

AGRONOMY AND SOILS

Planting Cotton Cultivar Mixtures to Enhance Fiber Quality

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ABSTRACT

Price discounts associated with inferior cotton (*Gossypium hirsutum* L.) fiber quality might be avoided through alternative planting strategies involving mixing cultivars of various yield and quality characteristics. A three-year field study was conducted to assess the potential of improving lint quality characteristics without sacrificing yield by using two alternative planting methods. The two alternative methods used were mixing equal volume of seed of two cultivars, Stoneville (ST) 474 mixed with Deltapine (DP) 5409 and Paymaster (PM) 1218BR mixed with either Sure-Grow (SG) 125BR or DP 436RR prior to planting, or planting seed of two cultivars in alternating rows and harvesting as a blend of lint from two cultivars. These methods were compared with monoculture plantings of conventional and transgenic cultivars used in the alternative strategies. Both alternative planting strategies produced yields similar (1195 kg ha^{-1}) to the higher yielding cultivar ST474 (1245 kg ha^{-1}) and significantly higher than the lower yielding DP 5409 (1133 kg ha^{-1}). Lint yields were not different among treatments utilizing either PM 1218BR and SG 125BR (1280 to 1400 kg ha^{-1}) or PM 1218BR and DP 436RR (1603 to 1687 kg ha^{-1}). Micronaire results were inconclusive. Fiber length from mixed seed and alternating row treatments typically were between the monoculture treatments. When DP 436RR was mixed with PM 1218BR, fiber length was increased from 2.69 to 2.79 cm and micronaire was reduced from 4.60 to 4.21. When SG 125BR was mixed with PM 1218BR, length was unchanged but micronaire reduced from 4.13 to 3.71. Fiber strength was not

enhanced as a result of cultivar mixing or alternate row planting. In most cases, the two alternative strategies were similar to each other in yield and fiber properties. This research demonstrated that in some cases using alternative planting strategies improved fiber quality and yield, but gains were of minimal economic or biological importance.

In addition to the cost associated with production, the overall profitability of a cotton producer is determined by not only yield but also lint quality. Since the value associated with obtaining optimum cotton quality without sacrificing yields is dependent upon numerous factors including cotton price and quality-based discounts, it varies annually. Fiber length has always been important to cotton manufacturing and since the introduction of rotor spinning technology to cotton manufacturing in 1970, micronaire and strength both have increased in importance relative to other quality characteristics (Deussen, 1986). Cotton yield and quality are influenced by both environmental conditions and genetics (Gipson and Joham, 1968; Ramey, 1986; Yfoulis and Fasoulas, 1978). Cotton producers may be able to enhance overall profitability through utilization of agronomic practices that optimize lint quality without sacrificing yields.

Agronomic practices affecting fiber quality and yield include timing of defoliation (Kelley and Boman 1999; 2000; Laferney et al., 1963), timing of harvest (Barnes and Herndon, 1997; Williford et al., 1995), irrigation (Lascano and Hicks, 1999; Marani and Amirav, 1971), use of plant growth regulators (Kerby, 1985), fertility (Pettigrew et al., 1996), tillage (Phipps and Clements, 1999), and cultivar selection (Bradow, 1999). With respect to the latter, twenty-three years of cotton-quality data from the lower Mississippi River Valley region indicated that inferior quality (grade, staple length, and micronaire) was highly correlated with the introduction of new cultivars (Barnes and Herndon, 1997).

Researchers have examined the potential for blended cultivar plantings in various crops to achieve certain desired characteristics (Akanda and Mundt,

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1996; Hancock et al., 1984). Blending seed or fiber from different cotton cultivars to achieve specific fiber quality has been examined in cotton production and processing. Simpson and Fiori (1974) examined the effects of mixing seed from cottons that differ in micronaire prior to milling and reported no effect on strength, strength variability, uniformity, and end breakage. Planting cottonseed mixtures of two cultivars has been examined for its effect on insect pests (Agi et al., 2001; Durant, 1995). In five experiments at three sites in Uganda, yields of mixing numerous upland cultivars did not exceed monoculture yields (Innes, 1977). Bridge et al. (1984) found that mixing two cultivars with similar yield potential, Stoneville 825 and Deltapine 41, did not result in yield potential or staple length differences, but possible additive effects increased micronaire and lint strength. In Arkansas, mixing seed of 'Deltapine 50' and 'Deltapine 90' or DP 50 and 'Hyperformer 46' increased strength values above DP 50 monocultures (McConnell et al., 1991).

Perceived declines in fiber quality, increased frequency of price discounts of lower quality cotton, and the introduction of new cultivars have renewed an interest in the potential of cultivar mixes to maintain yield and improve fiber quality. The objective of this research was to compare fiber yield and quality of conventional and transgenic cultivars planted in seed mixes with two cultivars and in alternate-row planting patterns. Both alternative planting strategies were aimed at achieving optimal fiber quality traits without sacrificing yield potential.

MATERIALS AND METHODS

Research was conducted in 1999, 2000, and 2001. In 1999, trials were conducted at the Central Crops Research Station in Clayton, NC, the Peanut Belt Research Station in Lewiston, NC, the Upper Coastal Plain Research Station in Rocky Mount, NC, and the Cherry Farm Unit in Goldsboro, NC. In 2000, trials were conducted at the Peanut Belt Research Station, the Upper Coastal Plain Research Station, and the Cherry Farm Unit in Goldsboro, NC. In 2001, trials were conducted at the Central Crops Research Station, the Peanut Belt Research Station, and the Upper Coastal Plain Research Station.

Blending cultivars requires the identification of cultivars that are similar in maturity. If maturities are not similar, yield and quality of the blends could be compromised. Many newer cotton cultivars dis-

playing superior lint quality characteristics are later maturing and would require a late-maturing blending partner. Cultivars in this study were selected from the North Carolina State University early-maturity cultivar trials (Bowman, 1996). In all three years (10 environments), ST 474 (Stoneville Pedigreed Seed Co., Collierville, TN) was selected for its high yielding capacity and DP 5409 (Delta Pine and Land Co., Scott, MS) for its superior quality characteristics. Treatments consisted of two monoculture treatments (100% ST 474 and 100% DP 5409), a hopper-box mix of equal volume of these cultivars, and the two cultivars planted in alternate rows. In 2000, the same arrangement of treatments was used at 3 sites with Paymaster (PM) 1218 BR and Sure-Grow (SG) 125 BR (Delta Pine and Land Co., Scott, MS). Because most fiber quality traits of SG 125BR in 2000 were inferior to PM 1218BR, SG 125BR was replaced with DP 436RR for the three sites based on 2001 North Carolina State University cultivar trials (Bowman, 2000).

At all locations and years, plots consisted of four rows, 15.2 m in length. Row spacing at the Goldsboro and Clayton sites was 97 cm and at the Rocky Mount and Lewiston sites was 91 cm. Treatments were arranged in a randomized complete block with four replications. North Carolina Cooperative Extension guidelines were followed for seeding rate, insect control, weed control, and soil fertilization (Anonymous, 2000). At each site, the targeted seeding rate was 11.5 seed m⁻¹ of row (3.5 seed per 30.5 cm). Plant growth regulator and harvest aid application decisions were based on average plant measurements in each test. Planting and harvest date for each site is shown in Table 1. Seed cotton yield was determined by mechanically harvesting the center two rows of each plot with a John Deere, two-row, spindle-type picker, modified for plot use. Subsamples from each plot were ginned on a 12-saw gin to determine lint yield. Fiber samples were analyzed using high volume instrumentation (HVI) analysis by Cotton Incorporated (Cary, NC). Discount levels are based on the 2001 USDA government loan program.

The legalities associated with combining cultivars of various transgenic cultivars change frequently and should be examined before implementing this management technique. In this study, data from treatments containing conventional cultivars (ST 474, DP 5409, or in combination) were analyzed separately from treatments that used transgenic cultivars [PM

1218BR and SG 125BR (2000) or DP 436RR (2001 cultivars)]. Analysis of variance was performed on all harvest data including lint yield, micronaire, staple length (UHM), and uniformity index (UI) using Proc GLM (SAS Institute release, 6.12, Cary, NC). Means were separated using Fisher’s protected LSD at $P = 0.05$. For the experimental variables tested (yield, micronaire, fiber length, fiber strength, and uniformity index), none of the treatment \times environment interactions were significant; therefore, all data from paired cultivars are reported as means across years, locations, and replications.

Air temperature and rain data were recorded in close proximity to all sites. Heat units (DD-16’s) were calculated using the following equation: (daily mean high air temperature + daily mean low air tem-

perature) / 2] – 16 °C. Any heat unit values less than 0 were treated as 0 for further calculations. Total rain accumulations from the time of planting through 1 October were measured for each year and are presented in Table 1.

RESULTS AND DISCUSSION

Similar to historical cultivar trial data in which DP 5409 produced 16% less lint yield than ST 474 (Bowman, 1996), the monoculture DP 5409 produced 9 % less lint yield than the ST 474 monoculture (Table 2). Yields in the two alternative planting strategy treatments were similar to ST 474 in monoculture. Lint in the monoculture of DP 5409 had lower micronaire values than either the ST 474 monoculture treatment

Table 1. Planting date, harvest date, accumulated heat units (DD-16’s), and rainfall from planting to 1 October for each year and location

Location	Year	Plant date	Harvest date	Heat units (°C) ^z	Cumulative rain (cm)
Clayton	1999	6-May	5-Nov.	1257	70
Goldsboro	1999	5-May	25-Oct.	1257	83
Lewiston	1999	4-May	5-Nov.	1161	67
R. Mount	1999	11-May	27-Oct.	1272	104
Goldsboro	2000	15-May	2-Nov.	1280	54
Lewiston	2000	3-May	1-Nov.	1202	55
R. Mount	2000	8-May	30-Nov.	1203	59
Clayton	2001	5-May	7-Nov.	1072	39
Lewiston	2001	1-May	25-Oct.	1111	68
R. Mount	2001	3-May	22-Oct.	1148	68

^z Heat units (DD-16’s) were calculated as follows:
 [(daily mean high air temperature + daily mean low air temperature) / 2] – 16 °C.

Table 2. Mean lint yield and fiber properties for Stoneville 474 (ST 474), Deltapine (DP 5409), the hopper-box mix of ST 474/DP 5409, and alternate-row planting of ST 474/DP 5409

Cultivar	Planting strategy	Yield and fiber properties ^z				
		Lint yield (kg ha ⁻¹)	Micronaire	Fiber length (cm)	Uniformity index (%)	Fiber strength (g tex ⁻¹)
ST 474	Alone	1245 a	3.95 a	2.69 d	81.9 a	26.9 c
DP 5409	Alone	1133 b	3.62 b	2.82 a	81.6 a	27.8 a
ST 474+ DP 5409 (50/50)	Hopper Mix	1197 a	3.81 a	2.77 b	81.6 a	27.3 b
ST 474 + DP 5409	Alternate-Row	1192 a	3.82 a	2.74 c	81.8 a	27.2 bc
LSD ($P \leq 0.05$)		53	0.20	0.03	0.5	0.5

^z All means are combined across 3 years and 10 sites. Means within a column followed by the same letter are not significantly different according to Fisher’s Protected LSD ($P \leq 0.05$).

or the alternative planting strategies. Similar to earlier findings with seed mixtures of ST 825 and DP 41 (Bridge et al., 1984), micronaire values in the seed mixture and alternate-row treatments were similar to the high yielding monoculture value. The fiber length from the monoculture of DP 5409 was longer than the monoculture of ST 474, which produced fiber length values above the discount level (<2.54 cm). Staple length in the mixed seed and alternate-row planting was increased above the monoculture of ST 474. These fiber lengths were above the discount level and between monocultures of ST 474 and DP 5409 indicating potential for economic gain (or absence of discount).

Similar to earlier findings with one high quality and one low quality conventional genotype (Innes 1977; Bridge et al., 1984), there were no significant yield differences among blends with transgenic cul-

tivars (Tables 3 and 4). In trials utilizing transgenic cultivars (2000 and 2001), micronaire values were highest in monocultures of PM 1218BR. In contrast to results when conventional cultivars were used, micronaire in the mixed seed and alternate-row planting strategies was similar to the lower micronaire of SG 125BR and DP 436RR in monoculture.

Blended treatments had significantly lower micronaire compared with the cultivar with the highest micronaire in two of three trials (Tables 2, 3, and 4). In this study, the reductions did not avoid a price discount, because high micronaire discounts currently begin at a micronaire reading of 5.0 and above, and micronaire in all of our treatments were 4.6 and below. Price discounts for high micronaire cotton are an annual problem for cotton producers. Blending of a high micronaire cultivar with a low micronaire cultivar appears to consistently result in

Table 3. Mean lint yield and fiber properties for Paymaster 1218BR (PM 1218BR), Sure-Grow 125BR (SG 125BR), the hopper-box mix of PM 1218BR/SG 125BR, and alternate-row planting of PM 1218BR/SG 125BR

Cultivar	Planting strategy	Yield and fiber properties ^z				
		Lint yield (kg ha ⁻¹)	Micronaire	Fiber length (cm)	Uniformity index (%)	Fiber strength (g tex ⁻¹)
SG 125BR	Alone	1379 a	3.66 b	2.72 a	82.2 b	26.5 b
PM 1218BR	Alone	1400 a	4.13 a	2.74 a	83.2 a	27.7 a
PM1218BR + SG 125BR (50/50)	Hopper Mix	1281 a	3.71 b	2.72 a	82.0 b	27.1 ab
PM 1218BR + SG 125BR	Alternate-Row	1342 a	3.71 b	2.74 a	82.7 ab	27.2 ab
LSD ($P \leq 0.05$)		127	0.30	0.05	1.0	0.3

^z All means are combined across 3 sites for the year 2000. Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

Table 4. Mean lint yield and fiber properties for cotton cultivars Paymaster 1218BR (PM1218BR), Deltapine 436RR (DP 436RR), the hopper-box mix of PM 1218BR/DP 436RR, and alternate-row planting of PM 1218BR/DP 436RR

Cultivar	Planting strategy	Yield and fiber properties ^z				
		Lint yield (kg ha ⁻¹)	Micronaire	Fiber length (cm)	Uniformity index (%)	Fiber strength (g tex ⁻¹)
DP 436RR	Alone	1603 a	4.12 b	2.84 a	82.8 a	26.6 a
PM 1218BR	Alone	1687 a	4.60 a	2.69 b	82.9 a	26.5 a
PM 1218BR + DP 436RR (50/50)	Hopper Mix	1617 a	4.21 b	2.79 a	82.8 a	26.5 a
PM 1218BR + DP 436RR	Alternate- Row	1605 a	4.30 b	2.77 a	83.4 a	26.4 a
LSD ($P \leq 0.05$)		100	0.20	0.02	0.8	0.7

^z All means are combined across 3 sites for the year 2001. Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

a lower overall micronaire compared with the highest micronaire cultivar. While this lower value may not be economically significant in every case, it could provide a measure of insurance against price discounts for high micronaire.

In 2000, fiber length was relatively low (<2.74) and the monoculture of PM 1218BR was in the acceptable range (>2.54 cm) (Table 3). Previous cultivar trials reported little difference between PM 1218BR and SG 125BR in fiber length (Bowman, 2000), and in this study differences among treatments were not significant for fiber length in 2000. In 2001, when DP 436RR was used, staple length was significantly increased in both alternative planting strategies compared with the monoculture of PM 1218BR (Table 4). Based on one year of data with three sites, DP 436RR appears to be a better mixing partner than SG 125 for enhancing PM 1218 fiber length characteristics.

Uniformity index values were unaffected by pairing ST 474 and DP 5409 (1999-2001) and PM 1218BR and DP 436RR in 2002 (Tables 2 and 4). The uniformity index value for PM 1218BR planted alone was higher than the SG 125BR planted alone or in either alternative planting strategy (Table 3). Thus, mixing resulted in a less desirable uniformity index in this situation.

Contrary to earlier findings (McConnell et al., 1991; Bridge et al., 1984), fiber strength was not enhanced above the cultivar with the best strength by any of the three mixes used in this study. The fiber strength from mixed seed planting strategy of the two conventional cultivars tested was higher than the monoculture of ST 474 and below the monoculture of DP 5409 (Table 2). Similarly, fiber strength from monoculture of PM 1218BR was higher than monoculture of SG 125BR, and the alternative planting strategies were intermediate (Table 3). There were no significant differences in fiber strength among treatments using PM 1218BR and DP 436RR (Table 4).

Few differences were observed between the 1:1 mixture of seed in the hopper and the alternate-row blends. Yield was the same in all trials (Tables 2, 3, and 4). Except for fiber length in the PM 1218BR/DPL 436RR trial, there were no differences between the alternative planting strategies (hopper vs. alternating rows) for the measured fiber properties. These data indicated that mixing cultivars will perform similarly, whether mixed in a 1:1 ratio, or planted in an alternate row configuration.

In some instances, the data demonstrated additive and deleterious effects associated with mixing of certain cultivars and alternating row planting strategy. For example, yields of both alternative strategies were closer to the higher yielding ST 474 than DP 5409. Theoretically, these effects could be due to phenotypic variations in cultivars that allow plants from the low yielding cultivar to exploit surroundings either more or less efficiently under mixed planting conditions. Additionally, micronaire values were reduced with mixed cultivars compared to high micronaire monocultures. Although not economically significant in these data, reduced overall micronaire can often be advantageous to producers. In summary, gains in yield and fiber quality were observed with selected seed mixes; however, these gains were of minimal economic and biological importance given the current pricing structure for United States cotton. The mixing of cotton cultivars appears to offer at most, a short-term approach to improving fiber quality. The best solution to avoiding fiber quality discounts is to continue breeding efforts aimed at developing cultivars with high yields and superior fiber properties.

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DISCLAIMER

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REFERENCES

- Agi, A. L., J. S. Mahaffey, J. R. Bradley, Jr., and J. W. Van Duyn. 2001. Efficacy of seed mixes of transgenic Bt and nontransgenic cotton against bollworms, *Helicoverpa zea* Boddie [Online]. J. Cot. Sci. 5(2): 74-80. Available at <http://journal.cotton.org/2001/issue02/toc.html>
- Akanda, S. I. and C. C. Mundt. 1996. Path coefficient analysis of the effects of stripe rust and cultivar mixtures on yield and yield components of winter wheat. Theor. Appl. Genet. 92 (6): 666-672.
- Anonymous. Cotton Information 2000. North Carolina Cooperative Extension Service. North Carolina State University, Raleigh, NC.

- Barnes, M., and C. W., Jr. Herndon. 1997. Causal factors of cotton quality discounts and premiums in the Mid-South: 1973-1995. p. 326-330. *In Proc. Beltwide Cotton Conf.*, New Orleans, LA, 7-10 Jan. 1997. Natl. Cotton Council Am., Memphis, TN.
- Bowman, D. T. 1996. North Carolina Measured Crop Performance, Soybean and Cotton 1996. Crop Science Research Report No. 163. North Carolina State University, Raleigh, NC.
- Bowman, D. T. 2000. North Carolina Measured Crop Performance, Soybean and Cotton. Crop Science Res. Report No. 188. North Carolina State University, Raleigh, NC.
- Bradow, J. M. 1999. How genotype and temperature modify yarn properties and dye uptake. p. 510-512. *In Proc. of the Beltwide Cotton Conf.*, Orlando, FL, 3-7 Jan. 1999. Natl. Cotton Council Am., Memphis, TN.
- Bridge, R. R., S. R. Miller, and S. M. Lane 1984. The influence of binary seed mixtures of 'Stoneville 825' and 'Deltapine 41' cotton cultivars on their performance. Bull # 931. Miss. Agric. For. Exp. Stn., Mississippi State, MS.
- Deussen, H. 1986. Stressing high strength, low micronaire may require a 'rethinking of breeding and marketing methods'. p. 32-36. *In W. Spencer (ed.) Cotton International 53rd Ed.* Meister Publishing Co., Memphis, TN.
- Durant, J. A. 1995. Efficacy of selected seed mixes of transgenic Bt and nontransgenic cotton against bollworms and tobacco budworms in South Carolina. p. 769-771. *In Proc. Beltwide Cotton Conf.*, San Antonio, TX, 4-7 Jan. 1995. Natl. Cotton Council Am., Memphis, TN.
- Gipson, J. R. and Joham, H. E. 1968. Influence of night temperature on growth and development of cotton (*Gossypium hirsutum* L.). I. Fruiting and boll development. *Agron. J.* 60: 292-295.
- Hancock, J. F., S. L. Krebs, M. Sakin, and T. P. Holtsford. 1984. Increasing blueberry yields through mixed cultivar plantings. *Annu. Rep. Mich. State Hortic. Soc.* 119: 130-133.
- Innes, N. L. 1977. Performance of seed mixtures and multilines of Upland cotton in Uganda. *J. Agric. Sci. (Cambridge)* 88: 47-54.
- Kelley, M. and R. Boman. 1999. High plains harvest-aid application timing studies. p. 606-607. *In Proc. Beltwide Cotton Conf.*, Orlando, FL, 3-7 Jan. 1999. Natl. Cotton Council Am., Memphis, TN.
- Kelley, M. and R. Boman. 2000. Harvest-aid combination and application timing effects on lint yield and quality of fiber and seed. p. 702. *In Proc. Beltwide Cotton Conf.*, San Antonio, TX, 6-9 Jan. 2000. Natl. Cotton Council of Am., Memphis, TN.
- Kerby, T. A. 1985. Cotton response to mepiquat chloride. *Agron. J.* 77: 515-518.
- Laferney, P. E., R. A. Mullikin, and W. E. Chapman. 1963. Effects of defoliation, harvesting, and ginning practices on micronaire reading, fiber properties, manufacturing performance, and product quality of El Paso area cotton, season 1960-1961. USDA-ARS Marketing Research Report No. 690. U.S. Gov. Print. Office, Washington, DC.
- Lascano, R. J. and S. K. Hicks. 1999. Cotton lint yield and fiber quality as a function of irrigation level and termination dates in the Texas high plains: 1996-1998. p. 570-571. *In Proc. Beltwide Cotton Conf.*, Orlando, FL, 3-7 Jan. 1999. Natl. Cotton Council Am., Memphis, TN.
- Marani, A. and A. Amirav. 1971. Effects of soil moisture stress on two cultivars of upland cotton in Israel. I. The coastal plain region. *Exp. Agric.* 7:213-224.
- McConnell, J. S., F. M. Bourland, B. S. Frizzel, and W. H. Baker. 1991. Enhanced cotton fiber strength through the use of blended cultivars. *Arkansas Farm Res.* 40 (5): 3.
- Pettigrew, W. T., J. J. Heitholt, and W. R. Meredith, Jr. 1996. Genotypic interactions with potassium and nitrogen in cotton of varied maturity. *Agron. J.* 88: 89-93.
- Phipps, B. J. and L. A. Clements. 1999. Effect of tillage upon lint yield and fiber quality. p. 576-577. *In Proc. Beltwide Cotton Conf.*, Orlando, FL, 3-7 Jan. 1999. Natl. Cotton Council Am., Memphis, TN.
- Ramey, H. H., Jr. 1986. Stress influences on fiber development. p. 351-359. *In: J. R. Mauney and J. McD. Stewart (eds.) Cotton Physiology.* Cotton Foundation, Memphis, TN.
- Simpson, J. and L. A. Fiori. 1974. Effect of mixing cottons differing in micronaire reading and carding variables on cotton sliver quality, yarn properties, and end breakage. *Textile Res. J.* 44 (5): 327-331.
- Williford, J. R., F. T. Cooke, Jr., D. F. Caillouet, and S. Anthony. 1995. Effect of harvest timing on cotton yield and quality. p. 633-638. *In Proc. Beltwide Cotton Conf.*, San Antonio, TX, 4-7 Jan. 1995. Natl. Cotton Council Am., Memphis, TN.
- Yfoulis, A. and Fasoulas, A. 1978. Role of minimum and maximum environmental temperature on maturation period of the cotton boll. *Agron. J.* 70: 421-425.