7890A Agilent Technologies gas chromatograph (Agilent Technologies, Santa Clara, CA) was fitted with a split injector, a flame-ionization detector (FID), and a Supelco SP-2380 capillary gas chromatography column (0.25 mm i.d. by 30 m by 0.20 µm film thickness). Injector and detector temperatures were set at 250°C. The oven was programmed to start at 170°C, which was held for 3 min, then increased at 1°C min⁻¹ to 180°C, and then increased at 4°C min⁻¹ to a final temperature of 240°C, which was held for 10 min. Helium was used as the carrier gas at a constant linear flow rate of 20 cm s⁻¹. The inlet was operated at a split ratio of 1:100 and a 1-µL injection volume was used. Eluted fatty acid methyl esters were identified by comparing peak elution times to those of known standards. Fatty acid distributions were determined from the integrated peak areas after correction for small FID response factor differences as recommended in the American Oil Chemists' Society (AOCS) Official Method Ce 1e-91 (AOCS, 1998).

Ratios of various fatty acids provide insight into the complex triacylglycerol biosynthesis pathway (Lukonge et al., 2007). The ratio of 16 carbon-atom fatty acids to 18 carbon-atom fatty acids (C16:C18) is thought to be an estimate of the activity of β-ketoacyl-ACP synthase II and was calculated as:

$$\text{C16:C18} = (\text{palmitic} + \text{palmitoleic acids}) / (\text{stearic} + \text{oleic} + \text{linoleic} + \text{linolenic acids}).$$

Oleic acid desaturation ratio (ODR), which relates to the activity of fatty acid desaturase II, was calculated as:

$$\text{ODR} = (\text{linoleic} + \text{linolenic acids}) / (\text{oleic} + \text{linoleic} + \text{linolenic acids}).$$

Linoleic acid desaturation ratio (LDR), relating to the effectiveness of the fatty acid desaturase III, was calculated as:

$$\text{LDR} = \text{linolenic acid} / (\text{linoleic} + \text{linolenic}).$$

Longer fatty acids with greater than 18 carbon atoms (C20–C24) represent the relative proportion of fatty acids not undergoing desaturation were calculated as the sum of arachidic, behenic, and lignoceric acids.

Gossypol

The gossypol enantiomers were detected by a slightly modified procedure based on AOCS Recommended Practice Ba 8a-99 (AOCS, 1998). Briefly, 100 mg of ground seed was weighed into a 12-mL screw-cap test tube. Two milliliters of a complexing reagent, consisting of 2/10/88 (v/v/v) *R*-(-)-2-amino-1-propanol (CAS #35320-23-1), glacial acetic acid (CAS #64-19-7), and dimethylformamide (CAS #68-12-2), were added, and the tube was heated at 95 to 100°C for 30 min to convert gossypol's aldehyde groups into Schiff's bases with the chiral amine. After allowing the solution to cool, 8 mL of high performance liquid chromatography (HPLC) mobile phase (described below) was added. This solution was vortex mixed then centrifuged to pellet the ground tissue and any suspended particles. An aliquot of the solution was then transferred into a HPLC vial for analysis.

The gossypol Schiff's bases were separated on a Hewlett-Packard Series 1100 HPLC system (Hewlett-Packard, Palo Alto, CA) fitted with a SGE cartridge column (SGE, Austin, TX) (4.0 mm i.d. by 100 mm) containing an Inertsil ODS-2 (5 μ m diam.) stationary phase. A photodiode array detector was used to detect and quantify the compounds. The mobile phase consisted of 78:22 (v:v) acetonitrile:phosphate buffer (10 mM, pH = 3.0) and was pumped at 1 mL min⁻¹. The gossypol complex was detected at 254 nm. A standard curve was constructed for each isomer with serial dilutions of racemic gossypol-acetic acid (1:1) in complexing reagent. Total gossypol was calculated from the sum of the individual (+)- and (-)-gossypol isomers. Percent (+) gossypol represents the amount of the (+) isomer divided by the sum of the (+) and (-) isomers, expressed as a percentage.

Nitrogen and Protein

Nitrogen was determined by combustion with a LECO Model FP-528 N analyzer (LECO Corp., St. Joseph, MI). Approximately 150 mg of each sample was weighed into a tin foil, which was then analyzed on the instrument. Nitrogen was converted to protein by multiplying the N content by 6.0, which is the conversion factor estimated for cottonseed proteins from reported amino acid distributions (Dowd and Wakelyn, 2010).

Soluble Carbohydrates

Approximately 50 mg of ground seed sample was weighed into a 5-mL Reacti-Vial (Pierce Biotechnology, Inc., Thermo-Fisher Scientific, Rockford, IL). An internal standard solution of methyl- β -D-glucopyranoside (CAS #709-50-2, Sigma-Aldrich Co.) was prepared in pyridine (CAS #110-86-1, Sigma-Aldrich Co.), and 250 μ L of this solution was weighed in to the sample. Then 750 μ L of additional pyridine, 1 mL of hexamethyldisilazane (CAS #999-97-3, Pierce Biotechnology, Inc.), and 100 μ L of trifluoroacetic acid (CAS #76-05-1, Pierce Biotechnology, Inc.) were added. The tube was capped and heated at 70°C for 45 min with occasional vortex mixing to convert the sugar hydroxyl groups to trimethylsilyl ethers. Upon cooling, approximately 1 mL of the supernatant was collected for chromatography.

To detect the sugars, the Agilent 7890A gas chromatograph was fitted with a J&W DB-5 (0.25 mm i.d. by 15 m by 0.1 μ L film thickness) capillary column (Agilent Technologies). The injector and detector temperatures were set at 360°C. Helium was used as the carrier gas at a linear velocity of \sim 30 cm s⁻¹. The injector was operated in split mode at a 1:100 split ratio and the injected sample volume was 2 μ L. The column oven was programmed to start at 170°C, which was held for 3 min, and then ramped at 10°C min⁻¹ to 360°C, which was held for 15 min. Peaks for the trimethylsilylated derivatives of sucrose, raffinose, and stachyose were identified by comparing elution times with silylated standards. Relative response factors were determined for each compound, and internal standardization was used to calculate the concentration of each component.

Statistical Analyses

Statistical analyses were performed by analysis of variance (PROC MIXED0; SAS Institute, 1996). Because all irrigation, planting date, and cultivar treatments remained in their original location each year of the study, years were treated as a repeated measurement when conducting a combined analysis across years. Random effects used in this model for the comparison across years were block \times water; replication \times water(block); block \times replication \times planting(water); block \times replication \times cultivar(water \times planting); and year \times block \times replication. Irrigation, planting date, and cultivar means were averaged across years and each other when statistically important interactions were not detected. Means were separated by use of a protected LSD at $p \leq 0.05$.

RESULTS

Dry matter partitioning, lint yield, yield components, and fiber quality data from these plots were published earlier (Pettigrew, 2010). Because that information is pertinent to the current research, those results will be reviewed here as they will help with the analysis of the seed results. Irrigation increased lint yield during three of the 4 yr (2006–2008). During 2 of the 4 yr (2006–2007), early planting increased lint yield by 13% when irrigation was applied, but it never increased lint yield under dryland conditions. Early planting actually decreased lint yield under dryland conditions for one of the 4 yr (2008) but not under irrigated conditions for that year. For two of the 4 yr (2006 and 2008), irrigation increased seed mass by 6% over that produced under dryland conditions.

Local weather data for the 3 yr that seed were collected represent three distinct growing environments (Table 1). The weather during the 2006 growing season was relatively typical for the Mississippi Delta. Rainfall during July in 2007 was unusually high compared with the rainfall for this month in the other years of the study. In contrast, June and July of 2008 were quite hot and dry, followed by an extraordinarily amount of precipitation during September of 2008 because of Hurricane Gustav. Years significantly interacted with planting date and irrigation regime for most of the traits due to the diverse growing environments prevailing during these years (Table 2). However, *F*-values for the yearly interactions were small relative to the main effects; consequently, the irrigation regime means, planting date means, and the irrigation \times planting date interaction means were averaged across years. After years, variety produced the second largest *F*-value relative to the other sources of variation. However, because variety differences in most seed composition traits has been previously well established via numerous earlier publications (Lukonge et al., 2007; Dowd et al., 2010; USDA, 2009), we did not dwell on the variety differences in this report. In addition, because the interactions involving varieties were generally small relative to

that of the main effects, the planting date and irrigation means were averaged across varieties.

Total seed gossypol concentration was significantly altered by both planting date and irrigation regime (Table 3). Seed from the irrigated plots had total gossypol concentrations 21% higher than did seed from the nonirrigated plots. Seed from the normal planting date also had 3% greater total gossypol concentration than did seed from the early planting date. The planting date response, however, was only significant under dryland and not irrigated conditions, leading to a significant planting date × irrigation interaction. The percentage of total seed gossypol in the (+) isomeric form was reduced from 62.1% for seed grown under dryland conditions to 59.7% for seed grown with irrigation. Although there was no planting date effect on % (+) gossypol, there was a significant interaction between planting date and irrigation. The proportion of (+) gossypol increased with early planting under dryland conditions, but it tended to decrease with early planting under irrigation.

Seed crude oil and protein levels were also impacted by irrigation regimes and planting dates (Table 3). Irrigation increased oil concentration by 7% but decreased protein concentration by 10% relative to dryland conditions. Early planting resulted in a modest 2% increase in the oil concentration, but protein levels were not impacted by the planting dates. The significant interaction between

Table 1. Monthly weather summary for 2006 to 2008 at Stoneville, MS.†

Month	2006	2007	2008
<u>Precipitation (cm)</u>			
Apr.	18.7	8.6	20.3
May	7.3	3.2	17.5
June	4.6	9.9	1.1
July	4.5	19.7	4.2
Aug.	4.0	8.7	15.3
Sept.	6.9	11.8	30.9
Oct.	21.9	10.7	4.8
<u>Thermal units‡</u>			
Apr.	174	85	89
May	239	253	211
June	337	346	348
July	392	342	400
Aug.	423	446	338
Sept.	229	296	245
Oct.	113	153	103
<u>Solar radiation (MJ m⁻²)</u>			
Apr.	592	615	550
May	687	698	668
June	760	718	731
July	720	634	781
Aug.	682	705	550
Sept.	596	516	485
Oct.	464	441	478

†All observations made by the NOAA Midsouth Agricultural Weather Service Center and the Delta Research and Extension Center Weather, both in Stoneville, MS.

‡[(Max. temperature + Min. temperature)/2] – 15.

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

Source of variation†	df	Total gossypol	Percent (+) gossypol	Crude oil	Protein	Total soluble carbohydrates	Saturated fatty acids	Unsaturated fatty acids
Block	2	1.21 (0.37)‡	0.52 (0.66)	2.89 (0.26)	2.90 (0.26)	5.66 (0.01)	1.83 (0.25)	0.22 (0.81)
Replication(block)	3	0.09 (0.96)	1.32 (0.27)	2.16 (0.14)	0.26 (0.85)	2.40 (0.11)	0.34 (0.80)	0.92 (0.50)
Water	1	112.72 (0.01)	183.93 (0.01)	27.75 (0.03)	74.19 (0.01)	56.31 (0.01)	74.55 (0.01)	75.88 (0.01)
Planting	1	7.49 (0.02)	0.69 (0.41)	20.16 (0.01)	2.01 (0.16)	55.49 (0.01)	5.62 (0.04)	3.91 (0.05)
Water × planting	1	18.32 (0.01)	20.70 (0.01)	20.76 (0.01)	15.53 (0.01)	17.00 (0.01)	0.01 (0.92)	1.23 (0.27)
Variety	5	280.68 (0.01)	553.14 (0.01)	139.55 (0.01)	103.36 (0.01)	66.61 (0.01)	945.00 (0.01)	937.85 (0.01)
Water × variety	5	7.66 (0.01)	4.04 (0.01)	1.04 (0.40)	0.39 (0.86)	2.10 (0.07)	4.26 (0.01)	4.66 (0.01)
Planting × variety	5	2.49 (0.04)	4.53 (0.01)	1.51 (0.19)	1.36 (0.24)	2.77 (0.02)	0.64 (0.67)	0.74 (0.59)
Water × planting × variety	5	1.22 (0.31)	1.37 (0.24)	0.43 (0.82)	0.57 (0.73)	0.89 (0.49)	2.02 (0.08)	2.09 (0.07)
Year	2	495.10 (0.01)	219.71 (0.01)	237.08 (0.01)	188.32 (0.01)	1338.89 (0.01)	1449.00 (0.01)	791.65 (0.01)
Year × water	2	20.41 (0.01)	3.75 (0.03)	13.83 (0.01)	41.29 (0.01)	69.72 (0.01)	1.65 (0.19)	4.56 (0.01)
Year × planting	2	43.72 (0.01)	59.11 (0.01)	1.75 (0.18)	11.90 (0.01)	92.17 (0.01)	6.28 (0.01)	3.01 (0.05)
Year × water × planting	2	10.72 (0.01)	29.40 (0.01)	0.85 (0.43)	0.91 (0.40)	8.23 (0.01)	3.22 (0.04)	0.90 (0.41)
Year × variety	10	8.10 (0.01)	8.57 (0.01)	2.03 (0.03)	2.03 (0.03)	9.85 (0.01)	5.06 (0.01)	6.02 (0.01)
Year × water × variety	10	2.57 (0.01)	5.07 (0.01)	1.97 (0.04)	1.37 (0.20)	0.76 (0.67)	1.46 (0.15)	1.28 (0.24)
Year × planting × variety	10	2.62 (0.01)	1.40 (0.18)	1.74 (0.07)	1.27 (0.25)	0.68 (0.74)	1.83 (0.06)	1.66 (0.09)
Year × water × planting × variety	10	0.89 (0.54)	0.86 (0.57)	1.46 (0.16)	0.65 (0.77)	0.47 (0.91)	0.76 (0.67)	0.71 (0.72)

†Random effects used in this model were block × water; replication × water(block); block × replication × planting(water); block × replication × variety(water × planting); year × block × replication. Nested effects denoted with parentheses (i.e., replication(block) denotes replication within block).

‡Values with parentheses represent $p > F$. Values less than 0.01 were rounded up.

Table 3. Effect of varying planting date and irrigation regimes on seed gossypol, crude oil, and protein concentrations.

Planting date	Irrigation regime	Percent (+)		Crude oil	Protein
		Total gossypol	gossypol		
		g kg ⁻¹	% [†]	g kg ⁻¹	
Early		11.6	61.0	330	400
Normal		12.0	60.9	323	403
LSD 0.05		0.3	0.3 NS [‡]	4	3 NS
	Dryland	10.7	62.1	316	422
	Irrigated	12.9	59.7	338	380
	LSD 0.05	0.5	0.8	18	20
Early	Dryland	10.2	62.5	316	424
	Irrigated	13.0	59.5	345	376
Normal	Dryland	11.1	61.7	316	420
	Irrigated	12.8	60.0	330	386
LSD 0.05 [§]		0.4	0.4	5	6
	LSD 0.05 [¶]	0.6	0.6	19	23

[†]Percentage of the total gossypol.

[‡]NS, not significantly different at the $p \leq 0.05$.

[§]LSD for comparing planting dates within irrigation regimes.

[¶]LSD for comparing irrigation regimes within planting dates.

planting date and irrigation for crude oil was due to two factors: (i) the planting date response was only manifested under irrigated conditions and not under dryland conditions and (ii) the irrigation response was only significant under early planted conditions and not under normal planting. The planting date main effect did not affect the protein concentration but there was a significant planting date \times irrigation interaction. Under irrigated conditions, early planting decreased protein concentration, but under dryland conditions, early planting did not affect protein concentration.

Irrigation increased the amount of soluble carbohydrates in the seed by 4% but early planting reduced the soluble carbohydrate concentration by 4% (Table 4). The planting date response was stronger for seed produced under dryland conditions than it was for seed grown with irrigation. Compared with the level of total sugar, individual sugars responded differently to the planting date and irrigation treatments. Early planting decreased the concentration of raffinose, the largest component among the soluble carbohydrates, by 9% compared with normal planting. In contrast, early planting increased sucrose concentration by 18%. Irrigation increased the concentration of raffinose by 9% but had no effect on the concentration of sucrose. A strong planting date \times irrigation interaction was detected for stachyose. Under dryland conditions, early planting decreased stachyose concentration, but under irrigated conditions stachyose concentration was increased by early planting. Furthermore, with normal planting, irrigation decreased the stachyose level compared with dryland conditions, but with early planting, irrigation had no significant affect on this sugar.

Table 4. Effect of varying planting date and irrigation regimes on various seed carbohydrate concentrations.

Planting date	Irrigation regime	Total soluble carbohydrates			
		Sucrose	Raffinose	Stachyose	
		g kg ⁻¹			
Early		63.9	11.0	43.2	9.7
Normal		66.7	9.3	47.7	9.7
LSD 0.05		0.8	0.2	0.8	0.1 NS [†]
	Dryland	63.9	10.5	43.4	10.0
	Irrigated	66.7	9.8	47.5	9.4
	LSD 0.05	0.8	0.9 NS	0.8	0.1
Early	Dryland	61.7	11.5	40.4	9.8
	Irrigated	66.1	10.5	46.0	9.6
Normal	Dryland	66.1	9.6	46.3	10.2
	Irrigated	67.3	9.0	49.1	9.2
LSD 0.05 [‡]		1.1	0.3	1.2	0.2
	LSD 0.05 [§]	1.1	0.8	1.2	0.2

[†]NS, not significantly different at the $p \leq 0.05$.

[‡]LSD for comparing planting dates within irrigation regimes.

[§]LSD for comparing irrigation regimes within planting dates.

The distribution of the seed oil's fatty acids was also impacted by planting date and irrigation treatment. Total saturated fatty acids increased slightly but significantly when the cotton was planted early and when the cotton was grown dryland (Table 5). Individually, only stearic, arachidic, and lignoceric acid levels were increased by early planting; the other saturated fatty acids were statistically unaffected by planting date. Irrigation decreased the level of all saturated fatty acids except for stearic acid, which was statistically unaffected. Although some significant interactions between planting date and irrigation were detected for the saturated fatty acids, there were no significant reversals in the trends exhibited by the overall treatment means for either planting date or irrigation regime. For instance, myristic, arachidic, behenic, and lignoceric acids all exhibited slight increases caused by early planting under dryland conditions. Under irrigated conditions, however, there were no statistical differences in the levels of these acids between planting dates.

The oil's level of unsaturated fatty acids was slightly increased by irrigation but was unaffected by planting date (Table 6). Within these main effect responses, individual unsaturated fatty acids behaved differently in response to irrigation or planting date. Although irrigation increased the level of total unsaturated fatty acids, the only individual unsaturated fatty acid that it increased was linoleic acid, which happens to be the most prevalent unsaturated fatty acid in the seed. The remaining unsaturated fatty acids, that is, oleic, palmitoleic, *cis*-vaccenic, and α -linolenic acids, had their levels reduced by irrigation. Early planting slightly decreased the level of linoleic acid but increased the levels of the other unsaturated fatty acids. Although interactions between planting date and irrigation were

Table 5. Effect of varying planting date and irrigation regimes on various seed saturated fatty acid distributions.

Planting date	Irrigation regime	Saturated fatty acids	Myristic acid, 14:0	Palmitic acid, 16:0	Stearic acid, 18:0	Arachidic acid, 20:0	Behenic acid, 22:0	Lignoceric acid, 24:0
		%†						
Early		29.1	0.830	25.1	2.60	0.299	0.138	0.112
Normal		29.0	0.823	25.0	2.56	0.295	0.136	0.109
LSD 0.05		0.1	0.013 NS‡	0.1 NS	0.02	0.003	0.002 NS	0.003
	Dryland	29.4	0.887	25.3	2.60	0.308	0.144	0.117
	Irrigated	28.7	0.767	24.8	2.56	0.285	0.130	0.104
	LSD 0.05	0.2	0.022	0.2	0.07 NS	0.005	0.004	0.003
Early	Dryland	29.5	0.897	25.3	2.62	0.313	0.147	0.120
	Irrigated	28.7	0.763	24.8	2.58	0.284	0.129	0.105
Normal	Dryland	29.3	0.876	25.2	2.58	0.303	0.142	0.115
	Irrigated	28.6	0.771	24.7	2.54	0.287	0.130	0.102
LSD 0.05§		0.2 NS	0.018	0.1 NS	0.02	0.004	0.003	0.004
	LSD 0.05¶	0.2	0.024	0.2	0.06 NS	0.005	0.004	0.004

†Percentage of the total fatty acid fraction.

‡NS, not significantly different at the $p \leq 0.05$.

§LSD for comparing planting dates within irrigation regimes.

¶LSD for comparing irrigation regimes within planting dates.

Table 6. Effect of varying planting date and irrigation regimes on various seed unsaturated fatty acid distributions.

Planting date	Irrigation regime	Unsaturated fatty acids	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	
		%†						
Early		70.5	0.590	0.777	16.1	53.1	0.146	
Normal		70.6	0.579	0.764	15.6	53.8	0.139	
LSD 0.05		0.10 NS‡	0.006	0.008	0.12	0.2	0.005	
	Dryland	70.2	0.598	0.803	16.6	52.3	0.146	
	Irrigated	70.9	0.571	0.738	15.0	54.6	0.138	
	LSD 0.05	0.2	0.010	0.018	0.4	0.6	0.005	
Early	Dryland	70.2	0.602	0.815	17.0	51.9	0.154	
	Irrigated	70.9	0.577	0.740	15.2	54.4	0.139	
Normal	Dryland	70.3	0.593	0.791	16.3	52.7	0.139	
	Irrigated	70.9	0.564	0.736	14.8	54.9	0.138	
LSD 0.05§		0.1	0.008	0.011	0.2	0.2	0.006	
	LSD 0.05¶	0.2	0.011	0.018	0.4	0.6	0.006	

†Percentage of the total fatty acid fraction.

‡NS, not significantly different at the $p \leq 0.05$.

§LSD for comparing planting dates within irrigation regimes.

¶LSD for comparing irrigation regimes within planting dates.

detected for all the unsaturated fatty acids, most of these trended in the same direction as the main effect trends, with differences existing in the degree of magnitude of the response. An exception to this general trend was for the total level of unsaturated fatty acids, where early planting slightly decreased the level of total unsaturated fatty acids under dryland conditions, but planting dates had no significant effect under irrigated conditions.

Cyclopropenoid fatty acids are undesirable minor components of cottonseed oil. These were also affected by varying planting dates and irrigation regimes (Table 7). Early planting decreased the levels of both malvalic and sterculic acid and correspondingly the level of total cyclopropenoid fatty acids. Irrigation had the opposite effect, increasing

the percentages of the individual and total cyclopropenoid fatty acids. Similar to the unsaturated fatty acid interactions, all planting date and irrigation regime interactions for the cyclopropenoid fatty acids were due to differences in the magnitude of the response rather than to any reversal of the trends exhibited by the main effects.

Oleic acid desaturation ratio, which estimates the efficiency of the desaturation reaction that converts oleic acid to linoleic acid, and LDR, which estimates the efficiency of converting linoleic into linolenic acid, were both impacted by varying planting dates and irrigation regimes (Table 8). Oleic acid desaturation ratio was 1% lower when the cotton was planted early, while LDR was increased 8% by early planting. Taken together these trends indicate that

Table 7. Effect of varying planting date and irrigation regimes on various seed cyclopropanoid fatty acid distributions.

Planting date	Irrigation regime	Cyclopropanoid fatty acids	Malvalic acid, cpe18:1	Sterculic acid, cpe19:1
			% [†]	
Early		0.718	0.411	0.307
Normal		0.763	0.444	0.319
LSD 0.05		0.020	0.014	0.006
	Dryland	0.690	0.391	0.300
	Irrigated	0.791	0.464	0.327
	LSD 0.05	0.039	0.029	0.011
Early	Dryland	0.673	0.378	0.295
	Irrigated	0.763	0.444	0.320
Normal	Dryland	0.708	0.404	0.304
	Irrigated	0.818	0.485	0.334
LSD 0.05 [‡]		0.029	0.021	0.009
	LSD 0.05 [§]	0.041	0.030	0.011

[†]Percentage of the total fatty acid fraction.

[‡]LSD for comparing planting dates within irrigation regimes.

[§]LSD for comparing irrigation regimes within planting dates.

linoleic acid should decrease and α -linolenic acid should increase with early planting, which was observed among the individual fatty acids. In addition, the amount of fatty acids with acyl carbon atoms greater than 18 was slightly higher in seed produced from early planting. Although irrigation increased ODR by 3% relative to dryland conditions, LDR was decreased 11%. These trends suggest that linoleic acid levels would increase and linolenic acid levels would decrease when irrigation was applied, as was also observed. Irrigation also slightly reduced both the percentage of longer chain fatty acids and the C16:C18 ratio. There were also significant and meaningful interactions between planting date and irrigation for LDR

and the amount of fatty acids with carbon atoms greater than 18. For both traits, early planting increased the trait expression under dryland conditions but when irrigation was applied planting dates did not differ in their response. Other interactions primarily followed the response of the main effects and differed only in the response magnitude.

DISCUSSION

Cottonseed composition is partially determined by whether the crop is irrigated or not and when the crop is planted. Significant and practical compositional differences were most pronounced when irrigation was applied, where gossypol, oil, and carbohydrate levels were all increased and protein levels were reduced. The irrigation results were in line with associations reported for cottonseed between rainfall distribution and seed protein, oil, and gossypol levels (Pons et al., 1953; Stansbury et al., 1953, 1956). In addition, the increased linoleic acid and total unsaturated fatty acid concentrations observed in this work under irrigated conditions paralleled the observation by Stansbury et al. (1953) that an increase in the oil's iodine value was associated with increased rainfall. Planting date effects were relatively minor in comparison to the irrigation effects even though a number of statistically significant differences were detected.

Some of these composition variations can be explained by the effects that irrigation regime and planting date had on yield development and yield components (Pettigrew, 2010). The increase in crude oil concentration and the decrease in protein concentration (Table 3) observed with irrigation were similar to trends observed with soybean [*Glycine max* (L.) Merr.], although the soybean response was genotype dependent (Bellaloui and Mengistu, 2008). Elevation of the protein level in dryland seeds can be

Table 8. Effect of varying planting date and irrigation regimes on various calculated seed fatty acid components.

Planting date	Irrigation regime	Oleic acid desaturation ratio (ODR)	Linoleic acid desaturation ratio (LDR)	C16:C18 fatty acid ratio	Total fatty acids w> C18
		% [†]			
Early		0.768	0.0027	0.358	0.549
Normal		0.776	0.0025	0.356	0.539
LSD 0.05		0.002	0.0001	0.002 NS [‡]	0.007
	Dryland	0.759	0.0028	0.362	0.569
	Irrigated	0.785	0.0025	0.351	0.519
	LSD 0.05	0.006	0.0001	0.003	0.012
Early	Dryland	0.754	0.0029	0.363	0.579
	Irrigated	0.782	0.0025	0.352	0.518
Normal	Dryland	0.764	0.0026	0.361	0.559
	Irrigated	0.788	0.0025	0.351	0.519
LSD 0.05 [§]		0.002	0.0001	0.003 NS	0.010
	LSD 0.05 [¶]	0.007	0.0001	0.004	0.012

[†]Percentage of the total fatty acid fraction.

[‡]NS, not significantly different at the $p \leq 0.05$.

[§]LSD for comparing planting dates within irrigation regimes.

[¶]LSD for comparing irrigation regimes within planting dates.

explained by the reduced yield and seed mass observed with dryland cotton (Pettigrew, 2010). The reduced overall reproductive sink size of the dryland crop indicates that a fixed amount of N (i.e., protein) is concentrated into fewer and smaller seeds. The crude oil component did not fit the same pattern that the protein component exhibited in dryland seed. A speculative explanation for the elevated levels of crude oil in irrigated seeds is that a greater total daily photosynthesis in irrigated cotton compared with drought stressed cotton (Pettigrew, 2004) allows for more assimilated carbon to be available to support not only increased yield but also increased production of reserve components. It is not unusual for seed oil and protein levels to move in opposite directions, as soybean breeders have commonly observed this inverse relationship when they have tried to increase the concentration of one or the other components (Wilcox and Cavins, 1995). This increased oil and decreased protein level pattern of response to irrigation was similar to trends reported by Stansbury et al. (1956) in their comparison of cottonseeds produced among locations across the U.S. Cotton Belt that varied in rainfall.

Irrigation increased the total seed gossypol concentration even though it led to a greater overall reproductive sink size and larger mass of the individual seeds. Because gossypol is generally thought to be a plant defensive compound that functions to reduce insect predation, the increased seed gossypol level that was found with irrigation might be beneficial from a production standpoint; however, it is not desirable from a commercial utilization standpoint. Furthermore, irrigation shifted the distribution toward (–) gossypol, which is considered to be the more toxic form (Stipanovic et al., 2005). Early planting decreased seed gossypol under dryland conditions with a bias away from the production of the less desirable (–) form (Table 3). Although these alterations in % (+) gossypol are small, they represent some of the first reported environmental or production effects related to % (+) gossypol, as this trait was generally thought to be under tight genetic control (Rayburn et al., 2000; Stipanovic et al., 2005).

Irrigation increased the total soluble carbohydrate level largely by increasing the concentration of raffinose, the predominant carbohydrate in cotton seed. Similar to the oil's response to irrigation, we speculate that the extra photoassimilates produced when cotton is grown under irrigated conditions would mean that more carbon is available for synthesis into carbohydrates. Consistent with this proposition, Pettigrew (2001) demonstrated reduced starch and soluble carbohydrate levels at various times during seed development when cotton was grown in shade as compared with full sunlight conditions. We are not aware of any other studies documenting production or environmental influences on soluble carbohydrate levels of mature cotton seeds.

The irrigation effect on the distribution of cottonseed fatty acids, where the linoleic acid level was increased but the oleic acid level was decreased, is similar to that seen in soybean (Dornbos and Mullen, 1992). Dowd et al. (2010) have also associated environments characterized by hot and dry conditions with lower levels of linoleic acid and higher levels of saturated fatty acids. The elevation of saturated fatty acids observed under the dryland conditions in this study fits this same pattern. In contrast to the Dornbos and Mullen (1992) soybean work that reported a decreased α -linolenic acid level in response to water deficit conditions, we found a slightly elevated α -linolenic acid levels in the dryland cotton seed. Although a statistical difference was observed here, this difference was not of practical importance as the level of α -linolenic acid in cottonseed oil is very low compared with the level of this acid in soybean oil. It appears that under moisture deficit stress, as could occur with dryland conditions, the cotton plant shifts some of the carbon assimilates away from linoleic acid production and into the production of the other unsaturated fatty acids, particularly oleic acid, and also into the increased production of longer chain saturated fatty acids. This cotton response to dry conditions is somewhat different than that seen in soybean, where the reduction in linoleic and α -linolenic acids appears to be entirely compensated by an increase in oleic acid.

Changes in the distribution of fatty acids in response to early planting was in many ways similar to distribution changes exhibited under the dryland conditions, although the magnitude of the differences were generally smaller. Linoleic acid was decreased while the levels of the remaining unsaturated and saturated fatty acids were increased. Similarities between the dryland effect and the early planting effects were most likely due to the fact that the planting date response was mostly exhibited under dryland conditions, with no response exhibited under irrigated conditions. Low June rainfall patterns would have had more effect on the early planted cotton, which would be further along in its reproductive growth, than the normal planted cotton. The effects that irrigation and planting date have on ODR and LDR and the estimates of desaturation are reflective of this redistribution of carbon and hydrogen atoms from linoleic acid to the other fatty acids under drier conditions.

Although both irrigation and early planting offer the potential for increased cotton yields, these practices will alter seed composition. Yield increases from irrigation also come with greater seed oil and gossypol levels and in lesser seed protein levels. Irrigation also results in a greater percentage of linoleic acid in the oil, which increases the level of unsaturated fatty acids. Given that the oil is the most valuable seed component, these trends could be considered positive. However, substantially higher gossypol levels might reduce the amounts of meal that can be incorporated

into animal diets, and higher levels of linoleic acid might necessitate increased hydrogenation resulting in the production of undesirable trans-fatty acids. With early planting, seed gossypol was reduced under dryland conditions and crude oil was increased when irrigation was applied. In contrast to the irrigation response, the oil's percentage of linoleic acid was decreased by planting early. These results highlight some of the effects that environmental and production factors can have in determining the composition of cottonseed. Although cotton will always be grown primarily for its lint, if the market price for cotton seed and cotton seed products remains high or increases in the future, then production decisions may eventually be made with an eye toward the composition of seed in addition to the quantity and quality of the lint.

References

- American Oil Chemists' Society (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
- Bellaloui, N., and A. Mengistu. 2008. Seed composition is influenced by irrigation regime and cultivar differences in soybean. *Irrig. Sci.* 26:261–268. doi:10.1007/s00271-007-0091-y
- Bernardi, L.C., and L.A. Goldblatt. 1980. Gossypol. p. 183–237. In I.E. Liener (ed.) *Toxic constituents of plant foodstuffs*. 2nd ed. Academic Press, New York, NY.
- Cherry, J.P., R.J. Kohel, L.A. Jones, and W.H. Powell. 1986. Food and feeding quality of cottonseed. p. 557–595. In J.R. Mauney and J.McD. Stewart (ed.) *Cotton physiology*. The Cotton Foundation, Memphis, TN.
- Cherry, J.P., and H.R. Leffler. 1982. Seed. p. 511–569. In R.J. Kohel and C.F. Lewis (ed.) *Cotton*. Agron. Monogr. 24. ASA, CSSA, and SSSA, Madison, WI.
- Dornbos, D.L., Jr., and R.E. Mullen. 1992. Soybean seed protein and oil contents and fatty acid composition adjustments by drought and temperature. *J. Am. Oil Chem. Soc.* 69:228–232. doi:10.1007/BF02635891
- 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 (ed.) *Cotton: technology for the 21st century*. ICAC Press, Washington, DC.
- Hunt, P.G., P.J. Bauer, C.R. Camp, and T.A. Matheny. 1998. Nitrogen accumulation in cotton grown continuously or in rotation with peanut using subsurface microirrigation and GOSSYM/COMAX management. *Crop Sci.* 38:410–415. doi:10.2135/cropsci1998.0011183X003800020023x
- Lukonge, E., M.R. 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. doi:10.1002/jsfa.2731
- O'Brien, R.D., and P.J. Wakelyn. 2005. Cottonseed oil: An oil for trans-free options. *Food Technol.* 16:677–679.
- Pettigrew, W.T. 2001. Environmental effects on cotton fiber carbohydrate concentration and quality. *Crop Sci.* 41:1108–1113. doi:10.2135/cropsci2001.4141108x
- Pettigrew, W.T. 2004. Physiological consequences of moisture deficit stress in cotton. *Crop Sci.* 44:1265–1272. doi:10.2135/cropsci2004.1265
- Pettigrew, W.T. 2010. Impact of varying planting dates and irrigation regimes on cotton growth and lint yield production. *Agron. J.* 102:1379–1387. doi:10.2134/agronj2010.0172
- 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. doi:10.1021/jf60018a007
- Rayburn, S.T., W.R. Meredith, Jr., C.W. Smith, R.G. Percy, and M.C. Calhoun. 2000. Variability of plus and minus gossypol in the 1998 National Cotton Variety tests. p. 536–537. In C.P. Dugger and D.A. Richter (ed.) *Beltwide Cotton Pro. Res. Conf.*, San Antonio, TX. 4–8 Jan. 2000. Natl. Cotton Council of America, Memphis, TN.
- Romano, G.B., and J.A. Scheffler. 2008. Lowering seed gossypol content in glanded cotton (*Gossypium hirsutum* L.) lines. *Plant Breed.* 127:619–624. doi:10.1111/j.1439-0523.2008.01545.x
- SAS Institute. 1996. SAS system for mixed models. SAS Institute, Cary, NC.
- Smith, N.E., L.S. Collar, D.L. Bath, W.L. Dunkley, and A.A. Franke. 1981. Digestibility and effects on whole cottonseed fed to lactating cows. *J. Dairy Sci.* 64:2209–2215. doi:10.3168/jds.S0022-0302(81)82831-7
- 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. doi:10.1007/BF02638664
- Stansbury, M.F., W.A. Pons, Jr., and G.T. Den Hartog. 1956. Relations between oil, nitrogen, and gossypol in cottonseed kernels. *J. Am. Oil Chem. Soc.* 33:282–286. doi:10.1007/BF02630862
- Stipanovic, R.D., L.S. Puckhaber, A.A. Bell, A.E. Percival, and J. Jacobs. 2005. Occurrence of (+) and (–) gossypol in wild species of cotton and in *Gossypium hirsutum* var. *marie-galante* (Watt). Hutchinson. *J. Agric. Food Chem.* 53:6266–6271. doi:10.1021/jf050702d
- 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. doi:10.2135/cropsci1976.0011183X001600030023x
- USDA. 2009. 2009 national cotton variety test. Available at <http://www.ars.usda.gov/SP2UserFiles/Place/64021500/2009NCVT.pdf> (verified 11 June 2011). USDA Agricultural Research Service, Crop Genetics Research Unit, Stoneville, MS.
- Wilcox, J.R., and J.F. Cavins. 1995. Backcrossing high seed protein to a soybean cultivar. *Crop Sci.* 35:1036–1041. doi:10.2135/cropsci1995.0011183X003500040019x
- Yunusova, S.G., S.D. Guskova, A.I. Glushenkova, U.K. Nadzhimov, Sh. Turabekov, and S.A. Musaev. 1991. A comparative investigation of the fatty acid compositions of the seeds of a number of lines of a genetic collection of *Gossypium hirsutum*. (In Russian.) *Khim. Prir. Soed.* 1991:173–176.