# EFFECT OF SUBTENDING LEAF REMOVAL ON THE YIELD AND FIBER QUALITY TRAITS OF UPLAND COTTON (GOSSYPIUM HIRSUTUM L.)

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(Received 12th Mar 2021; accepted 14th May 2021)

**Abstract.** Since the last decade cotton breeders have followed a trend towards emphasizing physiology of fruit development. Cotton plant can support development of leaves, stem and roots by themselves, but inefficient towards building fruit and young boll. Hence boll depends on and takes most of its food from its subtending leaf. In order to track the impact of subtending leaf on fiber quality and yield, a diverse set of cotton germplasm have been subjected to the removal of the subtending leaf at different stages. The results from the current study show that removal of subtending leaf later than 50 and 60 days post anthesis (DPA) has almost no impact as compared to the Control (No subtending leaf removal). Whereas subtending leaf removal at 35 days post anthesis (DPA) has maximum impact on yield traits as well as most of the fiber quality traits. There are several germplasm accessions which have been minimally impacted by the removal of the subtending leaf regardless of date showing the minimum dependency of boll formation on subtending leaf. The outcomes of this study will aid cotton breeders to develop varieties genetically strong enough to resist environmental influences, more input responsive and appropriate for mechanized farming.

Keywords: subtending leaf impact, cotton boll, fiber yield, fiber quality, upland cotton

### Introduction

Upland cotton (*Gossypium hirsutum* L.) is a world leading natural textile fiber, it is also considered an important oilseed crop. The genetic enhancement of cotton plants to increase seed cotton yield and fiber quality has been among the major objectives of cotton breeding programs across the globe for a long time. Continuous improvement of cotton for yield and quality resulted from numerous new cotton cultivars with desirable characters with the cost of decreasing genetic variability ultimately narrowing down genetics base of the available stock worldwide. Nowadays, enhancing cotton fiber quality became complicated and challenging provided the limited genetic basis of modern cotton cultivars (Ali et al., 2011; Zhang et al., 2011; Sarfraz et al., 2018; Jarwar

et al., 2019), as well as the potential negative genetic correlations among yield and fiber quality (Zeng and Meredith Jr, 2009; Campbell et al., 2011; Clement et al., 2012; Rahman et al., 2013). Genetic variation among most of the important agronomic traits in cotton is under polygenic control (Gapare et al., 2017). While considering fiber quality of cotton we usually consider fiber length, strength, finesses or micronaire, uniformity and color. Micronaire is actually an indirect measure related to fiber fineness and maturity. It is considered as an important parameter for fiber quality assessment behaving like an indicator for cotton production with profitability and sustainability (Luo et al., 2016). Gossypium genus has long been under extensive taxonomic and evolutionary studies by humans as found in history. Most of the attention and emphasis of such studies have been revolved around the four cultivated species which have been domesticated by human for their fiber. These include two tetraploid species (New world allopolyploids) G. hirsutum and G. barbadense with a chromosome number 2n = 52, and G. arboreum and G. herbaceum, two Old-World diploids with chromosome number 2n = 26. Originally, these cultivated species are assumed to have a considerable amount of genetic diversity, but unfortunately this diversity has been narrowed down as compared to that present in individuals of the genus as a whole, where there are almost 50 species having an overall geographic range, including most of the tropical as well as subtropical regions across the globe (Wendel et al., 2010). It is assumed that an important event in the cotton evolutionary history is the spontaneous evolvement of allopolyploid cotton, which was eventually gone through subsequent selection and domestication steps leading to current-day modern cotton cultivars. These cotton cultivars belonging to allotetraploid cotton have a wide range of similarities to 'AA' and 'DD' diploid species making them related to each other. This recorded polyploidization event is assumed to be happened around 1.5 MYA, whereas these allotetraploids (AADD) are noted to be further diverged into 5 tetraploid species distributed across New World as well as the rest of the world (Lee et al., 2007; Wendel, 1989; Wendel and Cronn, 2003). Since the last decade, an enhanced trend has been observed among cotton breeders towards emphasizing physiology of fruit development. Usually, researchers take into consideration fruit as well as base management decisions by counting number of squares and bolls with their perspective positions on plant during plant mapping. It is unfortunate that they make a mistake by overlooking value of leaves in the productivity enhancement. Basically, leaves are the basic building blocks of cotton productivity and must be recognized for and is necessary to be considered for management practices just like fruits. Sustaining the healthy young leaves must be considered important while making decisions based on understanding leaf influence on yield and quality benefits while considering them a sophisticated solution for the common plant problems including requirement for maximum sunlight absorption in order to fuel limited light harvesting mechanism for proper and optimum photosynthesis and the need to reduce water loss by enhancing carbon dioxide uptake (Hendrix and Grange, 1991; Oosterhuis et al., 1990). Cotton leaves can solve the light harvesting issue with the help of their large flat surfaces as well as their ability for dense stacking chlorophyll (light harvesting pigment) within the leaf through upper half portion. Stomatal pores are used for water conservation by controlling in and out air movement. These pores are called stomata and are located across the lower surface of leaves. Because stomata open throughout the day to enable the diffusion of CO<sub>2</sub> through the leaf, water vapor eventually diffuses outward. However, this water loss provides some benefits i.e. during the day the plant cools down to keep the leaf temperature far below

level of damage (100'F). In addition, water outflow in the form of vapors from leaf enables roots to take soil water along with various nutrients necessary for plants (Pace et al., 1999). Almost 60-87% of the carbon assimilates in boll are derived through CO<sub>2</sub> assimilation through boll development stage. Subtending leaf plays a vital role during this phase hence is considered as the most important contributor for accumulation of biomass in boll in the form of seed cotton (Oosterhuis et al., 1990; Ashley, 1972; Wullschleger and Oosterhuis, 1990). Subtending leaves along with their corresponding bolls act like source-sink during photosynthesis as well as photosynthate accumulation in boll. This source-sink relationship between subtending leave and boll exhibits a strong cooperation among vegetative-reproductive growth phases in cotton having a substantial impact on yield and fiber quality (Xie et al., 2003). Previous reports concluded that sink formation ability during early stages with a stronger potential for reproductive growth phase have substantial contribution and are important characteristics for high yielding varieties of cotton (Pace et al., 1999). Higher yield can be obtained not only by ensuring a strong photosynthesis within the functional leaf but also its proper and effective distribution across different reproductive organs (Richards, 2000; Wang, 2007). Hence, it has been suggested that an increase in the nitrogen-carbon partitioning in the reproductive meristem is needed to ensure enhanced seed size and number, and ultimately yield improvement (Richards, 2000). Structure and arrangement of leaves are very important to accomplish their critical task of photosynthesis and help them in the accumulation of photosynthate as storage of light energy and ultimately allow plants to fill bolls during shiny days as well as continue their vegetative growth during night time. Technically, photosynthesis is characterized to trap light energy within carbohydrate, latterly used to develop either leaf or may be transported through the plant to be utilized for growth in any other part (Oosterhuis et al., 1990; Xiangbin et al., 2012). The leaves producing carbohydrates excessively than their own needs are termed as "source", in comparison to plant "sink" that are those parts that usually dependent and receive these excessive nutrients or photosynthates from these source leaves. Usually, these "Sinks" are either roots, immature stems, bolls, or leaves. On the other hand, "Sources" are mostly leaves. It is worth considering that all the leaves are not "sources". Usually, middle-aged leaves act as "sources", as they can support the development of bolls. The term the strongest "source" is used for a recently expanded and fully illuminated leaf, whereas the strongest "sink" is usually a 20-30-day older boll which is rapidly comprehending a fast dry weight accumulation after gain. A relatively fewer amounts of photosynthate are provided through bracts and boll-walls as compared to leaves. An enhanced leaf area index and canopy-apparent photosynthesis during the development of boll also result in photosynthesis rate improvement as well as enhanced chlorophyll content (Oosterhuis et al., 1990; Wang et al., 2002; Pettigrew and Gerik, 2007). The efficacious management practices responsible for the maintenance of balance across "source-sinks" and the development of strong source include optimum irrigation scheduling, fertilization and some plant growth regulators. The successful consequences of aforementioned protocols build "healthy sinks" by developing bolls on the plant. The regrowth control concept is the base for the management decision that illustrates the use source-sink concept. Whether it is needed to control regrowth or may allow the plant to be green by producing fresh leaves helping to fill late setting bolls remains a question for the cotton growers (Pettigrew and Gerik, 2007). A young regrowth may require around 2-3 weeks prior to the ability of leaves to become a "source" through export of excessive carbohydrates to the boll as Sink. In addition, prior

to the availability of carbohydrate to support regrowth, an enhanced boll demand or "sinks" must be lower or lesser. Allowing the plant to regrow has no known benefit to the late setting boll, but causes risk by aggravating insect populations, lint staining as well as lowering down boll-opening during the late season of crop by creating hindrance of sunshine approaching plant parts and bolls making plant vulnerable to suffocation, disease incidence, bolls rot ultimately reducing yield and fiber quality (Oosterhuis et al., 1990; Liu et al., 2014). We are unable to visualize leaf growth and it is mostly hidden from our visual observations. At the time, we can observe the leaves, they have already reached their final development stage and only going through expansion. One day after planting we can see first true leaf with the help of the microscope only, and it has started the development on the shoot tip within folded cotyledon's inside seed. It may take at least 3-4 more weeks to become visible to be observed with naked eye. In the terminal, cell division is responsible for the development of leaves, whereas elongation as well as differentiation into small leaves with a normal shape may only have the requirement of expansion enabling it to push for the exposure of sunshine. Those leaves which are produced on fruiting branches that are formed similarly but the difference only is they develop from branching bud (Constable and Rawson, 1980). In fact, cotton is perennial and instead of growing across one season it grows for multiple growing seasons may be up to 10 years. Cotton plant similar to a shrub can support the development of leaves, stem and roots by themselves are assumed to be inefficient towards building fruit, and young boll derives most of its food from the subtending leaf (Reddy et al., 1992). Mostly, our emphasis during improvement as well as management efforts is focused on the encouragement of plants to partition major carbohydrate quantity towards bolls as compared to vegetation. Such management practices usually try to overcome many deficiencies in the way cotton grows. Squares usually supported by themselves by getting carbohydrates produced within the bracts, as far as boll is reached the age of 10 days, it is observed to have an enhanced need for carbohydrates as well as mineral nutrients. Usually, a younger boll caters for most of its food need through subtending leaf. In case this leaf is broken, malformed or shaded, may be due to harsh weather or dense growth, it will result in shedding of the young 4-7-day old boll. In cotton, during the boll filling period, leaves are rapidly aging along-with significant change in the day length, air quality and temperature deterioration ultimately resulting in reduction of supply of photosynthate to fill bolls (Oosterhuis et al., 1990; Reddy et al., 1992; Boquet and Clawson, 2009). So optimum management practices are essential in bringing better leaf output to cope with the boll demands. This can be accomplished maintaining leaf health and promoting earliness of boll retention time to avoid the extra management expense by growers ultimately misbalancing cost benefit ratios. So, it is necessary to sketch and know the optimum time duration required for the application of additional management practices (Oosterhuis et al., 1990; Ashley, 1972; Richards, 2000). Keeping in view above considerations, the current study has been planned to investigate the impact of subtending leaf removal on 355 upland cotton accessions during 2018 and 2019. The major objectives of the study were to find the impact of subtending leaf removal on yield and fiber quality traits as well as to estimate the optimum time for effective management practices to get maximum benefits within the shortest period of time.

## Materials and methods

A diverse collection of 355 upland cotton accessions obtained from the cotton germplasm collections gene bank of the Cotton Research Institute of the Chinese Academy of Agricultural Sciences (CRI-CAAS) has been used for the current study. These cotton accessions have been planted following triplicated randomized complete blocked design in factorial arrangement at field area of CRI, Anyang Henan during the sowing season 2018 and 2019. Sowing was carried out on the 30<sup>th</sup> April, during both the years viz. 2018 and 2019. Plot size was maintained as 8 m lengths of each accession. After germination, thinning was carried out to maintain plant population. The chemical control was applied at peak flowering and boll setting period. Inter-tillage was carried out 6 times during the whole growth season. All phosphorus fertilizer was applied at the time of sowing while nitrogen fertilizer was applied 3 times at planting, squaring/flowering stage and after topping with rapid release fertilizers. Furrow irrigation was applied as needed during each season to minimize moisture stress. Vegetative branches, old leaves and redundant buds and growth terminals of the main stem were manually removed. At start of blooming flower tagging in all plots of the experiment was carried out starting from 10th July onward to to10th August. Each flower during this period has been tagged for its blooming date. Subtending leaf removal has been carried out manually by hand for the tagged leaves as they reached 35 days' bolls, 40 days' bolls, 50 days' bolls, 60 days' bolls considering the four treatments, and one control kept as unremoved. The subtending leaf removal was carried out by hand on 20 bolls for each treatment on 5 plants. On maturity of five plants picking was carried out to pick 20 bolls from each plant for each treatment. Fiber quality for nine traits was recorded for the picked bolls using high-volume instrument (HVI) in the Laboratory of Quality & Safety Risk Assessment for Cotton Products (Anyang), Ministry of Agriculture, People's Republic of China. The traits considered for the current study included boll weight (BW), seed weight (SW), ginning outturn (GOT%), fiber weight (FW), fiber length (FL), fiber uniformity (FU), fiber fineness (MIC), fiber strength (FS), and fiber elongations (FE).

# Statistical analysis

Software SAS JMP Pro 15 (SAS Institute Inc., Cary, NC, 1989-2019) was used to calculate basic statistics and Pearson's correlations between traits and analyses of variances were calculated through mixed model and Tukey test (HSK) was performed for all pairwise comparisons.

#### Results

The first-order statistics for the measured nine traits of 355 cotton accessions, including means and ranges evaluated in the field trials for two consecutive years (2018-19) were given in *Table 1*. The average boll weight (BW) observed was 4.965 g and a range of minimum with 1.100 g and maximum with 8.600 g. Average fiber weight (FW) determined were 1.923 g with a range of minimum and maximum values 0.300 g and 3.778 g, respectively. Average ginning outturn (GOT) detected was 38.637% and a range of minimum with 20.212% and maximum with 49.675%. The average seed weight (SW) estimated was 2.981 g with a range of minimum and maximum values 0.600 g and 6.400 g, respectively. Fiber length (FL) exhibited a mean of 28.859 mm with a range of

minimum and maximum values 22.035 mm and 35.140 mm, respectively. Mean value estimated for fiber uniformity (FU) was 84.293 with a minimum and maximum range of values 75.800 and 88.650, respectively. Fiber micronaire (MIC) was calculated with a mean value of 4.681 µg/inch and minimum and maximum range of 2.105 µg/inch and 6.750 µg/inch, respectively. Fiber strength (FS) showed a mean value of 28.689 g/tex and a range of minimum value 22.900 g/tex and maximum value 41.880 g/tex. The mean value estimated for fiber elongation (FE) was 7.148 mm and range of minimum and maximum values observed were 2.633 mm and 9.700 mm, respectively.

Trait	Ν	DF	Mean	Std Dev	Sum	Minimum	Maximum
BW	3448	3447.00	4.9654	0.9125	17120.9	1.1000	8.6000
FW	3458	3446.16	1.9232	0.4296	6647.10	0.3000	3.7780
GOT	3447	3446.00	38.6372	4.5350	133193	20.2128	49.6753
SW	3457	3456.00	2.9805	0.5933	10301.3	0.6000	6.4000
FL	3045	3040.56	28.8596	1.6263	87907.2	22.0350	35.1400
FU	3045	3044.00	84.2934	1.5248	256723	75.8000	88.6500
MIC	3045	2955.13	4.6810	0.6638	14303.1	2.1050	6.7500
FS	3045	3018.69	28.6890	2.7286	87209.1	22.9000	41.8000
FE	3045	3024.61	7.1485	0.7663	21813.8	2.6333	9.7000

Table 1. Summary statistics agronomic and fiber quality related traits

Boll weight g (BW), seed weight g (SW), ginning outturn % (GOT), fiber weight g (FW), fiber length mm (FL), fiber uniformity % (FU), micronaire  $\mu$ g/inch (MIC), fiber strength g/tex (FS), fiber elongations mm (FE)

Results of analysis of variance (ANOVA) conducted via linear mixed model for 355 accessions with five treatments across two years have been indicated in *Table 2*. The outcomes indicated the high significant effects of years on all accessions regarding all the nine traits ( $\leq 0.0001$ ). Besides, treatments exhibited highly significant effects on accessions for BW, FW, GOT, SW and FL ( $\leq 0.0001$ ) while for MIC treatments depicted significant effects ( $\leq 0.01$ ). Additionally, all the accessions presented highly significant differences under all the treatments across two years for all the nine measured traits ( $\leq 0.0001$ ).

Correlation and its distribution related to nine studied traits were estimated to reveal the relationship between them presented in *Figure 1*. Upper triangle of the Correlogram depicted correlations among traits. However, lower triangle exhibited scatterplot matrix representing their distributions. All the traits displayed highly significant ( $\leq 0.0001$ ) positive correlations among themselves except two yield related traits i.e., BW and SW and two fiber quality traits i.e., FU and MIC which exhibited non-significant negative correlations with FL, FS and FE. Highly significant negative correlations were displayed by SW with GOT, MIC with FL, FS with FW and GOT, FE with SW, MIC and FS (*Fig. 1*).

All experimental accessions have been subjected to subtending leaf removal at different days after emergence of flower as treatments, i.e., 35D, 40D, 50D and 60D and a Control (C) with no subtending leaf removal. Data collected for all yield-related and fiber quality traits under all treatments have been subjected to statistical analysis to find out the impact of subtending leaf removal on different time intervals.

Sourc	e	Genotype	Treatment	year	
Nparr	n	354	4	1	
DFNu	m	354	4	1	
DW	F Ratio	5.557041	75.33325	25.42339	
DW	Prob > F	<.0001*	<.0001*	<.0001*	
	F Ratio	7.862929	658.4304	82.22511	
FW	Prob > F	<.0001*	<.0001*	<.0001*	
COT	F Ratio	18.43691	6.065353	3072.598	
GOI	Prob > F	<.0001*	<.0001*	<.0001*	
CW	F Ratio	6.348199	67.1953	201.1665	
5 W	Prob > F	> F <001* <00		<.0001*	
EI	F Ratio	15.63886	6.876369	552.8028	
ГL	Prob > F	<.0001*	<.0001*	<.0001*	
	F Ratio	3.704751	1.359658	59.3276	
FU	Prob > F	<.0001*	0.2455	<.0001*	
MIC	F Ratio	14.80133	2.95762	2814.855	
IVIIC	Prob > F	<.0001*	0.0188*	<.0001*	
EC	F Ratio	12.50008	1.543339	138.158	
гЗ	Prob > F	<.0001*	0.1869	<.0001*	
EE	F Ratio	7.347364	0.127697	1513.783	
ГЕ	Prob > F	<.0001*	0.9724	<.0001*	

 Table 2. Mixed model effect test

Boll weight g (BW), seed weight g (SW), ginning outturn % (GOT), fiber weight g (FW), fiber length mm (FL), fiber uniformity % (FU), micronaire  $\mu$ g/inch (MIC), fiber strength g/tex (FS), fiber elongations mm (FE)



*Figure 1.* Correlogram depicting correlations among nine studied traits in upper triangle and their scatterplot matrix in lower triangle. The legend on the top right corner is representing color gradient according to the positive and negative values of correlation

The 3-dimensional Surface Profiler Graph (Fig. 2) clearly showed that the subtending leaf removal across the years has highly significant differences for yield-related traits making a gesture to consider these characters more influenced by environmental factors. However, the fiber quality traits have minimum or non-significant effects of leaf removal treatments across years supporting the idea of a minimum effect of year or environmental effect on fiber quality.



**Figure 2.** A panel of 3-dimensional Surface profiling graphs based on linear mixed model depicting effect of subtending leaf removal having highly significant differences among fiber quality and yield related traits across two years being influenced by environmental factors

The results have revealed that most of the yield related traits including, BW, SW, LW and GOT% have shown a substantial influence of subtending leaf removal across different dates after flower initiation. Among all the treatments, 35D subtending leaf removal has shown maximum or reduction in these traits in comparison to the Control (no leaf removal). 40D and 50D also exhibited significant impact on yield traits but less than those in response to leaf removal after 35D. Minimum impact of leaf removal has been represented at 60D as compared to Control (C) showing a minimum decline in these traits under study.

As far as fiber quality characters are concerned most of the fiber quality traits have shown a non-significant effect of subtending leaf removal through various treatments. As far as 35D, 40D and 50D leaf removal are compared to the control (C), they presented a nominal decreasing trend with non-significant values but the subtending leaf removal at

60D has shown a prominent increase, although non-significant but is sufficient to conclude that at this time fiber quality got enhanced with this removal resulting from a stressed condition.

All pairwise comparisons using Tuckey (HSK) test as represented in (*Fig. 3a; Table A1*) it could be clearly comprehended that boll weight has been significantly affected by subtending leaf removal in all treatments at 35D, 40D, 50D, and 60D as compared to Control (C) across both years as a significant decrease in boll weight was observed during 2018 and 2019. During the year 2018, while comparing to Control (C), boll weight was significantly decreased by 10.73% at 35D, 9.57% at 40D. 7% at 50D and 3.04% at 60D. Similarly, during the second year a substantial decrease in boll weight has been observed as 11.7%, 10%, 8.2% and 7.9% at 35D, 50D, 40D, and 60D respectively, as compared to Control (C).



Figure 3. (a-i) Changes in trend of different fiber quality and yield related traits of cotton with effects imposed by removal of subtending leaf at different boll age treatments in comparison with control (C)

The cotton FW has shown to be significantly affected by removal of subtending leaf through all the treatments i.e. at 35D, 40D, 50D, and 60D during both the years 2018 and 2019 (*Fig. 3b; Table A2*). A significant decrease has been observed for fiber weight at 35D, 40D, 50D, and 60D during the first year as compared to the Control (C) as 12.5%, 8.9%, 12.1% and 7.1% respectively. During the second year a similar trend has been

observed for fiber weight decrease by 12.9%, 11.9%, 7.77%, and 5.69% for 35D, 40D, 50D, and 60D respectively as compared to Control (C).

The results for GOT presented a significant effect in it by removal of subtending leaf under different treatments, i.e., 35D, 40D, 50D and 60D across both years (*Fig. 3c; Table A3*). As shown in the results there were no significant differences observed during 2018 for GOT% with subtending leaf removal on 35D (36.3%), 40D (36.2%), 50D (36.5%) and with 60D it was 36.6%. A similar trend for GOT% has also been observed for second year with no substantial effect of subtending leaf removal at different time intervals as compared to the Control (C).

The results for SW also depicted a significant effect of subtending leaf removal under different treatments (*Fig. 3d; Table A4*). Also, the significant differences were observed for SW through the years 2018 as well as 2019. During these years, the highest decrease in SW as compared to Control (C) was observed under 35D treatment (11.3%), followed by 40D treatment (10.3%) with the lowest decrease. A similar trend in SW decrease has also been observed from Control (C) as during second year 2019 this decrease was 11.3%, 7.4% 8.4 and 7.7. It can be clearly perceived that removal of subtending leaf at 60D has the lowest seed weight decrease in both the years.

As far as FL was concerned, it has not been affected by subtending leaf removal under any treatment (*Fig. 3e; Table A5*) in comparison to Control (C) during both the years. Similar results were also depicted for FU with no significant effect of subtending leaf under all treatments as compared to Control (C) across the two years (*Fig. 3f; Table A6*). The results have also exhibited the non-effectiveness of all treatments of subtending leaf removal on MIC across both the years 2018 and 2019 as compared to the Control (C) with no subtending leaf removal (*Fig. 3g; Table A7*). Similarly, FS also has no significant impact of subtending leaf removal at different boll ages as treatments across both the years (*Fig. 3h; Table A8*). FE also revealed to be non-significantly affected in response to all treatments of subtending leaf removal across both years of investigation (*Fig. 3i; Table A9*).

#### Discussion

Cotton varieties with better adaptability and performance even after the removal of subtending leaf at some stages of boll development are crucial for sustainable cotton production. We analyzed comprehensive data of two years and four treatments in relation with fiber quality and yield-related traits to reveal their correlations, genetic variance, and their effects. As per cotton plant, approximately 60–87% of carbon is transferred from respective subtending leaf of a particular mature boll (immediate leaf below that boll) (Ashley, 1972; Wullschleger and Oosterhuis, 1990). Hence, there exists a distinct role of subtending leaf during developmental phase regarding improvement of cotton yield, specifically boll weight. Carbohydrates have to cross an ordered series of different boll parts during their distribution viz; from the boll wall to seed and then to fiber. Amassing of cellulose in fiber depends on every step of this pathway. Thus, transference and accretion of carbohydrates from leaf to boll parts directly affect development of the fiber and ultimately, its quality and yield (Liu et al., 2014). Highest demands for carbohydrates, nutrients and water in plant arise at the start of blooming phase and go on until boll opening. Thereby, shortages in any of the mentioned supplements during specific phases may cause a reduction in yield. In the previous study, it has been conveyed that with an increase of bolls and fruiting branch number, there occur a transition of plant growth from

vegetative to reproductive one and thus physiologically cotton plant gets matured (Liu et al., 2014). At this stage, high carbon gets accumulated in subtending leaf as its export to the boll slows down comparatively.

All the studied genotypes depicted considerable variations when analyzed through analysis of variance (ANOVA) for fiber quality traits FE, FU, FS, MIC and FL and yield related traits such as BW, FW, SW and GOT. The results indicated highly significant variation advocated in favor of diversity in genetic material and recommendation for further studies. Many other cotton researchers previously discovered variability of yield traits as well as fiber quality (Ahmad et al., 2012; Rahman et al., 2013; Haidar et al., 2012; Kitajima, 1996). Existence of variation in phenological traits strongly supports the concept of further research to plant breeders regarding improvement of these traits via conduction of effective breeding programs (Ahsan et al., 2015). To carry out further experimentations, numerous researchers executed mean performance of cotton genotypes prior to in-depth study of morphological, physiological and yield traits (Arshad et al., 1993).

The correlation matrix is used to investigate the dependence between multiple variables at the same time. Pearson correlation was analyzed for all traits to detect association among them (Farooq et al., 2014) and different levels of correlation got exhibited among all traits. The significant positive associations among fiber quality parameters, i.e., FU, FL, FS, FE and MIC observed in our study, have already been reported (Wan et al., 2007; Herring et al., 2004). Also, like earlier findings the current investigation too found negative correlations among yield and fiber quality related traits. For instance, highly significant negative correlations have been exhibited by FE with SW and FS with FW and GOT (Clement et al., 2012). However, GOT % revealed highly significant positive correlation with MIC as reported previously (Saeed et al., 2014; Farooq et al., 2014) MIC depicted significant negative correlation with FL found formerly (Rao and Mary, 1996; Desalegn et al., 2009). One of the liable mechanisms behind such negative correlations is repulsive linkage. In these instances, outstanding genotypes harboring desirable traits related to yield and fiber quality can be utilized as recurrent parents to overwhelm negative correlations in selection breeding programs (Clement et al., 2012). In earlier findings, FS and MIC revealed positive correlation with each other like current study. They have been reported in association with the developmental process of fibers and cellulose deposition within fibers (Wang et al., 2009). Micronaire formation and fiber strength could possibly be affected by altered relationship of carbohydrates source and reproductive sink i.e., boll that largely occur due to environmental conditions. (Lv et al., 2013) Although this fluctuated supply of photosynthetic assimilate to developing bolls, it primarily affects yield of fiber rather than its quality (Pettigrew et al., 2001). Subsequently, it is essentially important to get a true picture about consequent responses of subtending or single-leaf photosynthesis for the enhancement of fiber quality and yield (Peng, 2000; Sun et al., 2009). In current study, yield traits got highly influenced from removal of subtending leaf at different boll ages in both years depicting that these polygenic traits are more prone to surrounding environment and thus their heritability is comparatively less. However, fiber quality attributes got less influenced from environment in both years suggesting that these traits have more genetic effects and so, highly heritable. During boll development, quality of fiber is largely dependent on nutrition as well as surrounding environment. Photosynthetic assimilate produced by the leaf is utilized by its own self for growth during the first 16 days after its unfolding. From 16<sup>th</sup> day to 25<sup>th</sup> day, the leaf extents to peak regarding assimilate production and can

disseminate to developing bolls. Then, at age of 4 weeks, the production rate starts to decline until around 60<sup>th</sup> day and consequently no export of sugars takes place. This scenario is in congruence with current findings in which investigated traits got minimum or no effect from removal of subtending leaf at 60D elaborating the concept of slow or no production of carbohydrates by this immediate leaf of boll. Unfortunately, a cotton plant requires a vigorous canopy for bolls filling and maturation at the time when plant is leading to aging phase and environmental conditions are becoming unfavorable accounting lesser nutrient availability, day length, temperature and air quality. Influential management practices comprise of sufficient availability of water and midseason nutrients together with no chemical and insect damage to upper canopy.

# Conclusions

Regardless of the extent of growing season, management practices deliberately influence yield in a positive manner involving provision of healthy young leaves, particularly subtending leaf at the critical phase of boll filling. With the passage of improvements in production technologies and varieties, there will be a dire need to critically maintain young healthy leaves from blooming phase until boll filling for yield increment. Thus, defoliation at 60D can help to increase the efficiency of improved management practices as well as mechanized harvesting.

**Acknowledgments.** The study was financially supported by the Major Research Plan of the National Natural Science Foundation of China (grant no. 31690093) and the National Natural Science Foundation of China (grant no. 31701474).

Conflict of interests. The authors declare no conflict of interests.

#### REFERENCES

- Ahmad, K., Bhatti, I. A., Muneer, M., Iqbal, M., Iqbal, Z. (2012): Removal of heavy metals (Zn, Cr, Pb, Cd, Cu and Fe) in aqueous media by calcium carbonate as an adsorbent. – International Journal of Chemical and Biochemical Sciences 2: 48-53.
- [2] Ahsan, M. Z., Majidano, M. S., Bhutto, H., Soomro, A. W., Panhwar, F. H., Channa, A. R., Sial, K. B. (2015): Genetic variability, coefficient of variance, heritability and genetic advance of some Gossypium hirsutum L. accessions. Journal of Agricultural Science 7: 147.
- [3] Ali, B., Iqbal, M. S., Shah, M. K. N., Shabbir, G., Cheema, N. M. (2011): Genetic analysis for various traits in Gossypium hirsutum L. Pakistan Journal of Agricultural Research 24.
- [4] Arshad, S., Stevens, M., Hide, D. (1993): The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years. Clinical & Experimental Allergy 23: 504-511.
- [5] Ashley, D. A. (1972): C-labelled photosynthate translocation and utilization in cotton plants 1. Crop science 12: 69-74.
- [6] Boquet, D. J., Clawson, E. L. (2009): Cotton planting date: yield, seedling survival, and plant growth. Agronomy Journal 101: 1123-1130.
- [7] Campbell, B., Chee, P., Lubbers, E., Bowman, D., Meredith, W., Johnson, J., Fraser, D. (2011): Genetic improvement of the Pee Dee cotton germplasm collection following seventy years of plant breeding. – Crop science 51: 955-968.

- [8] Clement, J., Constable, G., Stiller, W., Liu, S. (2012): Negative associations still exist between yield and fibre quality in cotton breeding programs in Australia and USA. Field Crops Research 128: 1-7.
- [9] Constable, G., Rawson, H. (1980): Carbon production and utilization in cotton: inferences from a carbon budget. Functional Plant Biology 7: 539-553.
- [10] Desalegn, Z., Ratanadilok, N., Kaveeta, R. (2009): Correlation and heritability for yield and fiber quality parameters of Ethiopian cotton (Gossypium hirsutum L.) estimated from 15 (diallel) crosses. Agriculture and Natural Resources 43: 1-11.
- [11] Farooq, J., Anwar, M., Riaz, M., Farooq, A., Mahmood, A., Shahid, M., Rafiq, M., Ilahi, F. (2014): Correlation and path coefficient analysis of earliness, fiber quality and yield contributing traits in cotton (Gossypium hirsutum L.). – JAPS: Journal of Animal & Plant Sciences 24.
- [12] Gapare, W., Conaty, W., Zhu, Q.-H., Liu, S., Stiller, W., Llewellyn, D., Wilson, I. (2017): Genome-wide association study of yield components and fibre quality traits in a cotton germplasm diversity panel. – Euphytica 213.
- [13] Haidar, S., Aslam, M., Hassan, M., Hassan, H. M., Ditta, A. (2012): Genetic diversity among upland cotton genotypes for different economic traits and response to cotton leaf curl virus (CLCV) disease. – Pak. J. Bot 44: 1779-1784.
- [14] Hendrix, D. L., Grange, R. I. (1991): Carbon partitioning and export from mature cotton leaves. – Plant Physiology 95: 228-233.
- [15] Herring, A. D., Auld, D. L., Ethridge, M. D., Hequet, E. F., Bechere, E., Green, C. J., Cantrell, R. G. (2004): Inheritance of fiber quality and lint yield in a chemically mutated population of cotton. – Euphytica 136: 333-339.
- [16] Jarwar, A. H., Wang, X., Iqbal, M. S., Sarfraz, Z., Wang, L., Ma, Q., Shuli, F. (2019): Genetic divergence on the basis of principal component, correlation and cluster analysis of yield and quality traits in cotton cultivars. – Pak. J. Bot 51: 1143-1148.
- [17] Kitajima, K. (1996): Ecophysiology of Tropical Tree Seedlings. In: Mulkey, S. S. et al. (eds.) Tropical Forest Plant Ecophysiology. Springer, Boston.
- [18] Lee, J. J., Woodward, A. W., Chen, Z. J. (2007): Gene expression changes and early events in cotton fibre development. Oxford Journals 100: 1391-401.
- [19] Liu, J., Wang, Y., Chen, J., Lv, F., Ma, Y., Meng, Y., Chen, B., Zhou, Z. (2014): Sucrose metabolism in the subtending leaf to cotton boll at different fruiting branch nodes and the relationship to boll weight. – The Journal of Agricultural Science 152: 790.
- [20] Luo, Q., Bange, M., Johnston, D. (2016): Environment and cotton fibre quality. Climatic Change 138: 207-221.
- [21] Lv, F., Liu, J., Ma, Y., Chen, J., Keyoumu Abudurezikekey, A., Wang, Y., Chen, B., Meng, Y., Zhou, Z. (2013): Effect of shading on cotton yield and quality on different fruiting branches. – Crop Science 53: 2670-2678.
- [22] Oosterhuis, D., Kerby, T., Hake, K. (1990): Leaf physiology and management. Cotton Physiology Today, May 1990. https://www.cotton.org/tech/physiology/cpt/plantphysiology/upload/CPT-May90-REPOP-144.pdf.
- [23] Pace, P., Cralle, H. T., Cothren, J. T., Senseman, S. A. (1999): Photosynthate and dry matter partitioning in short-and long-season cotton cultivars. Crop Science 39: 1065-1069.
- [24] Peng, S. (2000): Single-leaf and canopy photosynthesis of rice. Studies in Plant Science 7: 213-228.
- [25] Pettigrew, A. M., Woodman, R. W., Cameron, K. S. (2001): Studying organizational change and development: challenges for future research. – Academy of Management Journal 44: 697-713.
- [26] Pettigrew, W., Gerik, T. (2007): Cotton leaf photosynthesis and carbon metabolism. Advances in Agronomy 94: 209-236.

- [27] Rahman, S. A., Iqbal, M. S., Riaz, M., Mahmood, A., Shahid, M. R., Abbas, G., Farooq, J. (2013): Cause and effect estimates for yield contributing and morphological traits in upland cotton (Gossypium hirsutum L.). – J. Agric. Res 51.
- [28] Rao, K., Mary, T. (1996): Variability, correlation and path analysis of yield and fibre traits in upland cotton. J. Res. APAU 24: 66-70.
- [29] Reddy, K., Hodges, H., Reddy, V. (1992): Temperature effects on cotton fruit retention. Agronomy Journal 84: 26-30.
- [30] Richards, R. (2000): Selectable traits to increase crop photosynthesis and yield of grain crops. Journal of Experimental Botany 51: 447-458.
- [31] Saeed, F., Farooq, J., Mahmood, A., Hussain, T., Riaz, M., Ahmad, S. (2014): Genetic diversity in upland cotton for cotton leaf curl virus disease, earliness and fiber quality. – Pakistan Journal of Agricultural Research 27.
- [32] Sarfraz, Z., Iqbal, M. S., Pan, Z., Jia, Y., He, S., Wang, Q., Qin, H., Liu, J., Liu, H., Yang, J. (2018): Integration of conventional and advanced molecular tools to track footprints of heterosis in cotton. BMC genomics 19: 1-19.
- [33] Sun, C., Qi, H., Hao, J., Miao, L., Wang, J., Wang, Y., Liu, M., Chen, L. (2009): Single leaves photosynthetic characteristics of two insect-resistant transgenic cotton (Gossypium hirsutum L.) varieties in response to light. – Photosynthetica 47: 399-408.
- [34] Wan, Q., Zhang, Z., Hu, M., Chen, L., Liu, D., Chen, X., Wang, W., Zheng, J. (2007): T 1 locus in cotton is the candidate gene affecting lint percentage, fiber quality and spiny bollworm (Earias spp.) resistance. – Euphytica 158: 241-247.
- [35] Wang, W. (2007): The Associations of photosynthesis and grain filling during grain filling period in flag leaves of wheat species. Doctoral Thesis, Ocean University of China.
- [36] Wang, K., Li, S., Song, G., Chen, G., Cao, S. (2002): Studies on cultivated physiological indexes for high-yielding cotton in Xinjiang. Scientia Agricultura Sinica 35: 638-644.
- [37] Wang, Y., Shu, H., Chen, B., Mcgiffen, M. E., Zhang, W., Xu, N., Zhou, Z. (2009): The rate of cellulose increase is highly related to cotton fibre strength and is significantly determined by its genetic background and boll period temperature. – Plant Growth Regulation 57: 203-209.
- [38] Wendel, J. F. (1989): New World tetraploid cottons contain Old World cytoplasm. Proceedings of the National Academy of Sciences 86: 4132-4136.
- [39] Wendel, J. F., Cronn, R. C. (2003): Polyploidy and the evolutionary history of cotton. Advances in Agronomy 78: 139.
- [40] Wendel, J. F., Brubaker, C. L., Seelanan, T. (2010): The Origin and Evolution of Gossypium. – In: Stewart J. M., Oosterhuis D. M., Heitholt J. J., Mauney J. R. (eds.) Physiology of Cotton. Springer, Dordrecht.
- [41] Wullschleger, S., Oosterhuis, D. (1990): Photosynthetic carbon production and use by developing cotton leaves and bolls. Crop Science 30: 1259-1264.
- [42] Xiangbin, G., Youhua, W., Zhiguo, Z., Oosterhuis, D. M. (2012): Response of cotton fiber quality to the carbohydrates in the leaf subtending the cotton boll. – Journal of Plant Nutrition and Soil Science 175: 152-160.
- [43] Xie, Z., Jiang, D., Cao, W., Dai, T., Jing, Q. (2003): Relationships of endogenous plant hormones to accumulation of grain protein and starch in winter wheat under different post-anthesis soil water statusses. Plant Growth Regulation 41: 117-127.
- [44] Zeng, L., Meredith Jr, W. R. (2009): Associations among lint yield, yield components, and fiber properties in an introgressed population of cotton. Crop Science 49: 1647-1654.
- [45] Zhang, Y., Wang, X., Li, Z., Zhang, G., Ma, Z. (2011): Assessing genetic diversity of cotton cultivars using genomic and newly developed expressed sequence tag-derived microsatellite markers. – Genet. Mol. Res 10: 1462-1470.

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## APPENDIX

Year	Treatment	Year	Treatment	Difference	Std error	t ratio	Prob> t	Lower 95%	Upper 95%
Y18	35D	Y18	40D	-0.130779	0.0474891	-2.75	0.1530	-0.281119	0.019561
Y18	35D	Y18	50D	-0.147869	0.0469833	-3.15	0.0528	-0.296608	0.000870
Y18	35D	Y18	60D	-0.303904	0.0473452	-6.42	<.0001*	-0.453789	-0.154020
Y18	35D	Y18	Control	-0.600009	0.0469664	-12.78	<.0001*	-0.748694	-0.451324
Y18	35D	Y19	35D	-0.114382	0.0299192	-3.82	0.0052*	-0.209100	-0.019665
Y18	35D	Y19	40D	-0.245162	0.0558842	-4.39	0.0005*	-0.422079	-0.068244
Y18	35D	Y19	50D	-0.262252	0.0557818	-4.70	0.0001*	-0.438845	-0.085658
Y18	35D	Y19	60D	-0.418286	0.0558375	-7.49	<.0001*	-0.595056	-0.241517
Y18	35D	Y19	Control	-0.714391	0.0557334	-12.82	<.0001*	-0.890831	-0.537952
Y18	40D	Y18	50D	-0.017090	0.0474409	-0.36	1.0000	-0.167277	0.133097
Y18	40D	Y18	60D	-0.173125	0.0477949	-3.62	0.0110*	-0.324433	-0.021817
Y18	40D	Y18	Control	-0.469230	0.0474235	-9.89	<.0001*	-0.619362	-0.319097
Y18	40D	Y19	35D	0.016397	0.0563711	0.29	1.0000	-0.162062	0.194855
Y18	40D	Y19	40D	-0.114382	0.0299192	-3.82	0.0052*	-0.209100	-0.019665
Y18	40D	Y19	50D	-0.131472	0.0564105	-2.33	0.3692	-0.310056	0.047111
Y18	40D	Y19	60D	-0.287507	0.0564618	-5.09	<.0001*	-0.466253	-0.108762
Y18	40D	Y19	Control	-0.583612	0.0563621	-10.35	<.0001*	-0.762042	-0.405182
Y18	50D	Y18	60D	-0.156035	0.0472964	-3.30	0.0331*	-0.305765	-0.006305
Y18	50D	Y18	Control	-0.452140	0.0469159	-9.64	<.0001*	-0.600665	-0.303614
Y18	50D	Y19	35D	0.033487	0.0556199	0.60	0.9999	-0.142593	0.209567
Y18	50D	Y19	40D	-0.097292	0.0557624	-1.74	0.7696	-0.273824	0.079239
Y18	50D	Y19	50D	-0.114382	0.0299192	-3.82	0.0052*	-0.209100	-0.019665
Y18	50D	Y19	60D	-0.270417	0.0557152	-4.85	<.0001*	-0.446799	-0.094035
Y18	50D	Y19	Control	-0.566522	0.0556097	-10.19	<.0001*	-0.742570	-0.390474
Y18	60D	Y18	Control	-0.296105	0.0472792	-6.26	<.0001*	-0.445780	-0.146429
Y18	60D	Y19	35D	0.189522	0.0561750	3.37	0.0260*	0.011684	0.367359
Y18	60D	Y19	40D	0.058743	0.0563123	1.04	0.9896	-0.119530	0.237015
Y18	60D	Y19	50D	0.041653	0.0562142	0.74	0.9992	-0.136309	0.219614
Y18	60D	Y19	60D	-0.114382	0.0299192	-3.82	0.0052*	-0.209100	-0.019665
Y18	60D	Y19	Control	-0.410487	0.0561657	-7.31	<.0001*	-0.588295	-0.232679
Y18	Control	Y19	35D	0.485626	0.0556399	8.73	<.0001*	0.309483	0.661770
Y18	Control	Y19	40D	0.354847	0.0557819	6.36	<.0001*	0.178254	0.531440
Y18	Control	Y19	50D	0.337757	0.0556784	6.07	<.0001*	0.161492	0.514023
Y18	Control	Y19	60D	0.181722	0.0557349	3.26	0.0374*	0.005278	0.358167
Y18	Control	Y19	Control	-0.114382	0.0299192	-3.82	0.0052*	-0.209100	-0.019665
Y19	35D	Y19	40D	-0.130779	0.0474891	-2.75	0.1530	-0.281119	0.019561
Y19	35D	Y19	50D	-0.147869	0.0469833	-3.15	0.0528	-0.296608	0.000870
Y19	35D	Y19	60D	-0.303904	0.0473452	-6.42	<.0001*	-0.453789	-0.154020
Y19	35D	Y19	Control	-0.600009	0.0469664	-12.78	<.0001*	-0.748694	-0.451324
Y19	40D	Y19	50D	-0.017090	0.0474409	-0.36	1.0000	-0.167277	0.133097
Y19	40D	Y19	60D	-0.173125	0.0477949	-3.62	0.0110*	-0.324433	-0.021817
Y19	40D	Y19	Control	-0.469230	0.0474235	-9.89	<.0001*	-0.619362	-0.319097
Y19	50D	Y19	60D	-0.156035	0.0472964	-3.30	0.0331*	-0.305765	-0.006305
Y19	50D	Y19	Control	-0.452140	0.0469159	-9.64	<.0001*	-0.600665	-0.303614
Y19	60D	Y19	Control	-0.296105	0.0472792	-6.26	<.0001*	-0.445780	-0.146429

**Table A1.** Tukey (HSD) test for all pairwise comparisons representing pairwise differences for boll weight under all treatments across two years

Year	Treatment	Year	Treatment	Difference	Std error	t ratio	Prob> t	Lower 95%	Upper 95%
Y18	35D	Y18	40D	-0.056921	0.0215215	-2.64	0.1973	-0.125053	0.011211
Y18	35D	Y18	50D	-0.050014	0.0212990	-2.35	0.3580	-0.117442	0.017414
Y18	35D	Y18	60D	-0.126987	0.0214560	-5.92	<.0001*	-0.194912	-0.059062
Y18	35D	Y18	Control	-0.260676	0.0212687	-12.26	<.0001*	-0.328008	-0.193344
Y18	35D	Y19	35D	-0.262687	0.0135672	-19.36	<.0001*	-0.305637	-0.219736
Y18	35D	Y19	40D	-0.319607	0.0253096	-12.63	<.0001*	-0.399732	-0.239483
Y18	35D	Y19	50D	-0.312700	0.0252634	-12.38	<.0001*	-0.392678	-0.232722
Y18	35D	Y19	60D	-0.389674	0.0252882	-15.41	<.0001*	-0.469730	-0.309617
Y18	35D	Y19	Control	-0.523362	0.0252380	-20.74	<.0001*	-0.603260	-0.443465
Y18	40D	Y18	50D	0.006907	0.0215367	0.32	1.0000	-0.061273	0.075087
Y18	40D	Y18	60D	-0.070067	0.0216897	-3.23	0.0411*	-0.138731	-0.001402
Y18	40D	Y18	Control	-0.203755	0.0215067	-9.47	<.0001*	-0.271841	-0.135670
Y18	40D	Y19	35D	-0.205766	0.0255717	-8.05	<.0001*	-0.286720	-0.124812
Y18	40D	Y19	40D	-0.262687	0.0135672	-19.36	<.0001*	-0.305637	-0.219736
Y18	40D	Y19	50D	-0.255779	0.0255947	-9.99	<.0001*	-0.336806	-0.174753
Y18	40D	Y19	60D	-0.332753	0.0256172	-12.99	<.0001*	-0.413851	-0.251655
Y18	40D	Y19	Control	-0.466442	0.0255696	-18.24	<.0001*	-0.547389	-0.385494
Y18	50D	Y18	60D	-0.076974	0.0214713	-3.58	0.0126*	-0.144947	-0.009000
Y18	50D	Y18	Control	-0.210662	0.0212839	-9.90	<.0001*	-0.278042	-0.143282
Y18	50D	Y19	35D	-0.212673	0.0252428	-8.43	<.0001*	-0.292586	-0.132760
Y18	50D	Y19	40D	-0.269594	0.0253123	-10.65	<.0001*	-0.349727	-0.189461
Y18	50D	Y19	50D	-0.262687	0.0135672	-19.36	<.0001*	-0.305637	-0.219736
Y18	50D	Y19	60D	-0.339660	0.0252908	-13.43	<.0001*	-0.419725	-0.259595
Y18	50D	Y19	Control	-0.473349	0.0252405	-18.75	<.0001*	-0.553255	-0.393443
Y18	60D	Y18	Control	-0.133689	0.0214412	-6.24	<.0001*	-0.201567	-0.065811
Y18	60D	Y19	35D	-0.135699	0.0254828	-5.33	<.0001*	-0.216372	-0.055027
Y18	60D	Y19	40D	-0.192620	0.0255497	-7.54	<.0001*	-0.273505	-0.111735
Y18	60D	Y19	50D	-0.185713	0.0255058	-7.28	<.0001*	-0.266459	-0.104967
Y18	60D	Y19	60D	-0.262687	0.0135672	-19.36	<.0001*	-0.305637	-0.219736
Y18	60D	Y19	Control	-0.396375	0.0254807	-15.56	<.0001*	-0.477041	-0.315709
Y18	Control	Y19	35D	-0.002011	0.0252170	-0.08	1.0000	-0.081842	0.077821
Y18	Control	Y19	40D	-0.058931	0.0252866	-2.33	0.3693	-0.138983	0.021120
Y18	Control	Y19	50D	-0.052024	0.0252401	-2.06	0.5554	-0.131929	0.027880
Y18	Control	Y19	60D	-0.128998	0.0252651	-5.11	<.0001*	-0.208982	-0.049014
Y18	Control	Y19	Control	-0.262687	0.0135672	-19.36	<.0001*	-0.305637	-0.219736
Y19	35D	Y19	40D	-0.056921	0.0215215	-2.64	0.1973	-0.125053	0.011211
Y19	35D	Y19	50D	-0.050014	0.0212990	-2.35	0.3580	-0.117442	0.017414
Y19	35D	Y19	60D	-0.126987	0.0214560	-5.92	<.0001*	-0.194912	-0.059062
Y19	35D	Y19	Control	-0.260676	0.0212687	-12.26	<.0001*	-0.328008	-0.193344
Y19	40D	Y19	50D	0.006907	0.0215367	0.32	1.0000	-0.061273	0.075087
Y19	40D	Y19	60D	-0.070067	0.0216897	-3.23	0.0411*	-0.138731	-0.001402
Y19	40D	Y19	Control	-0.203755	0.0215067	-9.47	<.0001*	-0.271841	-0.135670
Y19	50D	Y19	60D	-0.076974	0.0214713	-3.58	0.0126*	-0.144947	-0.009000
Y19	50D	Y19	Control	-0.210662	0.0212839	-9.90	<.0001*	-0.278042	-0.143282
Y19	60D	Y19	Control	-0.133689	0.0214412	-6.24	<.0001*	-0.201567	-0.065811

**Table A2.** Tukey (HSD) test for all pairwise comparisons representing pairwise differences for fiber weight under all treatments across two years

Year	Treatment	Year	Treatment	Difference	Std error	t ratio	Prob> t	Lower 95%	Upper 95%
Y18	35D	Y18	40D	-0.082525	0.0307417	-2.68	0.1802	-0.179846	0.014797
Y18	35D	Y18	50D	-0.123911	0.0304240	-4.07	0.0019*	-0.220226	-0.027595
Y18	35D	Y18	60D	-0.190826	0.0306599	-6.22	<.0001*	-0.287889	-0.093764
Y18	35D	Y18	Control	-0.366209	0.0303807	-12.05	<.0001*	-0.462387	-0.270031
Y18	35D	Y19	35D	0.225648	0.0193827	11.64	<.0001*	0.164287	0.287009
Y18	35D	Y19	40D	0.143123	0.0361543	3.96	0.0031*	0.028667	0.257580
Y18	35D	Y19	50D	0.101737	0.0360884	2.82	0.1303	-0.012511	0.215985
Y18	35D	Y19	60D	0.034822	0.0361256	0.96	0.9941	-0.079544	0.149187
Y18	35D	Y19	Control	-0.140561	0.0360521	-3.90	0.0039*	-0.254694	-0.026428
Y18	40D	Y18	50D	-0.041386	0.0307635	-1.35	0.9430	-0.138776	0.056004
Y18	40D	Y18	60D	-0.108302	0.0309934	-3.49	0.0173*	-0.206420	-0.010184
Y18	40D	Y18	Control	-0.283684	0.0307207	-9.23	<.0001*	-0.380939	-0.186430
Y18	40D	Y19	35D	0.308173	0.0365288	8.44	<.0001*	0.192531	0.423814
Y18	40D	Y19	40D	0.225648	0.0193827	11.64	<.0001*	0.164287	0.287009
Y18	40D	Y19	50D	0.184262	0.0365616	5.04	<.0001*	0.068516	0.300008
Y18	40D	Y19	60D	0.117346	0.0365955	3.21	0.0442*	0.001493	0.233199
Y18	40D	Y19	Control	-0.058036	0.0365258	-1.59	0.8538	-0.173669	0.057596
Y18	50D	Y18	60D	-0.066915	0.0306816	-2.18	0.4702	-0.164047	0.030216
Y18	50D	Y18	Control	-0.242298	0.0304024	-7.97	<.0001*	-0.338545	-0.146051
Y18	50D	Y19	35D	0.349559	0.0360589	9.69	<.0001*	0.235404	0.463713
Y18	50D	Y19	40D	0.267034	0.0361582	7.39	<.0001*	0.152565	0.381503
Y18	50D	Y19	50D	0.225648	0.0193827	11.64	<.0001*	0.164287	0.287009
Y18	50D	Y19	60D	0.158732	0.0361294	4.39	0.0005*	0.044355	0.273110
Y18	50D	Y19	Control	-0.016650	0.0360557	-0.46	1.0000	-0.130794	0.097494
Y18	60D	Y18	Control	-0.175383	0.0306387	-5.72	<.0001*	-0.272378	-0.078387
Y18	60D	Y19	35D	0.416474	0.0364194	11.44	<.0001*	0.301179	0.531770
Y18	60D	Y19	40D	0.333950	0.0365148	9.15	<.0001*	0.218352	0.449547
Y18	60D	Y19	50D	0.292563	0.0364523	8.03	<.0001*	0.177164	0.407963
Y18	60D	Y19	60D	0.225648	0.0193827	11.64	<.0001*	0.164287	0.287009
Y18	60D	Y19	Control	0.050265	0.0364164	1.38	0.9334	-0.065021	0.165552
Y18	Control	Y19	35D	0.591857	0.0360222	16.43	<.0001*	0.477819	0.705895
Y18	Control	Y19	40D	0.509332	0.0361215	14.10	<.0001*	0.394980	0.623685
Y18	Control	Y19	50D	0.467946	0.0360552	12.98	<.0001*	0.353803	0.582089
Y18	Control	Y19	60D	0.401031	0.0360927	11.11	<.0001*	0.286769	0.515292
Y18	Control	Y19	Control	0.225648	0.0193827	11.64	<.0001*	0.164287	0.287009
Y19	35D	Y19	40D	-0.082525	0.0307417	-2.68	0.1802	-0.179846	0.014797
Y19	35D	Y19	50D	-0.123911	0.0304240	-4.07	0.0019*	-0.220226	-0.027595
Y19	35D	Y19	60D	-0.190826	0.0306599	-6.22	<.0001*	-0.287889	-0.093764
Y19	35D	Y19	Control	-0.366209	0.0303807	-12.05	<.0001*	-0.462387	-0.270031
Y19	40D	Y19	50D	-0.041386	0.0307635	-1.35	0.9430	-0.138776	0.056004
Y19	40D	Y19	60D	-0.108302	0.0309934	-3.49	0.0173*	-0.206420	-0.010184
Y19	40D	Y19	Control	-0.283684	0.0307207	-9.23	<.0001*	-0.380939	-0.186430
Y19	50D	Y19	60D	-0.066915	0.0306816	-2.18	0.4702	-0.164047	0.030216
Y19	50D	Y19	Control	-0.242298	0.0304024	-7.97	<.0001*	-0.338545	-0.146051
Y19	60D	Y19	Control	-0.175383	0.0306387	-5.72	<.0001*	-0.272378	-0.078387

*Table A3. Tukey (HSD) test for all pairwise comparisons representing pairwise differences for seed weight under all treatments across two years* 

Year	Treatment	Year	Treatment	Difference	Std error	t ratio	Prob> t	Lower 95%	Upper 95%
Y18	35D	Y18	40D	-0.04202	0.2137274	-0.20	1.0000	-0.71863	0.63460
Y18	35D	Y18	50D	0.23273	0.2113697	1.10	0.9847	-0.43642	0.90188
Y18	35D	Y18	60D	-0.17825	0.2129979	-0.84	0.9980	-0.85256	0.49605
Y18	35D	Y18	Control	-0.34110	0.2112937	-1.61	0.8415	-1.01001	0.32781
Y18	35D	Y19	35D	-4.44016	0.1346230	-32.98	<.0001*	-4.86635	-4.01398
Y18	35D	Y19	40D	-4.48218	0.2514381	-17.83	<.0001*	-5.27818	-3.68618
Y18	35D	Y19	50D	-4.20743	0.2509645	-16.77	<.0001*	-5.00193	-3.41293
Y18	35D	Y19	60D	-4.61842	0.2512146	-18.38	<.0001*	-5.41371	-3.82313
Y18	35D	Y19	Control	-4.78126	0.2507463	-19.07	<.0001*	-5.57507	-3.98745
Y18	40D	Y18	50D	0.27475	0.2135108	1.29	0.9567	-0.40118	0.95068
Y18	40D	Y18	60D	-0.13624	0.2151017	-0.63	0.9998	-0.81720	0.54473
Y18	40D	Y18	Control	-0.29908	0.2134327	-1.40	0.9272	-0.97476	0.37660
Y18	40D	Y19	35D	-4.39815	0.2537409	-17.33	<.0001*	-5.20143	-3.59486
Y18	40D	Y19	40D	-4.44016	0.1346230	-32.98	<.0001*	-4.86635	-4.01398
Y18	40D	Y19	50D	-4.16541	0.2539184	-16.40	<.0001*	-4.96926	-3.36156
Y18	40D	Y19	60D	-4.57640	0.2541478	-18.01	<.0001*	-5.38098	-3.77182
Y18	40D	Y19	Control	-4.73924	0.2537004	-18.68	<.0001*	-5.54240	-3.93608
Y18	50D	Y18	60D	-0.41099	0.2127781	-1.93	0.6474	-1.08460	0.26262
Y18	50D	Y18	Control	-0.57383	0.2110664	-2.72	0.1664	-1.24202	0.09436
Y18	50D	Y19	35D	-4.67290	0.2502355	-18.67	<.0001*	-5.46509	-3.88070
Y18	50D	Y19	40D	-4.71491	0.2508902	-18.79	<.0001*	-5.50918	-3.92065
Y18	50D	Y19	50D	-4.44016	0.1346230	-32.98	<.0001*	-4.86635	-4.01398
Y18	50D	Y19	60D	-4.85115	0.2506642	-19.35	<.0001*	-5.64470	-4.05760
Y18	50D	Y19	Control	-5.01399	0.2501900	-20.04	<.0001*	-5.80604	-4.22195
Y18	60D	Y18	Control	-0.16284	0.2127008	-0.77	0.9990	-0.83621	0.51052
Y18	60D	Y19	35D	-4.26191	0.2527332	-16.86	<.0001*	-5.06201	-3.46181
Y18	60D	Y19	40D	-4.30393	0.2533635	-16.99	<.0001*	-5.10602	-3.50183
Y18	60D	Y19	50D	-4.02918	0.2529095	-15.93	<.0001*	-4.82983	-3.22852
Y18	60D	Y19	60D	-4.44016	0.1346230	-32.98	<.0001*	-4.86635	-4.01398
Y18	60D	Y19	Control	-4.60301	0.2526914	-18.22	<.0001*	-5.40297	-3.80304
Y18	Control	Y19	35D	-4.09907	0.2503259	-16.37	<.0001*	-4.89154	-3.30659
Y18	Control	Y19	40D	-4.14108	0.2509778	-16.50	<.0001*	-4.93562	-3.34654
Y18	Control	Y19	50D	-3.86633	0.2504989	-15.43	<.0001*	-4.65936	-3.07331
Y18	Control	Y19	60D	-4.27732	0.2507528	-17.06	<.0001*	-5.07115	-3.48349
Y18	Control	Y19	Control	-4.44016	0.1346230	-32.98	<.0001*	-4.86635	-4.01398
Y19	35D	Y19	40D	-0.04202	0.2137274	-0.20	1.0000	-0.71863	0.63460
Y19	35D	Y19	50D	0.23273	0.2113697	1.10	0.9847	-0.43642	0.90188
Y19	35D	Y19	60D	-0.17825	0.2129979	-0.84	0.9980	-0.85256	0.49605
Y19	35D	Y19	Control	-0.34110	0.2112937	-1.61	0.8415	-1.01001	0.32781
Y19	40D	Y19	50D	0.27475	0.2135108	1.29	0.9567	-0.40118	0.95068
Y19	40D	Y19	60D	-0.13624	0.2151017	-0.63	0.9998	-0.81720	0.54473
Y19	40D	Y19	Control	-0.29908	0.2134327	-1.40	0.9272	-0.97476	0.37660
Y19	50D	Y19	60D	-0.41099	0.2127781	-1.93	0.6474	-1.08460	0.26262
Y19	50D	Y19	Control	-0.57383	0.2110664	-2.72	0.1664	-1.24202	0.09436
Y19	60D	Y19	Control	-0.16284	0.2127008	-0.77	0.9990	-0.83621	0.51052

**Table A4.** Tukey (HSD) test for all pairwise comparisons representing pairwise differences for ginning outturn percentage% under all treatments across two years

Year	Treatment	Year	Treatment	Difference	Std error	t ratio	Prob> t	Lower 95%	Upper 95%
Y18	35D	Y18	40D	0.18020	0.0916220	1.97	0.6227	-0.10988	0.470280
Y18	35D	Y18	50D	-0.11913	0.0892518	-1.33	0.9456	-0.40171	0.163443
Y18	35D	Y18	60D	0.08109	0.0911403	0.89	0.9968	-0.20746	0.369650
Y18	35D	Y18	Control	0.03519	0.0880274	0.40	1.0000	-0.24351	0.313886
Y18	35D	Y19	35D	-0.81720	0.0578384	-14.13	<.0001*	-1.00032	-0.634078
Y18	35D	Y19	40D	-0.63700	0.1067681	-5.97	<.0001*	-0.97503	-0.298964
Y18	35D	Y19	50D	-0.93633	0.1065840	-8.78	<.0001*	-1.27378	-0.598880
Y18	35D	Y19	60D	-0.73610	0.1068373	-6.89	<.0001*	-1.07436	-0.397850
Y18	35D	Y19	Control	-0.78201	0.1064617	-7.35	<.0001*	-1.11908	-0.444948
Y18	40D	Y18	50D	-0.29933	0.0913562	-3.28	0.0356*	-0.58857	-0.010094
Y18	40D	Y18	60D	-0.09910	0.0931238	-1.06	0.9880	-0.39394	0.195730
Y18	40D	Y18	Control	-0.14501	0.0902143	-1.61	0.8449	-0.43064	0.140610
Y18	40D	Y19	35D	-0.99740	0.1099106	-9.07	<.0001*	-1.34538	-0.649414
Y18	40D	Y19	40D	-0.81720	0.0578384	-14.13	<.0001*	-1.00032	-0.634078
Y18	40D	Y19	50D	-1.11653	0.1099122	-10.16	<.0001*	-1.46452	-0.768543
Y18	40D	Y19	60D	-0.91630	0.1100916	-8.32	<.0001*	-1.26486	-0.567746
Y18	40D	Y19	Control	-0.96221	0.1098379	-8.76	<.0001*	-1.30996	-0.614459
Y18	50D	Y18	60D	0.20023	0.0908689	2.20	0.4545	-0.08747	0.487924
Y18	50D	Y18	Control	0.15432	0.0877265	1.76	0.7609	-0.12343	0.432067
Y18	50D	Y19	35D	-0.69806	0.1061234	-6.58	<.0001*	-1.03406	-0.362072
Y18	50D	Y19	40D	-0.51786	0.1063099	-4.87	<.0001*	-0.85445	-0.181282
Y18	50D	Y19	50D	-0.81720	0.0578384	-14.13	<.0001*	-1.00032	-0.634078
Y18	50D	Y19	60D	-0.61697	0.1063759	-5.80	<.0001*	-0.95376	-0.280178
Y18	50D	Y19	Control	-0.66288	0.1059822	-6.25	<.0001*	-0.99842	-0.327333
Y18	60D	Y18	Control	-0.04591	0.0897045	-0.51	1.0000	-0.32992	0.238101
Y18	60D	Y19	35D	-0.89829	0.1090388	-8.24	<.0001*	-1.24352	-0.553069
Y18	60D	Y19	40D	-0.71809	0.1091536	-6.58	<.0001*	-1.06368	-0.372506
Y18	60D	Y19	50D	-1.01743	0.1090370	-9.33	<.0001*	-1.36264	-0.672209
Y18	60D	Y19	60D	-0.81720	0.0578384	-14.13	<.0001*	-1.00032	-0.634078
Y18	60D	Y19	Control	-0.86311	0.1089486	-7.92	<.0001*	-1.20804	-0.518169
Y18	Control	Y19	35D	-0.85238	0.1041831	-8.18	<.0001*	-1.18223	-0.522534
Y18	Control	Y19	40D	-0.67218	0.1044197	-6.44	<.0001*	-1.00278	-0.341586
Y18	Control	Y19	50D	-0.97152	0.1041645	-9.33	<.0001*	-1.30131	-0.641727
Y18	Control	Y19	60D	-0.77129	0.1044729	-7.38	<.0001*	-1.10206	-0.440522
Y18	Control	Y19	Control	-0.81720	0.0578384	-14.13	<.0001*	-1.00032	-0.634078
Y19	35D	Y19	40D	0.18020	0.0916220	1.97	0.6227	-0.10988	0.470280
Y19	35D	Y19	50D	-0.11913	0.0892518	-1.33	0.9456	-0.40171	0.163443
Y19	35D	Y19	60D	0.08109	0.0911403	0.89	0.9968	-0.20746	0.369650
Y19	35D	Y19	Control	0.03519	0.0880274	0.40	1.0000	-0.24351	0.313886
Y19	40D	Y19	50D	-0.29933	0.0913562	-3.28	0.0356*	-0.58857	-0.010094
Y19	40D	Y19	60D	-0.09910	0.0931238	-1.06	0.9880	-0.39394	0.195730
Y19	40D	Y19	Control	-0.14501	0.0902143	-1.61	0.8449	-0.43064	0.140610
Y19	50D	Y19	60D	0.20023	0.0908689	2.20	0.4545	-0.08747	0.487924
Y19	50D	Y19	Control	0.15432	0.0877265	1.76	0.7609	-0.12343	0.432067
Y19	60D	Y19	Control	-0.04591	0.0897045	-0.51	1.0000	-0.32992	0.238101

**Table A5.** Tukey (HSD) test for all pairwise comparisons representing pairwise differences for fiber length under all treatments across two years

Year	Treatment	ear	Treatment	Difference	Std error	t ratio	Prob> t	Lower 95%	Upper 95%
Y18	35D	Y18	40D	0.104507	0.1572491	0.66	0.9997	-0.393352	0.602366
Y18	35D	Y18	50D	-0.046936	0.1531811	-0.31	1.0000	-0.531916	0.438044
Y18	35D	Y18	60D	0.093357	0.1564223	0.60	0.9999	-0.401885	0.588599
Y18	35D	Y18	Control	-0.119142	0.1510798	-0.79	0.9987	-0.597469	0.359185
Y18	35D	Y19	35D	0.768874	0.0992670	7.75	<.0001*	0.454590	1.083159
Y18	35D	Y19	40D	0.873381	0.1832440	4.77	<.0001*	0.293221	1.453542
Y18	35D	Y19	50D	0.721938	0.1829281	3.95	0.0032*	0.142778	1.301099
Y18	35D	Y19	60D	0.862231	0.1833628	4.70	0.0001*	0.281694	1.442768
Y18	35D	Y19	Control	0.649732	0.1827182	3.56	0.0140*	0.071236	1.228228
Y18	40D	Y18	50D	-0.151443	0.1567929	-0.97	0.9940	-0.647858	0.344972
Y18	40D	Y18	60D	-0.011150	0.1598266	-0.07	1.0000	-0.517170	0.494870
Y18	40D	Y18	Control	-0.223649	0.1548331	-1.44	0.9131	-0.713859	0.266561
Y18	40D	Y19	35D	0.664368	0.1886374	3.52	0.0158*	0.067131	1.261604
Y18	40D	Y19	40D	0.768874	0.0992670	7.75	<.0001*	0.454590	1.083159
Y18	40D	Y19	50D	0.617432	0.1886402	3.27	0.0360*	0.020186	1.214677
Y18	40D	Y19	60D	0.757724	0.1889481	4.01	0.0025*	0.159504	1.355945
Y18	40D	Y19	Control	0.545225	0.1885126	2.89	0.1081	-0.051616	1.142067
Y18	50D	Y18	60D	0.140293	0.1559566	0.90	0.9965	-0.353474	0.634060
Y18	50D	Y18	Control	-0.072206	0.1505633	-0.48	1.0000	-0.548898	0.404486
Y18	50D	Y19	35D	0.815810	0.1821374	4.48	0.0003*	0.239153	1.392468
Y18	50D	Y19	40D	0.920317	0.1824576	5.04	<.0001*	0.342647	1.497988
Y18	50D	Y19	50D	0.768874	0.0992670	7.75	<.0001*	0.454590	1.083159
Y18	50D	Y19	60D	0.909167	0.1825709	4.98	<.0001*	0.331138	1.487197
Y18	50D	Y19	Control	0.696668	0.1818951	3.83	0.0051*	0.120778	1.272558
Y18	60D	Y18	Control	-0.212499	0.1539581	-1.38	0.9334	-0.699939	0.274941
Y18	60D	Y19	35D	0.675518	0.1871412	3.61	0.0116*	0.083018	1.268017
Y18	60D	Y19	40D	0.780025	0.1873381	4.16	0.0013*	0.186902	1.373148
Y18	60D	Y19	50D	0.628582	0.1871381	3.36	0.0273*	0.036092	1.221071
Y18	60D	Y19	60D	0.768874	0.0992670	7.75	<.0001*	0.454590	1.083159
Y18	60D	Y19	Control	0.556375	0.1869863	2.98	0.0865	-0.035634	1.148385
Y18	Control	Y19	35D	0.888017	0.1788075	4.97	<.0001*	0.321902	1.454131
Y18	Control	Y19	40D	0.992524	0.1792135	5.54	<.0001*	0.425124	1.559924
Y18	Control	Y19	50D	0.841081	0.1787754	4.70	0.0001*	0.275068	1.407094
Y18	Control	Y19	60D	0.981374	0.1793047	5.47	<.0001*	0.413685	1.549062
Y18	Control	Y19	Control	0.768874	0.0992670	7.75	<.0001*	0.454590	1.083159
Y19	35D	Y19	40D	0.104507	0.1572491	0.66	0.9997	-0.393352	0.602366
Y19	35D	Y19	50D	-0.046936	0.1531811	-0.31	1.0000	-0.531916	0.438044
Y19	35D	Y19	60D	0.093357	0.1564223	0.60	0.9999	-0.401885	0.588599
Y19	35D	Y19	Control	-0.119142	0.1510798	-0.79	0.9987	-0.597469	0.359185
Y19	40D	Y19	50D	-0.151443	0.1567929	-0.97	0.9940	-0.647858	0.344972
Y19	40D	Y19	60D	-0.011150	0.1598266	-0.07	1.0000	-0.517170	0.494870
Y19	40D	Y19	Control	-0.223649	0.1548331	-1.44	0.9131	-0.713859	0.266561
Y19	50D	Y19	60D	0.140293	0.1559566	0.90	0.9965	-0.353474	0.634060
Y19	50D	Y19	Control	-0.072206	0.1505633	-0.48	1.0000	-0.548898	0.404486
Y19	60D	Y19	Control	-0.212499	0.1539581	-1.38	0.9334	-0.699939	0.274941

**Table A6.** Tukey (HSD) test for all pairwise comparisons representing pairwise differences for fiber strength under all treatments across two years

Year	Treatment	Year	Treatment	Difference	Std error	t ratio	Prob> t	Lower 95%	Upper 95%
Y18	35D	Y18	40D	-0.053676	0.0319068	-1.68	0.8057	-0.154694	0.0473432
Y18	35D	Y18	50D	-0.013314	0.0310814	-0.43	1.0000	-0.111719	0.0850913
Y18	35D	Y18	60D	-0.031399	0.0317390	-0.99	0.9929	-0.131887	0.0690884
Y18	35D	Y18	Control	-0.038957	0.0306550	-1.27	0.9600	-0.136013	0.0580982
Y18	35D	Y19	35D	0.735010	0.0201419	36.49	<.0001*	0.671240	0.7987806
Y18	35D	Y19	40D	0.681335	0.0371813	18.32	<.0001*	0.563617	0.7990530
Y18	35D	Y19	50D	0.721696	0.0371172	19.44	<.0001*	0.604181	0.8392115
Y18	35D	Y19	60D	0.703611	0.0372054	18.91	<.0001*	0.585817	0.8214057
Y18	35D	Y19	Control	0.696053	0.0370746	18.77	<.0001*	0.578673	0.8134334
Y18	40D	Y18	50D	0.040361	0.0318142	1.27	0.9605	-0.060364	0.1410871
Y18	40D	Y18	60D	0.022276	0.0324298	0.69	0.9996	-0.080398	0.1249509
Y18	40D	Y18	Control	0.014718	0.0314166	0.47	1.0000	-0.084748	0.1141849
Y18	40D	Y19	35D	0.788686	0.0382757	20.61	<.0001*	0.667503	0.9098687
Y18	40D	Y19	40D	0.735010	0.0201419	36.49	<.0001*	0.671240	0.7987806
Y18	40D	Y19	50D	0.775372	0.0382762	20.26	<.0001*	0.654187	0.8965565
Y18	40D	Y19	60D	0.757287	0.0383387	19.75	<.0001*	0.635904	0.8786692
Y18	40D	Y19	Control	0.749729	0.0382503	19.60	<.0001*	0.628626	0.8708313
Y18	50D	Y18	60D	-0.018085	0.0316445	-0.57	0.9999	-0.118273	0.0821033
Y18	50D	Y18	Control	-0.025643	0.0305502	-0.84	0.9980	-0.122367	0.0710804
Y18	50D	Y19	35D	0.748324	0.0369568	20.25	<.0001*	0.631317	0.8653317
Y18	50D	Y19	40D	0.694649	0.0370217	18.76	<.0001*	0.577436	0.8118618
Y18	50D	Y19	50D	0.735010	0.0201419	36.49	<.0001*	0.671240	0.7987806
Y18	50D	Y19	60D	0.716925	0.0370447	19.35	<.0001*	0.599640	0.8342110
Y18	50D	Y19	Control	0.709367	0.0369076	19.22	<.0001*	0.592515	0.8262187
Y18	60D	Y18	Control	-0.007558	0.0312390	-0.24	1.0000	-0.106463	0.0913464
Y18	60D	Y19	35D	0.766409	0.0379721	20.18	<.0001*	0.646188	0.8866312
Y18	60D	Y19	40D	0.712734	0.0380120	18.75	<.0001*	0.592386	0.8330822
Y18	60D	Y19	50D	0.753095	0.0379714	19.83	<.0001*	0.632876	0.8733151
Y18	60D	Y19	60D	0.735010	0.0201419	36.49	<.0001*	0.671240	0.7987806
Y18	60D	Y19	Control	0.727452	0.0379407	19.17	<.0001*	0.607330	0.8475744
Y18	Control	Y19	35D	0.773968	0.0362811	21.33	<.0001*	0.659100	0.8888357
Y18	Control	Y19	40D	0.720292	0.0363635	19.81	<.0001*	0.605163	0.8354210
Y18	Control	Y19	50D	0.760654	0.0362746	20.97	<.0001*	0.645806	0.8755011
Y18	Control	Y19	60D	0.742568	0.0363820	20.41	<.0001*	0.627381	0.8577560
Y18	Control	Y19	Control	0.735010	0.0201419	36.49	<.0001*	0.671240	0.7987806
Y19	35D	Y19	40D	-0.053676	0.0319068	-1.68	0.8057	-0.154694	0.0473432
Y19	35D	Y19	50D	-0.013314	0.0310814	-0.43	1.0000	-0.111719	0.0850913
Y19	35D	Y19	60D	-0.031399	0.0317390	-0.99	0.9929	-0.131887	0.0690884
Y19	35D	Y19	Control	-0.038957	0.0306550	-1.27	0.9600	-0.136013	0.0580982
Y19	40D	Y19	50D	0.040361	0.0318142	1.27	0.9605	-0.060364	0.1410871
Y19	40D	Y19	60D	0.022276	0.0324298	0.69	0.9996	-0.080398	0.1249509
Y19	40D	Y19	Control	0.014718	0.0314166	0.47	1.0000	-0.084748	0.1141849
Y19	50D	Y19	60D	-0.018085	0.0316445	-0.57	0.9999	-0.118273	0.0821033
Y19	50D	Y19	Control	-0.025643	0.0305502	-0.84	0.9980	-0.122367	0.0710804
Y19	60D	Y19	Control	-0.007558	0.0312390	-0.24	1.0000	-0.106463	0.0913464

**Table A7.** Tukey (HSD) test for all pairwise comparisons representing pairwise differences for micronaire value under all treatments across two years

Year	Treatment	Year	Treatment	Difference	Std error	t ratio	Prob> t	Lower 95%	Upper 95%
Y18	35D	Y18	40D	0.095721	0.0883384	1.08	0.9863	-0.183963	0.375405
Y18	35D	Y18	50D	0.045163	0.0860531	0.52	1.0000	-0.227286	0.317612
Y18	35D	Y18	60D	0.015593	0.0878740	0.18	1.0000	-0.262621	0.293807
Y18	35D	Y18	Control	-0.041408	0.0848727	-0.49	1.0000	-0.310119	0.227304
Y18	35D	Y19	35D	-0.343875	0.0557656	-6.17	<.0001*	-0.520432	-0.167318
Y18	35D	Y19	40D	-0.248154	0.1029417	-2.41	0.3196	-0.574073	0.077765
Y18	35D	Y19	50D	-0.298712	0.1027642	-2.91	0.1040	-0.624070	0.026645
Y18	35D	Y19	60D	-0.328282	0.1030085	-3.19	0.0469*	-0.654413	-0.002152
Y18	35D	Y19	Control	-0.385283	0.1026463	-3.75	0.0068*	-0.710267	-0.060299
Y18	40D	Y18	50D	-0.050558	0.0880821	-0.57	0.9999	-0.329431	0.228315
Y18	40D	Y18	60D	-0.080128	0.0897864	-0.89	0.9967	-0.364397	0.204141
Y18	40D	Y18	Control	-0.137129	0.0869812	-1.58	0.8596	-0.412516	0.138259
Y18	40D	Y19	35D	-0.439596	0.1059715	-4.15	0.0014*	-0.775108	-0.104084
Y18	40D	Y19	40D	-0.343875	0.0557656	-6.17	<.0001*	-0.520432	-0.167318
Y18	40D	Y19	50D	-0.394433	0.1059731	-3.72	0.0077*	-0.729950	-0.058916
Y18	40D	Y19	60D	-0.424003	0.1061461	-3.99	0.0027*	-0.760068	-0.087939
Y18	40D	Y19	Control	-0.481004	0.1059014	-4.54	0.0002*	-0.816294	-0.145714
Y18	50D	Y18	60D	-0.029570	0.0876123	-0.34	1.0000	-0.306956	0.247815
Y18	50D	Y18	Control	-0.086570	0.0845825	-1.02	0.9909	-0.354363	0.181223
Y18	50D	Y19	35D	-0.389038	0.1023201	-3.80	0.0057*	-0.712989	-0.065087
Y18	50D	Y19	40D	-0.293317	0.1024999	-2.86	0.1170	-0.617837	0.031204
Y18	50D	Y19	50D	-0.343875	0.0557656	-6.17	<.0001*	-0.520432	-0.167318
Y18	50D	Y19	60D	-0.373445	0.1025636	-3.64	0.0103*	-0.698167	-0.048723
Y18	50D	Y19	Control	-0.430445	0.1021839	-4.21	0.0011*	-0.753965	-0.106925
Y18	60D	Y18	Control	-0.057000	0.0864896	-0.66	0.9997	-0.330831	0.216831
Y18	60D	Y19	35D	-0.359468	0.1051310	-3.42	0.0224*	-0.692318	-0.026617
Y18	60D	Y19	40D	-0.263747	0.1052417	-2.51	0.2654	-0.596948	0.069454
Y18	60D	Y19	50D	-0.314305	0.1051293	-2.99	0.0831	-0.647150	0.018540
Y18	60D	Y19	60D	-0.343875	0.0557656	-6.17	<.0001*	-0.520432	-0.167318
Y18	60D	Y19	Control	-0.400875	0.1050440	-3.82	0.0054*	-0.733451	-0.068300
Y18	Control	Y19	35D	-0.302467	0.1004494	-3.01	0.0783	-0.620496	0.015561
Y18	Control	Y19	40D	-0.206746	0.1006774	-2.05	0.5608	-0.525497	0.112004
Y18	Control	Y19	50D	-0.257305	0.1004314	-2.56	0.2364	-0.575276	0.060667
Y18	Control	Y19	60D	-0.286875	0.1007287	-2.85	0.1212	-0.605788	0.032038
Y18	Control	Y19	Control	-0.343875	0.0557656	-6.17	<.0001*	-0.520432	-0.167318
Y19	35D	Y19	40D	0.095721	0.0883384	1.08	0.9863	-0.183963	0.375405
Y19	35D	Y19	50D	0.045163	0.0860531	0.52	1.0000	-0.227286	0.317612
Y19	35D	Y19	60D	0.015593	0.0878740	0.18	1.0000	-0.262621	0.293807
Y19	35D	Y19	Control	-0.041408	0.0848727	-0.49	1.0000	-0.310119	0.227304
Y19	40D	Y19	50D	-0.050558	0.0880821	-0.57	0.9999	-0.329431	0.228315
Y19	40D	Y19	60D	-0.080128	0.0897864	-0.89	0.9967	-0.364397	0.204141
Y19	40D	Y19	Control	-0.137129	0.0869812	-1.58	0.8596	-0.412516	0.138259
Y19	50D	Y19	60D	-0.029570	0.0876123	-0.34	1.0000	-0.306956	0.247815
Y19	50D	Y19	Control	-0.086570	0.0845825	-1.02	0.9909	-0.354363	0.181223
Y19	60D	Y19	Control	-0.057000	0.0864896	-0.66	0.9997	-0.330831	0.216831

**Table A8.** Tukey (HSD) test for all pairwise comparisons representing pairwise differences for fiber uniformity under all treatments across two years

Year	Treatment	Year	Treatment	Difference	Std error	t ratio	Prob> t	Lower 95%	Upper 95%
Y18	35D	Y18	40D	0.002603	0.0386538	0.07	1.0000	-0.119777	0.124983
Y18	35D	Y18	50D	0.008834	0.0376538	0.23	1.0000	-0.110380	0.128048
Y18	35D	Y18	60D	0.032471	0.0384506	0.84	0.9979	-0.089266	0.154208
Y18	35D	Y18	Control	0.014072	0.0371373	0.38	1.0000	-0.103507	0.131650
Y18	35D	Y19	35D	-0.741942	0.0244011	-30.41	<.0001*	-0.819198	-0.664687
Y18	35D	Y19	40D	-0.739340	0.0450437	-16.41	<.0001*	-0.881950	-0.596729
Y18	35D	Y19	50D	-0.733109	0.0449660	-16.30	<.0001*	-0.875473	-0.590744
Y18	35D	Y19	60D	-0.709472	0.0450729	-15.74	<.0001*	-0.852175	-0.566768
Y18	35D	Y19	Control	-0.727871	0.0449144	-16.21	<.0001*	-0.870072	-0.585669
Y18	40D	Y18	50D	0.006231	0.0385416	0.16	1.0000	-0.115794	0.128256
Y18	40D	Y18	60D	0.029868	0.0392874	0.76	0.9991	-0.094518	0.154254
Y18	40D	Y18	Control	0.011469	0.0380599	0.30	1.0000	-0.109031	0.131969
Y18	40D	Y19	35D	-0.744545	0.0463694	-16.06	<.0001*	-0.891353	-0.597737
Y18	40D	Y19	40D	-0.741942	0.0244011	-30.41	<.0001*	-0.819198	-0.664687
Y18	40D	Y19	50D	-0.735711	0.0463701	-15.87	<.0001*	-0.882522	-0.588901
Y18	40D	Y19	60D	-0.712074	0.0464458	-15.33	<.0001*	-0.859124	-0.565024
Y18	40D	Y19	Control	-0.730474	0.0463387	-15.76	<.0001*	-0.877185	-0.583763
Y18	50D	Y18	60D	0.023637	0.0383361	0.62	0.9998	-0.097737	0.145011
Y18	50D	Y18	Control	0.005238	0.0370103	0.14	1.0000	-0.111939	0.122415
Y18	50D	Y19	35D	-0.750776	0.0447717	-16.77	<.0001*	-0.892526	-0.609027
Y18	50D	Y19	40D	-0.748174	0.0448503	-16.68	<.0001*	-0.890172	-0.606175
Y18	50D	Y19	50D	-0.741942	0.0244011	-30.41	<.0001*	-0.819198	-0.664687
Y18	50D	Y19	60D	-0.718305	0.0448782	-16.01	<.0001*	-0.860392	-0.576218
Y18	50D	Y19	Control	-0.736705	0.0447121	-16.48	<.0001*	-0.878266	-0.595144
Y18	60D	Y18	Control	-0.018399	0.0378448	-0.49	1.0000	-0.138218	0.101420
Y18	60D	Y19	35D	-0.774413	0.0460016	-16.83	<.0001*	-0.920057	-0.628770
Y18	60D	Y19	40D	-0.771811	0.0460500	-16.76	<.0001*	-0.917608	-0.626014
Y18	60D	Y19	50D	-0.765579	0.0460009	-16.64	<.0001*	-0.911221	-0.619938
Y18	60D	Y19	60D	-0.741942	0.0244011	-30.41	<.0001*	-0.819198	-0.664687
Y18	60D	Y19	Control	-0.760342	0.0459636	-16.54	<.0001*	-0.905865	-0.614819
Y18	Control	Y19	35D	-0.756014	0.0439531	-17.20	<.0001*	-0.895172	-0.616856
Y18	Control	Y19	40D	-0.753411	0.0440529	-17.10	<.0001*	-0.892885	-0.613937
Y18	Control	Y19	50D	-0.747180	0.0439452	-17.00	<.0001*	-0.886313	-0.608047
Y18	Control	Y19	60D	-0.723543	0.0440753	-16.42	<.0001*	-0.863088	-0.583998
Y18	Control	Y19	Control	-0.741942	0.0244011	-30.41	<.0001*	-0.819198	-0.664687
Y19	35D	Y19	40D	0.002603	0.0386538	0.07	1.0000	-0.119777	0.124983
Y19	35D	Y19	50D	0.008834	0.0376538	0.23	1.0000	-0.110380	0.128048
Y19	35D	Y19	60D	0.032471	0.0384506	0.84	0.9979	-0.089266	0.154208
Y19	35D	Y19	Control	0.014072	0.0371373	0.38	1.0000	-0.103507	0.131650
Y19	40D	Y19	50D	0.006231	0.0385416	0.16	1.0000	-0.115794	0.128256
Y19	40D	Y19	60D	0.029868	0.0392874	0.76	0.9991	-0.094518	0.154254
Y19	40D	Y19	Control	0.011469	0.0380599	0.30	1.0000	-0.109031	0.131969
Y19	50D	Y19	60D	0.023637	0.0383361	0.62	0.9998	-0.097737	0.145011
Y19	50D	Y19	Control	0.005238	0.0370103	0.14	1.0000	-0.111939	0.122415
Y19	60D	Y19	Control	-0.018399	0.0378448	-0.49	1.0000	-0.138218	0.101420

**Table A9.** Tukey (HSD) test for all pairwise comparisons representing pairwise differences for fiber elongation under all treatments across two years