ABSTRACT

RIAR, RANJIT SINGH. Physiological Studies in Cotton (*Gossypium hirsutum*). (Under the direction of Dr. Randy Wells).

Cotton production requires intensive management of inputs and production decisions to increase yield, reduce cost and improve profit margins for the growers while optimizing the utilization of available natural resources like soil, space and light. Four experiments were conducted from 2006 to 2008 to evaluate various inputs and production techniques for their influence on cotton yield and physiological parameters. In the first experiment, two row spacings of 38 and 97 cm and two varieties with two different leaf morphologies were evaluated at three population densities for their influence on light interception, canopy closure, lint yield and fiber properties. Narrow row spacing and higher population density had higher light interception and canopy closure. Varieties with okra leaf and normal leaf shape did not differ for lint yield, while narrow row had higher lint yield compared to wide rows. Lint yields were stable across three population densities of 7, 12 and 18 plants m^{-2}. In the second experiment, two plant growth regulators Mepiquat Chloride and MC plus cyclanilide were compared for their effect on cotton leaf physiology and lint yield. MC plus cyclanilide had lower leaf area and fresh and dry weight compared to MC alone. Both PGRs had higher chlorophyll content compared to control. However, there was no effect of PGRs on cotton lint yield. The third experiment evaluated the effect of early fruit loss on lint yield and fiber properties. Removing three weeks of fruit load caused a redistribution of bolls on the plant on higher and outer positions. This redistribution along with favorable growing conditions later in the season led to stable yield in fruit removal treatment over control. Thus early fruit loss had no negative impact on lint yield. Another experiment was conducted in

which two leaves per plant were framed in opaque plastic frames and inverted to expose the abaxial side of leaves to direct sunlight. These inverted and normal framed leaves were evaluated for leaf growth and pigment concentration. The exposure of lower leaf surface to direct sunlight resulted in an increased anthocyanin concentration and reduced chlorophyll content. Excessive sunlight has potential to damage photosynthetic organs in the leaves, thus reduction in chlorophyll content. Anthocyanins are synthesized in stressed leaves to protect the components from damage due to excessive sunlight and reactive oxygen species.

Physiological Studies in Cotton (*Gossypium hirsutum*)

by
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DEDICATION

This work is dedicated to the growers, agricultural education, extension and support personnel who contribute to the largest primary occupation of the world, provide food and other raw materials to make human life possible as we see it today and will see in the future.

BIOGRAPHY

Since early childhood, Ranjit Singh Riar was fascinated with agriculture and farm life. Being born and raised in an agricultural family, he developed an interest and inclination towards agriculture in his early years, spending time on the farm, looking and learning about crops and management practices from his father and grandfather. That inclination was so strong that it always motivated him to pursue agriculture for his entire life and choose agriculture as a career over other professions.

After graduating high school, he spent about six months actively participating and managing the day to day activities on the farm full time, while planning what to do in future. Next year, he started his Bachelors in Agriculture at Khalsa College, Amritsar, one of the oldest and most reputed institutes of higher education in Punjab, India. There he learned new concepts of crop production and management and understood the science behind the daily decisions of farm management. His weekends and breaks were spent working on the farm, experimenting new methods and crop rotations to improve soil and environmental health while maintaining or improving yields compared to conventional practices which were detrimental to the environment and unsustainable.

After finishing his BS in Agriculture, he competed and was selected for Masters in Agronomy at Punjab Agricultural University, Ludhiana, a premier research, education and extension institute for agriculture in India. There he was exposed to advanced concepts of Agronomy and Crop Science while the extension component of the university incited an aggressive adoption of new techniques like no-till planting and crop residue management.

Academically, he was motivated to move one step ahead and started his PhD in crop physiology and management with Dr. Randy Wells at NC State University. There he learned about cotton production and management in a totally mechanized production system and the problems and decision making choices faced by growers of North Carolina. His training in the classroom and research experience in the fields across North Carolina trained him to understand the problems faced by the growers and provide an amicable solution to those problems based on applied unbiased research. His passion for agriculture will always motivate him to work for the improvement in crop production and farm management techniques so as to improve the quality of life of people engaged in agriculture.

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The Cotton team will always hold a special place in my heart and memories. My life here in the US and the transition into Southern culture was made easy and enjoyable by the company of my team mates James Lanier, Gary Hamm, Guy Collins, and Andrew Hunt, who were later joined by Bill Foote, and Seth Holt. The company and help of these fabulous persons is sincerely appreciated, without their help and tremendous hard work, this work could have not been completed. The time spent in the field on those long, hot days was made enjoyable and memorable by the humor and fun we all had together. The early morning breakfast at "Bohangles" during the planting season and late night snacks at "Takobell" during the harvest season, driving to and back from the field after long days would persist happily in my memory. Sincere thanks are also due to undergrad summer workers Matt Schimidt, Logan Watson, Luke O'Neal and James Atkins who helped tremendously during the peak of work period.

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And last, but not the least, I am thankful to the Almighty for this good life and for guiding me to what and where I am today.

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Row Spacing, Leaf Morphology and Plant Population Influence Yield and Light Interception of Cotton (*Gossypium hirsutum*)

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Abstract

Cotton yield is partly determined by crop geometry which is a function of row width and plant population. Field experiments were conducted at two locations in 2007 and 2008 to evaluate the effect of row width and plant populations on canopy closure, light interception, yield, and fiber quality of cotton. Okra-leaf (FiberMax 800 BR) and normal-leaf (FiberMax 960 BR) cultivars were planted at 38 and 97-cm row spacing, with populations of 7, 12, and 18 plants m^{-2}. Plant population did not affect cotton lint yield. However, cultivar and row spacing did influence lint yield. Normal-leaf cotton had higher lint yield versus okra-leaf cotton. Cotton grown at 38-cm row spacing yielded more than cotton grown at 97-cm row spacing regardless of cultivar. Similarly light interception and canopy closure was higher in 38 cm row spacing and for normal leaf-type compared to 97-cm row spacing and okra-leaf type. Higher plant population increased light interception and canopy closure early in the season. However, with time these differences were reduced and were abated by the last observation. Light interception and canopy closure were highly correlated over time and factors. Narrow row spacing with normal-leaf cotton had the highest light interception, canopy closure and lint yield.

Abbreviations:

DAP: days after planting, LI: light interception, CC: canopy closure, PPFD: photosynthetic photon flux density, LAI: leaf area index, NAWF: nodes above white flower, HNR: height to node ratio. UNR: Ultra narrow row.

Introduction

Cotton (*Gossypium hirsutum* L.) is conventionally grown at row widths of 76 to 97 cm for ease of cultivation, weed management, and convenient application of agricultural chemicals. Wide-row spacings may result in poor canopy closure if growth conditions are less than optimum, thus resulting in less than maximal light interception. This situation in association with greater weed proliferation in a more favorable light environment often results in reduced lint yield and lower economic return for the producer.

Two technological improvements have made narrow-row cotton more attractive to the producer. The first is the development of herbicide resistant cotton that allows weed control without cultivation (Wilson and York, 2006). The second is the development of a narrow–row (38 cm) spindle harvester that results in higher quality lint than found in previously utilized stripper type harvesters (Karnei, 2005).

Jost and Cothren (2000) reported that narrow row cotton grown in 38-cm row spacing can have 37% higher lint yield compared to cotton in 76-cm rows. In addition, faster growth, early fruiting, reduced weed interference, increased canopy light interception and faster canopy closure were observed in the narrower row configuration. Cotton planted in narrow rows matured 12 days earlier than the wider-row cotton. They also reported that narrow row spacing resulted in reduced evapotranspiration compared to wide row due to less soil surface area being exposed to sunlight. Heitholt et al. (1993) also reported that narrow-row cotton had enhanced earliness in normal leaf cultivars and influenced lint fiber properties due to

earlier anthesis and boll maturity than wide row cotton. They theorized that narrow-row cotton has the potential to alter the plant growth regulator and defoliation dynamics when compared to wide-row cotton due to differences in plant height and fruiting characteristics. Wilson et al. (2007) reported that cotton grown in 38-cm rows had a greater number of bolls per unit area, greater boll retention at the first position on the sympodial branches, shorter plant height and 10% higher lint yield compared to cotton planted in 97–cm rows. Alternatively, Clawson et al. (2006) reported inconsistent yield responses to row spacing and different population configurations regardless of nitrogen levels.

Okra-leaf morphology cotton has an altered trait (L_2^0 L_2^0) which reduces the leaf area and alters leaf morphology to a more deeply lobed shape compared to the normal leaf morphology (l_2 l_2) (Heitholt and Meredith, 1998). Okra-leaf cotton is early maturing (Heitholt and Meredith, 1998), produces more flowers per season compared to normal leaf isolines (Wells and Meredith, 1986), has reduced trash content in lint at harvest (Novick et al., 1990), and improves penetration coverage of applied pesticides (James and Jones, 1985). It also has reduced incidence of boll rot due to deeper light penetration in the canopy and improved air movement within the canopy profile (Andries et al., 1969). However, Wilson (1986) reported reduced lint yield in okra-leaf cotton compared to normal leaf cotton in Arizona. Heitholt and Meredith (1998) reported that okra-leaf type cotton had higher yield and earlier maturity than normal leaf cotton, although these results were not consistent over years. Pettigrew (2004) compared six normal-leaf and two okra-leaf varieties under varying light conditions and reported that okra leaf lines had higher rate of photosynthesis compared

to normal leaf lines at light saturation and the okra-leaf lines maintained higher rate of photosynthesis per unit leaf area even when light intensity was the major limiting factor for photosynthesis.

There are conflicting reports in the literature regarding the effect of various plant populations on final lint yield, with some researchers reporting higher lint yield at higher plant populations while others reporting inconsistent response in different environments. Wrather et al. (2008) compared four populations ranging from 24,000 to 136,000 plants per hectare in Mississippi for four years and reported that the lowest population had lowered lint yield in two of the four years. In contrast, Bednarz et al. (2000) reported no yield response to increasing population densities, while Jones and Wells (1997) reported stable yields across two populations of 2 and 12 plants m^{-2} mainly due to increased number of main stem bolls on the outer sympodial positions and higher number of monopodial bolls in the lower population. Siebert and Stewart (2006) reported more bolls produced on outer sympodial positions and on lower monopodial branches at lower population densities.

The effect of leaf morphology has not been studied in respect to varying row width and plant populations. Therefore, the objective of this research is to determine the effect of two varieties with okra leaf and normal leaf morphologies on the light environment and fiber yield with respect to varying row width and plant populations.

Materials and Methods

The experiment was conducted in North Carolina at two locations, the Central Crops Research Station near Clayton and a grower's field near Beulaville during 2007 and 2008. Soil at Beulaville was Norfolk fine sandy loam (fine-loamy, siliceous, thermic, Typic Paleudult). Soil at Clayton was Dothan loamy. The experiment design was a randomized complete block with four replications, with two row spacings of 38 cm and 97 cm, three populations and two leaf morphologies. Three plant populations of 7, 12, and 18 plants m^{-2} were planted with okra leaf (FiberMax 800 BR) and normal leaf (FiberMax 960 BR) varieties on 29 Apr. 2007 and 14 May 2008 at Clayton and 3 May 2007 and 6 May 2008 at Beulaville. The wide row plots were planted with a Case White vacuum planter with variable settings to obtain the desired plant populations with 8, 16 and 25 seeds per meter of row length in a 97 cm row width. The narrow row plots were planted with a John Deere vacuum planter with 3.3, 6.6 and 9.9 seeds per meter row length in a 38 cm row width. Stand counts were taken 30 DAP when the crop was properly established by counting from 3 m row length of one wide row and two narrow rows. Crop production, pest management, fertilization and defoliation were based according to the current recommendations for the region. Irrigation was applied in August 2007 (6.6 cm) and July (1.9 cm) and August (4.8 cm) 2008.

The plots were harvested with John Deere spindle picker fitted with adjustable heads to harvest row widths of 38 cm and 102 cm (Lanier et al., 2005). Approximately 200 g of seed cotton was taken from each plot for determining lint percentage and fiber properties by High Volume Instrumentation. Final lint yield was calculated from lint percentage and seed

cotton yield.

Canopy light interception and canopy closure readings were taken at 43, 58, 72, 84, and 98 DAP. All measurements were taken from the center two rows of the 97-cm plots and middle four rows of the 38-cm plots, considered treatment rows using a LICOR-1000 line quantum sensor of 1 m length (LICOR, Lincoln, NE). The readings were taken on clear sunny days within one hour of solar noon. One reading was taken at top of canopy to measure total incident PPFD and three readings were taken at the bottom of canopy from representative positions among treatment rows. Light interception was calculated as a percentage of incident light reaching ground level.

Canopy closure readings were taken same day as light interception readings with a Kodak EasyShare DX6490 digital camera (Kodak, Rochester NY) with a spatial resolution of 2304 x 1728 pixels. The camera was mounted on an aluminum frame at 2.13 m height. The camera's lens was positioned perpendicular to the ground, and the field of view for the camera was adjusted to 97 cm wide by placing a meter stick on the ground for reference. Three images were taken from each plot at random locations from treatment rows. The images were analyzed using Adobe Photoshop 5 (Adobe Systems, San Jose CA) by converting color images to black and white. Green pixels from the canopy were converted to black and inter row space and shaded areas were converted into white pixels. The percent of white and black pixels was determined by Pixel Counter (Stewart et al., 2007). Percent canopy closure for each plot was then determined from the ratio of white to dark pixels.

Prior to harvest, six plants from treatment rows of each plot were cut at ground level,

bundled, tagged for box mapping analysis. From these plants, height, nodes, first sympodial boll, number of monopodial bolls, monopodial boll weight, number of sympodial bolls, sympodial boll positions, and sympodial boll weight were determined.

Data for canopy closure, light interception, lint yield and yield parameters were analyzed using Proc Mixed in SAS. Means of significant main effects and interactions were separated using Fisher's Protected LSD at $p \leq 0.05$.

Results and Discussion

Light Interception (LI) and Canopy Closure (CC)

Canopy closure and light interception were affected by all three treatments, i.e. row spacing, variety and populations for most dates of observation (Table 1.1). The mean LI for the 38 cm rows was higher than the 97 cm rows at all observed dates (Figure 1.1A). Light interception at 43, 58, 72, 84, and 98 DAP was 17, 19, 15, 8 and 3% higher in 38 cm compared to 97 cm row spacings, respectively (Figure 1.1A). Similarly, narrow row spacing of 38 cm had higher CC during the season compared to wide row spacing, with 21, 12, 14, 10, 4% higher CC at 43, 58, 72, 84, and 98 DAP, respectively (Figure 1.2A). Normal leaf variety had higher LI all along the season compared to okra leaf, except at 84 DAP, with 13, 5, 4, 2 and 3% higher LI at 43, 58, 72, 84, and 98 DAP, respectively (Figure 1.1B). Normal leaf had higher CC than okra leaf at all dates except 72 DAP (Figure 1.2B).

Light interception differed among plant populations on all dates except at 84 DAP (Figure 1.1C). At 43 and 58 DAP, the difference in LI was largest amongst the plant populations with the increasing populations displaying LI percentages of 32, 46, and 56%,

respectively. At 72 DAP, the intermediate population was statistically equal with both the lowest and highest population, which were different from each other, with the highest population having highest LI (Figure 1.1C). However, later during the season at 84 DAP, LI for all populations was similar. At 98 DAP, the highest and the intermediate population had equal LI, and both were higher than the lowest population. In fact, the highest population had higher LI compared to the lowest population during the entire season (Figure 1.1C). The lowest population had the lowest CC, followed by the intermediate population while the highest population density had the highest CC at 43, 58 and 72 DAP. At 84 DAP, highest and lowest population had higher CC compared to intermediate population (Figure 1.2C). Final CC at 98 DAP was similar for all populations (Figure 1.2C). Narrow row spacing places the plants in closer proximity to each other and have a higher LI and CC early in the season as the plants cover more of the available ground area than do plants in wide row spacing, which have a lot of empty, unutilized space between two rows of plants. The light incident on the vacant space is wasted in the wide row plots whereas the same is absorbed and utilized by the leaves of plants in narrow row spacing. These results prove that regardless of leaf type and population density, the plants in narrow rows had a head start early in the season with higher LI and CC than that of wide rows. The higher LI and CC was maintained to the end of the observation period at 98 DAP where the narrow rows had higher LI and CC compared to wide rows. Thus plants in narrow rows better utilized the incident light (PPFD) all season long by intercepting a higher percentage of incident light compared to wide rows and were efficient in converting this additional light energy into higher yield especially for normal leaf

variety (Table 1.5).

Between two varieties, normal leaf had higher LI (Figure 1.1B) and CC (Figure 1.2B) compared to okra leaf because of higher leaf area. As the leaf shape of okra leaf permits more light penetration into the canopy, the cleft and deeply lobed leaves of okra leaves intercept only a portion of total light incident on the canopy, whereas normal leaves which have a higher leaf area than okra leaves are able to absorb most of the light incident on the canopy and very small fraction of the incident light penetrates the canopy, which is shown by higher LI and CC for normal leaves compared to okra leaves.

Among population densities, the highest population had higher LI (Figure 1.1C) and CC (Figure 1.2C) early in the season compared to intermediate and lowest populations, as more plants per unit area translates to more leaf area early in the season. This additional leaf area is instrumental in harvesting a larger proportion of incident light and reducing light penetration deeper into the canopy early in the season, thus the higher values for LI and CC. As the plants grow and mature over time, developing more nodes, branches and leaves, LI increases with addition of more light harvesting surface and closing or diminishing the gaps in the canopy reducing the proportion of light reaching the ground surface after penetrating the canopy. The advantage of higher LI and CC the highest population has early in the season is diminished with time as the plants at lower population also grow more leaves and branches and harvest comparable amount of light incident on the canopy. Lateral growth of plants at lower population almost compensates for the lower plant density so that by the end of the observation period, the differences in LI and CC due to population density are greatly

diminished, if not entirely non-existent.

Similar results were reported by Heitholt (1994) where higher population density and narrow row spacing resulted in higher PPFD interception and LAI. They also reported that plants were more efficient in utilizing PPFD at lower population compared to higher population and normal leaf type required lower LAI and density for higher yield compared to okra leaf. LAI greater than 5 caused yield depression in normal leaf cotton. Higher Light Interception was reported at 51 cm compared to 102 cm row spacing. Bednarz et al. (2000) reported increase LAI in response to increasing population density. Jost and Cothren (2000) reported faster canopy closure in UNR cotton at 19 and 38 cm compared to conventional row cotton at 76 and 102 cm.

Overall, averaged over two varieties and row spacings and three population densities, the correlation coefficient was very high between LI and CC at r^2 0.84. Due to variation of row spacing, leaf shape and population density, some data points for corresponding observations were scattered (Figure 1.6). Therefore, to correctly compare both methods, r^2 from the data were separated for the three treatments, i.e. row spacing, leaf shape and population density.

The r^2 for LI and CC measured in narrow rows at 38 cm over different dates was very high at 0.99, while for wide rows at 97 cm, it was 0.98. For normal leaf, r^2 was 0.98 while for okra leaf, it was 0.99. At a population of 7, and 18 plants m^{-2}, r^2 was 0.99, while the intermediate population at 12 plants m^{-2} had r^2 of 0.98.

Plant growth

Differences in final plant height were observed in response to all three treatments (Tables 1.3and 1.4). Cotton planted at 38 cm row spacing had lower (9.5 cm) plant height compared to 97 cm row spacing and normal leaf cotton had lower height than okra leaf. The highest population of 18 plants m^{-2} had lower plant height compared to other lower populations which were at par with each other (Table 1.4). More plants per unit area in higher populations and in narrow rows might have resulted in increased demand for water and nutrients from given volume of soil. This increased demand for similar availability could be responsible for reduction in plant height.

Additionally, a higher population resulted in a lower number of bolls per plant (Table 1.5) while the lowest population had higher number of bolls per plant (Figure 1.4A). This means that the lower population had a longer flowering and fruiting period which might have resulted in extended periods of vegetative and reproductive growth, thus taller plants with more bolls per plant. Conversely, higher population density had lower number of bolls per plant, which by virtue of limiting the flowering and fruiting period, might have reduced the vegetative growth of the plants while translocating more photosynthates towards developing bolls instead of vegetative growth.

Jost and Cothren (2000) reported a significant reduction in plant height in cotton planted at 38 cm compared to cotton planted at 76.2 cm. Similarly, Clawson et al. (2006) reported taller plants when cotton was grown at 76 cm compared to 38 cm and 19 cm. As row spacing increased from 19 to 76 cm, plant height increased in response to increasing row

spacing. Vories and Glover (2006) reported reduced plant height in ultra narrow row cotton grown at 19 cm compared to conventional row cotton grown at 97 cm. Bednarz et al. (2000) reported inverse relationship of plant population density with plant height and total nodes per plant.

Row spacing and variety did not influence total nodes per plant. Wells and Meredith (1986) also reported no difference in the number of nodes per plant irrespective of planting or harvesting dates. Increasing plant population reduced total nodes per plant (Table 4). Jost and Cothren (2000) reported no difference in nodes for cotton grown at 38.1, 76.2 and 101.6 cm, while that grown at 19 cm had lower nodes per plant. Gerik (1998) reported no difference in number of nodes due to different row spacing. However, Vories and Glover (2006) reported increased number of nodes per plant in cotton planted under conventional row spacing compared to cotton at UNR and Clawson et al. (2006) reported that UNR cotton had fewer nodes per plant. Bednarz et al. (2000) reported increased number of main stem nodes at lower populations and vice versa.

Narrow row at 38 cm had a lower height to node ratio (HNR) against wide row cotton at 97 cm and normal leaf had lower HNR compared to okra leaf cotton. Conversely from total nodes, higher plant population resulted in higher HNR compared to lower plant population (Table 1.4). Similar results were reported by Siebert and Stewart (2006). A lower HNR means a compact canopy with shorter internode length. Thus higher plant population resulted in plants that were taller with similar number of fruiting positions having longer internode distances. This scenario is mostly consistent with rank growth and demands

aggressive plant growth management using PGRs. Thus one disadvantage of using higher populations, among others would be higher cost on PGR use and negative effects of rank cotton.

Row spacing did not have an effect on number of bolls per plant at nodes 3-5, 6-10, 11-15, 16-20 and 21-25, and total bolls per plant (data not shown). Both leaf types had similar results for these observations and were not different from each other except for node 6-10 and 16-20, where normal leaf had higher number of bolls than okra leaf (data not shown). However, boll distribution and individual boll characteristics were highly influenced by plant populations.

The number of bolls per plant from position 3-5, 6-10, 11-15, and 16-20 and total bolls per plant decreased with increasing population, with the lowest population having the highest number of bolls at the respective position compared to intermediate and highest population. The lowest population had more than double the number of bolls per plant than the highest population. The number of bolls at position 21-25 was not different among the three populations (Figure 1.4A). Clawson et al. (2006) reported that higher plant population resulted in reduced number of bolls per plant and more bolls per unit area. Reduced number of bolls resulted in earlier maturity due to shorter flowering and fruiting window. More plants per unit area at the higher populations means that individual plants produce lower number of flowers and bolls compared to lower population, which, due to increased availability of space and resources, produces more flowers towards late in the season, retain more bolls per plant and produce similar lint yields from the lower population due to

compensatory reproductive growth in terms of higher number of bolls per plant (Figure 1.4A) and higher weight per boll (Figure 1.5).

Similar to number of bolls per plant by node position, average seed cotton weight by node position and total weight per plant did not differ between row spacing and leaf types, except for nodes 16-20, where normal leaf had higher (1.93 g) seed cotton weight per plant compared to 1.22 g for okra leaf (data not shown).However, the effect of population density was significant on average seed cotton weight per plant (data not shown). Figure 4B shows that total seed cotton weight per plant and for all node sections except 21-25, the lowest population had higher seed cotton weight than the higher populations. As the population increased, the seed cotton weight per plant declined. This higher seed cotton weight per plant in lower population, in addition to higher number of bolls per plant contributed to attainment of similar yield across all populations.

Individual boll weight by node was not different for both row spacings and leaf morphology. Increasing population reduced single boll weight for all positions except nodes 21-25 where no differences were observed (Figure1.5). Overall average single boll weight per plant was highest for the lowest population, while other two populations were equal. Bednarz et al. (2000) reported reduced boll number and weight with increasing population density due to increased LAI, mutual shading and reduced PPFD utilization efficiency resulting in lower mean net assimilation rate. They also reported inverse relationship of boll size with population density. Thus the cumulative effect of more bolls per plant, higher seed cotton weight per plant and higher average boll weight in lowest population compared to

higher populations resulted in yield stability across populations.

Average number and weight of monopodial bolls per plant and average single boll weight per plant were not different for both the row spacings and leaf types (data not shown). However, increasing population resulted in decreased number and weight for monopodial bolls as was the case with sympodial bolls. The lowest population had the highest number and weight for monopodial bolls than higher populations (data not shown). Bednarz et al. (2000) reported higher number of monopodial nodes, increased boll retention at nodes 6-14 and increased fruiting site production and retention at lower populations.

Leaf type did not have any effect on lint percent. Statistically, wide row had higher lint percent than narrow row but these differences were not important for marketing and quality assessment. Clawson et al. (2006) reported increase in lint percent in response to narrow row spacing compared to wide row. Thus these results differ for lint percent.

Lint yield (Kg ha^{-1})

Lint yield did not differ between two varieties with different leaf types (Data not shown). This is contrary to earlier reports where okra leaf was reported to have lower lint yield than normal leaf isolines (Wilson, 1986,). Bednarz et al. (2000) reported that okra leaf had higher optimal plant population than normal leaf isolines for similar yield. But in this study, yield was found to be stable between leaf types and plant populations. Meredith (1985) reported similar yield for 6 different okra leaf and normal leaf isolines. Differences in boll distribution and individual boll characteristics imparted yield stability at all populations resulting in no difference in lint yield across all populations. These results are in agreement

with Jones and Wells (1998) and Bednarz et al. (2000). Wrather et al. (2008) reported no difference in lint yield due to population in Mississippi. In that report, only one population density of 24,000 plants per hectare had lower yield than the higher populations for one year, which is much lower than the lowest population studied in this experiment. Similar populations in both studies did not differ for lint yield. Siebert and Stewart (2006) reported similar yield in one year for all populations and higher yield in the lowest population of 51,000 plants ha^{-1} in the second year compared to 102,000 and 153,000 plants ha^{-1}.

However, row spacing did affect lint yield and there were significant row by environment interactions (data not shown). Clayton in 2007 and 2008 did not differ for lint yield, although one year had drastically lower average yield than the other. On the other hand, at Beulaville with higher average rainfall and moisture availability and a different soil type, narrow row had higher yield than wide row averaged across leaf types and populations (Figure 1.7). Overall, lint yield for narrow row was higher than wide row spacing (data not shown). These results contradict the findings of Clawson et al. (2006) wherein it was reported that lint yield was not different for 19, 38 and 76 cm. Jost and Cothren (2000) reported increased yield at 19 and 38 cm compared to 76 and 102 cm in one of the two years of study. The greatest determinant for getting a difference in yield between narrow and wide row is the availability of moisture during the growing season.

Uniformity index and strength were affected by row spacing (Table 1.2). Uniformity index and strength was higher for wide row cotton planted at 97 cm. There were no differences for micronaire and length. Micronaire, fiber length, uniformity index and strength

were affected by leaf type. Normal leaf had higher micronaire than okra leaf. Whereas converse was true for length, uniformity index and strength, which were higher for okra leaf compared to normal leaf. Different populations did not have any effect on fiber quality characteristics.

Conclusions

From these results, we can conclude that although higher populations did increase canopy light interception and canopy closure early in the season, differences diminished as the season progressed and lower population had same levels of canopy closure and light interception as higher populations. Lint yield was not different among different populations which mean that growers can save money by planting cotton at lower population while obtaining similar lint yield thus improving their profit margin. Narrow row cotton had higher yield than wide row cotton especially in high growth, moisture rich environment which favored efficient utilization of space and other inputs. With the availability of improved planting and harvesting equipment for narrow row cotton grown at 38 cm, the growers should shift from wide row to narrow row cotton to keep cotton production profitable and properly utilize available irrigation resources for enhanced yield. Okra leaf and normal leaf cotton did not differ in their yield potential and yield determining traits even though okra leaf had lower LI and CC during the entire growing season compared to normal leaf. However, fiber quality was different for leaf types.

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Table 1.1. Analysis of variance summary for light interception to ground and canopy closure measured at different days after planting in response to main effects of environment, leaf morphology, and plant population and their interactions.

Source	Light Interception					Canopy Closure				
	Days after planting									
	43	58	72	84	98	43	58	72	84	98
	---------- p > F ----------									
Environment (Env)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004
Row width (Row)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Env*Row	0.5775	<0.0001	<0.0001	0.0008	0.0005	0.0668	0.0147	<0.0001	<0.0001	<0.0001
Leaf morphology (Leaf)	0.0005	<0.0001	0.0067	0.1244	0.0009	<0.0001	<0.0001	0.4541	0.0065	0.0007
Env*Leaf	0.6429	0.7020	0.0180	0.8205	0.8190	0.1405	0.0073	0.0087	0.0023	0.0010
Row*Leaf	0.5137	0.3883	0.4857	0.0662	0.7508	0.0026	0.3382	0.0196	0.0940	0.0913
Env*Row*leaf	0.9080	0.4215	0.6545	0.0016	0.3221	0.1909	0.0249	0.0002	<0.0001	0.0020
Plant Population (Pop)	<0.0001	<0.0001	0.0174	0.0841	0.0021	<0.0001	<0.0001	<0.0001	<0.0001	0.1799
Env*Pop	0.0150	0.1216	0.8602	0.8740	0.4509	0.0007	0.0058	0.1344	0.0120	0.2651
Row*Pop	0.0152	0.6521	0.5888	0.8568	0.2862	<0.0001	0.0027	0.0029	0.0289	0.0154
Env*Row*Pop	0.6618	0.1750	0.3201	0.0588	0.3390	0.8762	0.1254	0.2618	0.0020	0.1534
Leaf*Pop	0.7105	0.0130	0.8916	0.4852	0.9242	0.5709	0.0010	0.1818	<0.0001	0.9529
Env*Leaf*Pop	0.6345	0.0007	0.1688	0.0752	0.9919	0.7666	0.5624	0.5591	<0.0001	0.0043
Row*Leaf*Pop	0.4073	0.4440	0.1750	0.2463	0.8083	0.9184	0.1985	0.0989	0.0001	0.1215
Env*Row*Leaf*Pop	0.3471	0.0553	0.2189	0.2384	0.7434	0.0471	0.0063	<0.0001	<0.0001	0.4743

Table 1.2. Analysis of variance summary for fiber yield, micronaire (MIC), fiber length, uniformity index (UI), and fiber strength in response to main effects of environment, leaf morphology, and plant population and their interactions.

Source	Yield	MIC	Length	UI	Strength
	----------	----------	p > F	----------	----------
Environment	0.0056	0.0002	<0.0001	<0.0001	<0.0001
Row width (Row)	<0.0001	0.2339	0.0865	0.0271	0.0042
Env*Row	0.0052	0.0014	0.8595	0.4694	0.7856
Leaf morphology (leaf)	0.3323	<0.0001	<0.0001	<0.0001	0.0001
Env*Leaf	0.1924	0.1433	0.0839	0.0506	0.8245
Row*Leaf	0.2202	0.2960	0.8032	0.3767	0.5099
Env*Row*leaf	0.9542	0.5380	0.2711	0.1697	0.3220
Population (Pop)	0.4090	0.6124	0.1981	0.4603	0.3823
Env*Pop	0.8677	0.0466	0.8942	0.8076	0.3239
Row*Pop	0.1197	0.0281	0.1402	0.5100	0.9394
Env*Row*Pop	0.9906	0.4601	0.2672	0.2956	0.6168
Leaf*Pop	0.3469	0.9197	0.6891	0.7177	0.1302
Env*Leaf*Pop	0.3853	0.3662	0.6737	0.1936	0.2803
Row*Leaf*Pop	0.9025	0.1833	0.2467	0.3787	0.1668
Env*Row*Leaf*Pop	0.9280	0.5979	0.9434	0.6363	0.8720

Table 1.3. Analysis of variance summary for main effects of environment, leaf morphology, and plant population and their interactions.

Source	Plant Height	Node Number	Height to node ratio
	p > F		
Environment (Env)	0.0049	0.0001	0.0025
Row width (Row)	0.0012	0.1774	0.0006
Env*Row	0.4924	0.8696	0.1800
Leaf Morphology (Leaf)	0.0018	0.0717	<0.0001
Env*Leaf	0.0004	0.0201	0.0509
Row*Leaf	0.1857	0.9007	0.0943
Env*Row*leaf	0.0840	0.7665	0.1470
Population	0.0370	<0.0001	0.0127
Env*Pop	0.1955	0.5334	0.6904
Row*Pop	0.3919	0.5488	0.7191
Env*Row*Pop	0.9279	0.6229	0.5014
Leaf*Pop	0.9317	0.7141	0.5637
Env*Leaf*Pop	0.0486	0.5497	0.0092
Row*Leaf*Pop	0.0343	0.4267	0.0043
Env*Row*Leaf*Pop	0.0282	0.0635	0.9237

Table 1.4. Response of plant height, total nodes, and intermodal length per plant to row width, leaf morphology and plant population.

Source	Plant Height	Total nodes	Height to node ratio
	cm	number/plant	
Row 38 cm	82.5 b†	19.7 a	4.2 b
Row 97 cm	92.0 a	20.1 a	4.6 a
Leaf Normal	85.4 b	20.1 a	4.3 b
Leaf Okra	89.1 a	19.6 b	4.6 a
Population 7 m^{-2}	88.9 a	20.8 a	4.3 c
Population 12 m^{-2}	87.8 a	19.9 b	4.4 b
Population 18 m^{-2}	85.0 b	19.0 c	4.5 a

† Means within a column followed by the different letters are different at $p \leq 0.05$.

Table 1.5. Fiber yield, bolls per plant and weight per boll in response to row width, leaf morphology, and plant population in four environments.

Row width	Leaf morphology	Plants m^{-2}	Lint yield				Bolls/plant			Weight / boll		
			C-07†	B-07	C-08	B- 08	B-07	C-08	B-08	B-07	C-08	B- 08
38 cm	Normal	7	987	1086	1357	1332	8.0	14.0	8.5	3.7	4.7	5.9
		12	1043	1160	1278	1331	5.4	7.4	5.9	3.6	4.4	5.1
		18	1080	1277	1284	1658	3.7	6.3	5.0	3.8	4.1	5.1
38 cm	Okra	7	667	1069	1184	1422	6.7	11.5	7.5	3.3	5.2	5.8
		12	834	1109	1349	1557	4.9	9.2	6.8	4.0	4.5	5.2
		18	841	1104	1352	1435	2.1	6.5	3.6	3.6	4.8	4.8
97 cm	Normal	7	969	964	1357	1184	8.2	11.9	9.6	5.1	4.9	5.3
		12	923	954	1205	1148	5.7	10.8	3.3	4.1	4.5	4.8
		18	957	1009	1164	1218	3.1	6.6	5.3	4.1	4.7	5.1
97 cm	Okra	7	802	952	1290	1350	9.0	10.8	10.0	3.9	4.6	6.0
		12	887	973	1318	1280	5.9	7.2	5.7	3.5	4.7	5.8
		18	910	962	1219	1233	3.5	4.8	3.9	3.7	5.3	5.6

† C-07, B-07, C-08, and B-08 correspond to Central Crops Research Station and Beulaville, NC in 2007 and 2008.

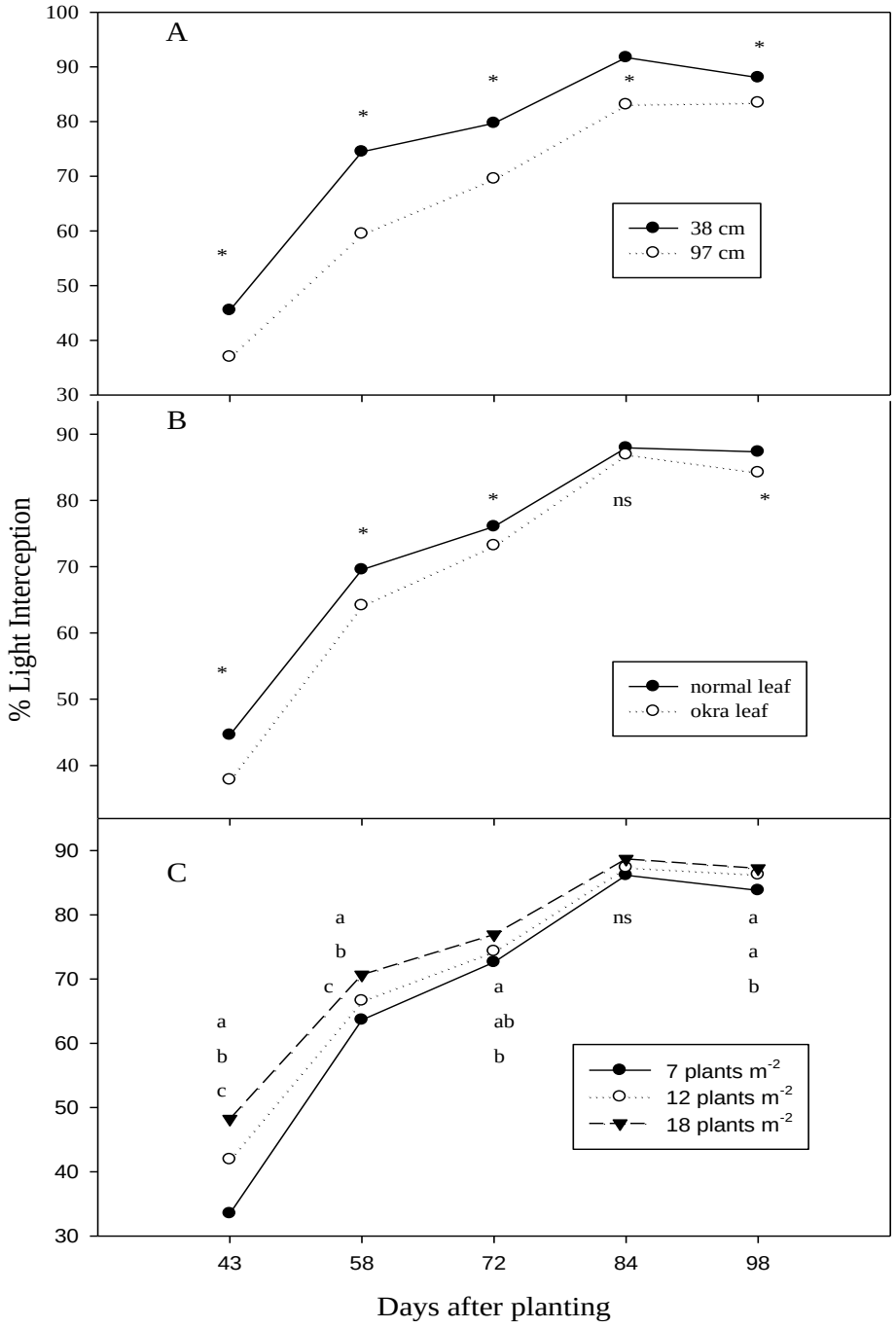

Figure 1.1. Light interception in response to row spacing (A), leaf morphology (B) and plant population (C). Values are averaged over two years with two locations each. Data points with * or different letters are different at $p \leq 0.05$.

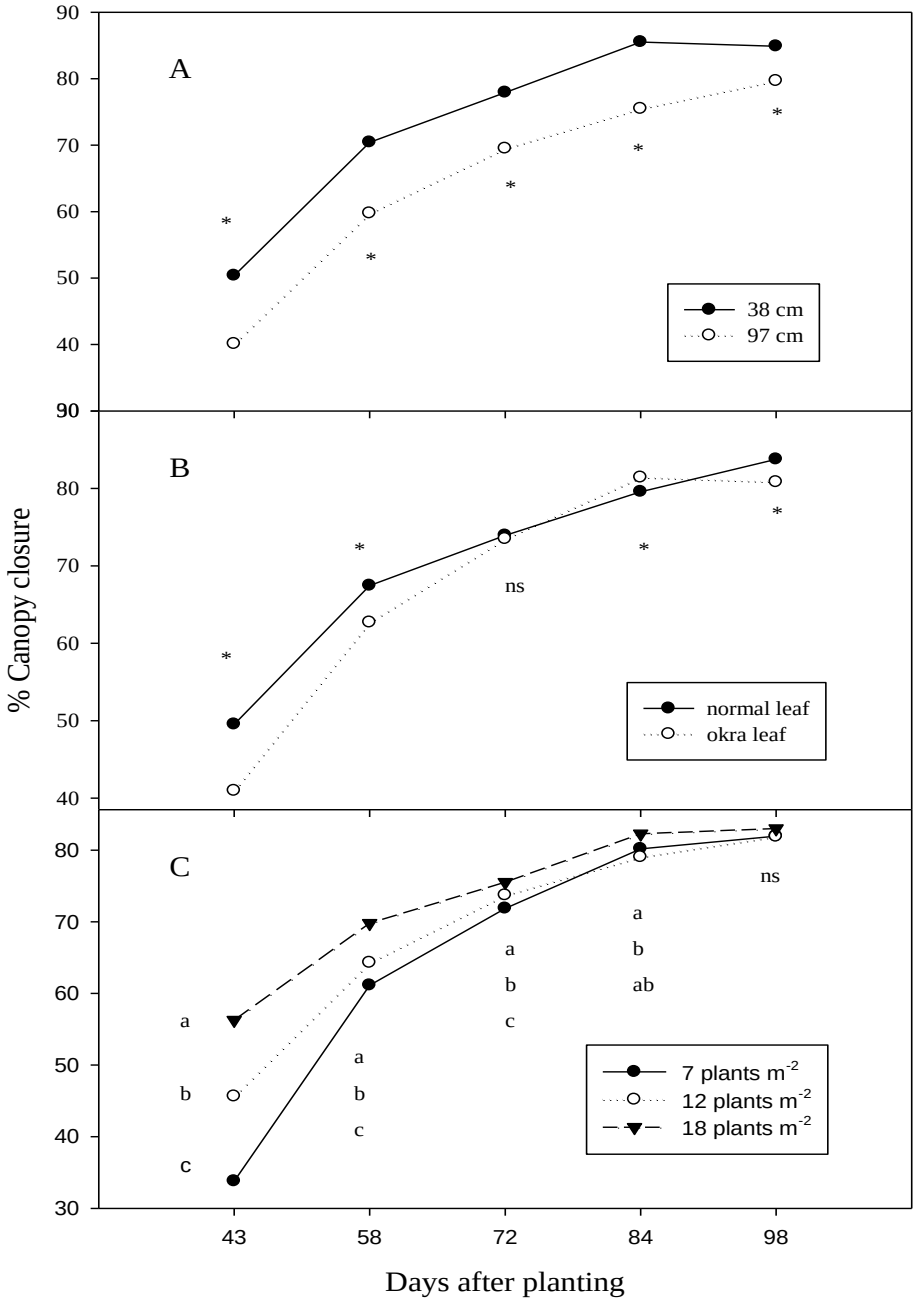

Figure 1.2. Canopy closure in response to row spacing (A), leaf morphology (B) and plant population (C). Values are averaged over two years with two locations each. Data points with * or different letters are different at $p \leq 0.05$.

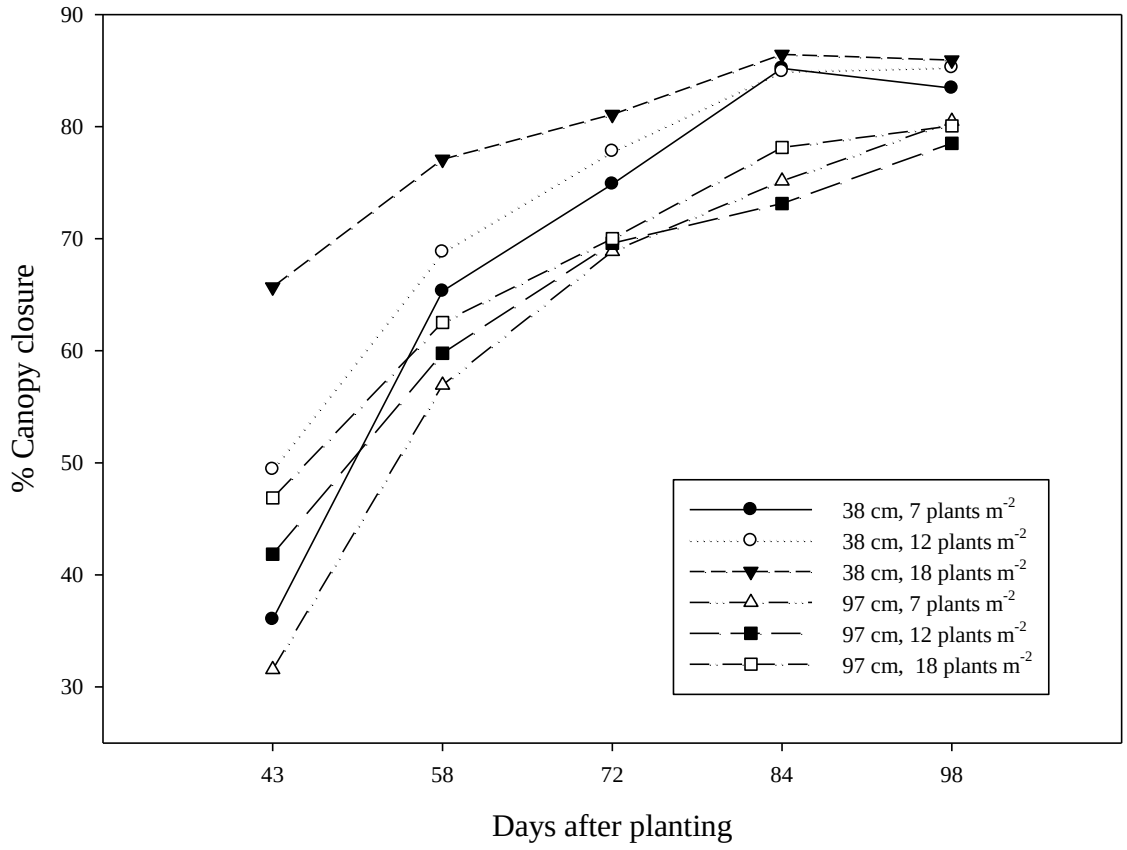

Figure 1.3. Interaction effects of row spacing and population on canopy closure. Data averaged over leaf morphology and environments.

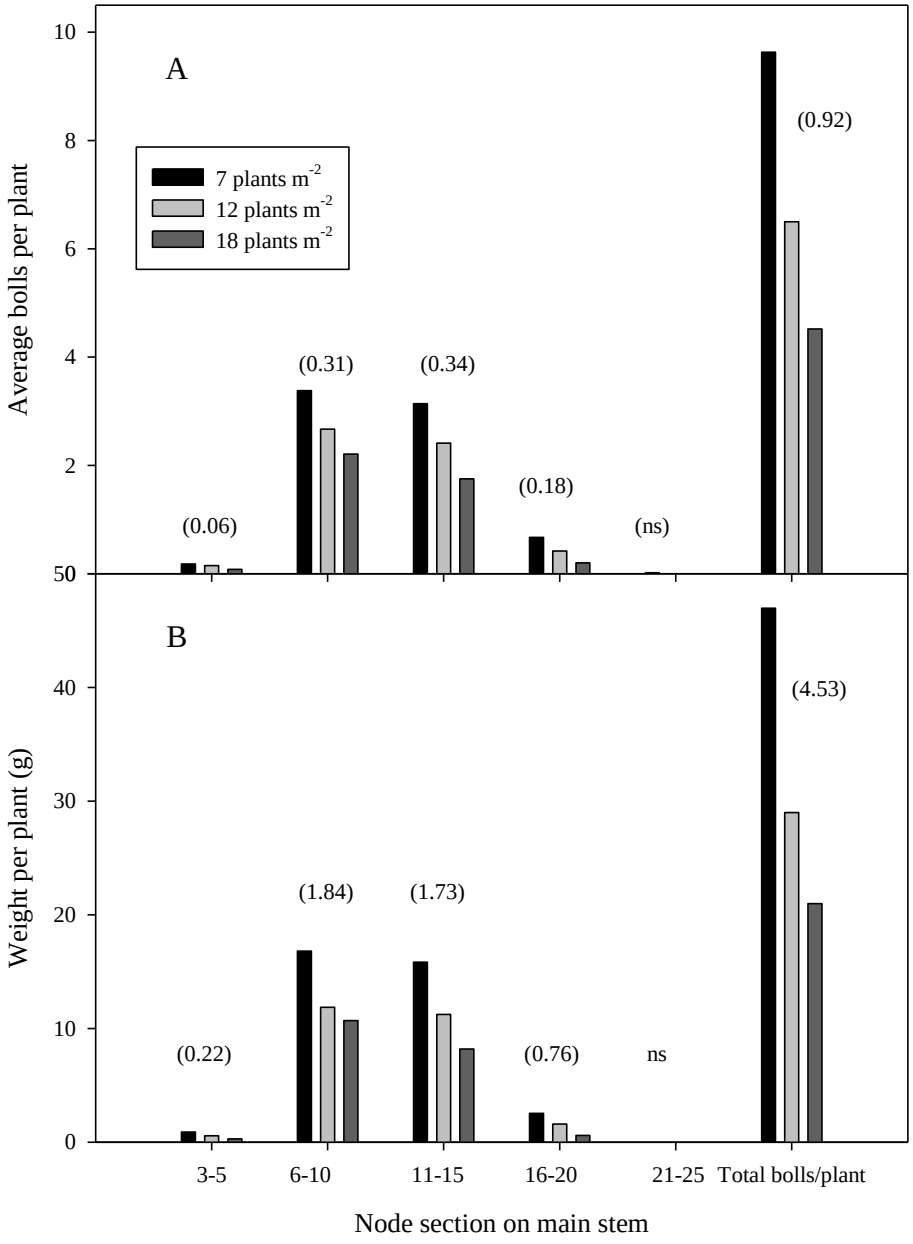

Figure 1.4. Number of bolls per plant (A) and seed cotton weight (g) per plant (B) in response to three populations. Values are averaged over years, locations, row spacing and leaf morphology. Figures in parenthesis represent LSD for respective set of means.

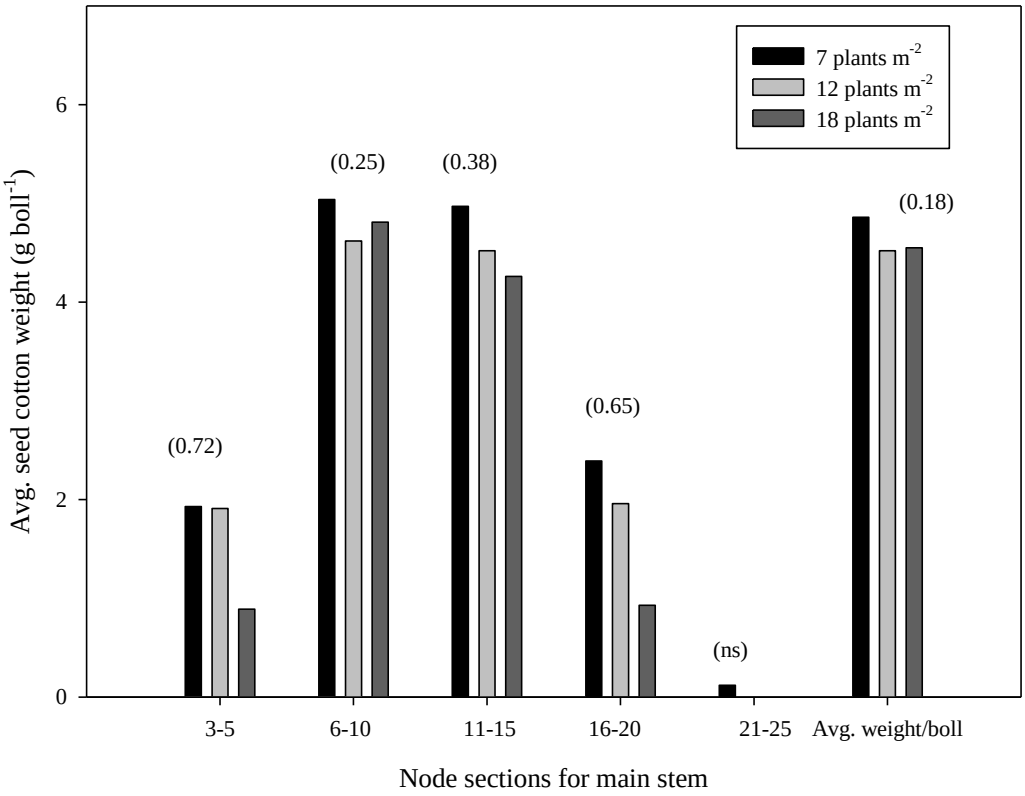

Figure 1.5. Seed cotton weight (g) per boll in response to population. Values are averaged over years, locations, row spacing and leaf morphology. Figures in parenthesis represent LSD for respective set of means.

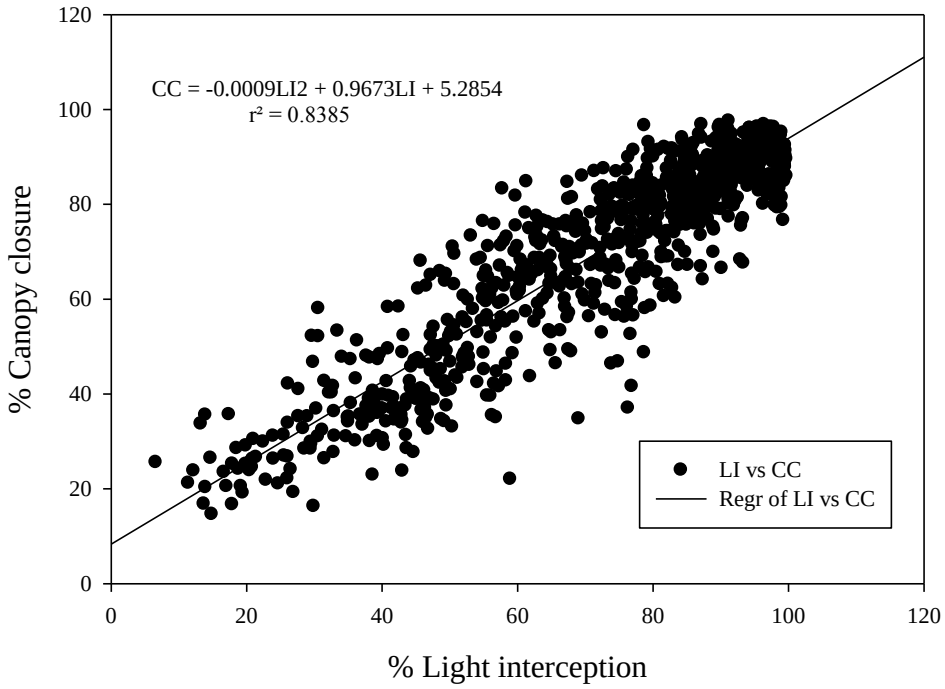

Figure 1.6. Relationship between light interception and canopy closure. Each data point corresponds to light interception and canopy closure for one specific observation. Values represent 912 data points over row spacing, leaf morphology and populations over two years and two locations.

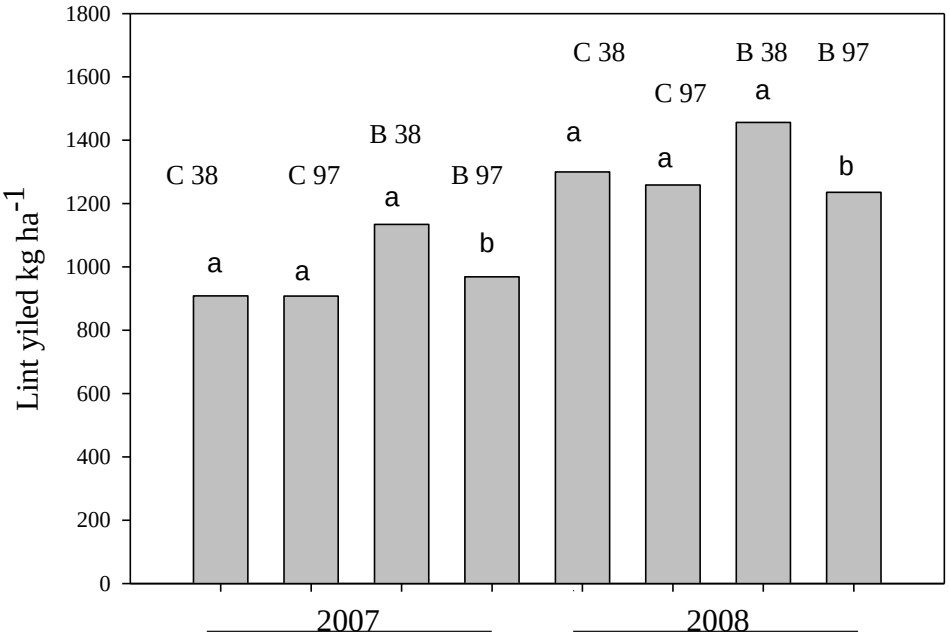

Figure 1.7. Lint yield (Kg ha^{-1}) at two row spacings for four environments over two years. Data averaged over leaf morphology and populations. B and C denote locations Beulaville and Clayton, numbers 38 and 97 denote row spacing in cm.

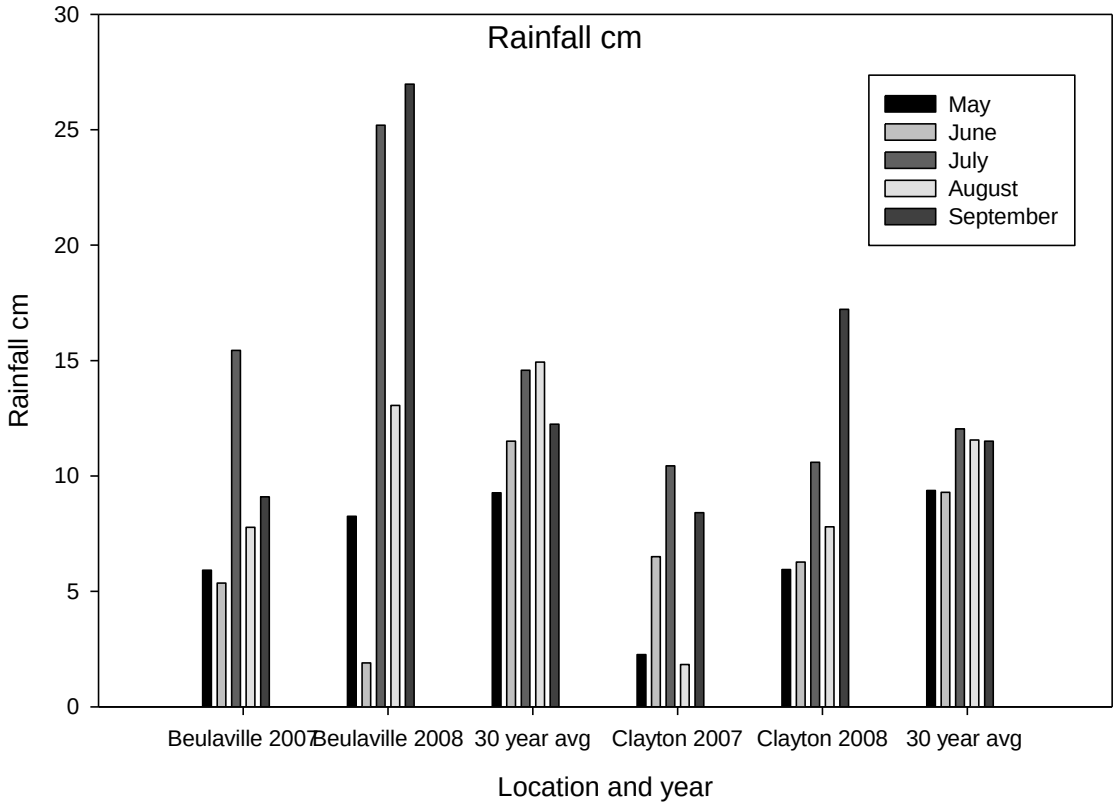

Figure 1.8. Seasonal rainfall (cm) for four growth environments over two years with 30 years average for each location.

Leaf Growth and Yield of Cotton (*Gossypium hirsutum*) as affected by Mepiquat Chloride and Mepiquat Chloride plus Cyclanilide

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Abstract

Plant growth regulators that inhibit vegetative growth can be a very important management tool for efficient and profitable cotton production in the United States. While effects of mepiquat chloride (MC) and MC plus cyclanilide (MCC) on plant stature has been studied, the effects on individual leaf characteristics have not been assessed. This experiment was conducted to compare the effect of MC and MCC on leaf characteristics and lint yield of cotton. Recommended rates of MC and MCC were evaluated for their effect on leaf area; fresh, dry and specific leaf weight; leaf thickness; chlorophyll and anthocyanin content; and lint yield for two years on a Wagram loamy sand (Fine-loamy, kaolinitic, thermic Typic Kandiudults) in 2006 and Goldsboro fine sandy loam (Fine-loamy, siliceous, subactive, thermic, Aquic paleudult) in 2007. PGRs had a significant impact on leaf area, chlorophyll, fresh and dry weight while there was no effect on anthocyanin, leaf thickness, SLW and lint yield. MCC caused a reduction in leaf area, fresh weight and dry weight compared to MC alone because of synergistic activity of cyclanilide added to MC. The effect of MCC was more pronounced on suppression of leaf growth as compared to MC alone or control, even under growing conditions where response to MC is not expected and MC use is not warranted. Addition of cyclanilide to MC increases the efficacy compared to MC alone.

Abbreviations:

PGR, plant growth regulator; MC, 1, 1-dimethylpiperidinium chloride; GA, gibberellic acid; NPA, 2-naphthalen-1-ylcarbamoyl; TIBA, 2,3,5-triiodobenzoic acid; IAA, indole acetic acid; MC+C, mepiquat chloride plus cyclanilide; DAT, days after treatment; DAP, days after platning; DMF, dimethylformamide; Chl, chlorophyll; Ant, anthocyanin; UTC, untreated control; SLW, specific leaf weight; UHM, upper half mean length.

Introduction

Cotton production in the United States involves intensive management and extensive inputs. The indeterminate growth habit and production in the northeastern area of the cotton production region adds to the challenges of effective crop management. Poor management decisions can have significant effects on crop growth, yield and net returns. These input decisions include the proper choice and application of plant growth regulators (PGR).

Mepiquat chloride (1, 1-dimethylpiperidinium chloride) has long been used as a PGR in cotton for reducing plant growth and stature. It inhibits gibberellic acid (GA) biosynthesis by blocking the synthesis of the GA-precursor *ent*-copalyl diphosphate (Halmann, 1990). Mepiquat chloride (MC) has also been documented to reduce stem growth, plant height, number of main stem nodes, internode length, and leaf area (Reddy et al., 1990). Alternatively, it causes an increase in specific leaf weight and imparts heat tolerance to treated plants (Reddy et al., 1990).

Fernandez et al. (1992) reported that MC inhibited leaf area expansion in plants growing without moisture stress. In water stressed plants, the effect of MC on the reduction in leaf area was confounded by moisture deficit. Mepiquat chloride reduced daily transpiration rate early in the season, thus slowed the rate of soil moisture depletion and delayed the onset of moisture stress in treated plants. Reduced light interception due to a compact foliar canopy, lower leaf area, decreased water use efficiency and reduced gross daily carbon uptake have also been reported in MC treated plants (Fernandez et al., 1992).

Xu and Taylor (1992) treated cotton with MC and observed increased total root

length and faster rate of root growth in response to seed and seedling MC treatments. In addition, resistance to wilting, decreased stem elongation, reduced shoot and root weight and increased survival under drought stress of treated seedlings was observed. Similar findings were reported by Hodges et al. (1991).

Wilson et al. (2007) reported reduction in plant height, main stem nodes, number of effective sympodia and total bolls per plant in response to MC compared to the control. Similar reduction in plant height and total nodes in plants treated with MC has been reported by Nichols et al. (2003), Pettigrew and Johnson (2005) and Nuti et al. (2006). Heilman (1985) reported reduced vegetative growth under high moisture and nitrogen environment in response to MC treatment, in addition to increased calcium uptake by leaves. Lint yield and quality, however was not affected by MC treatments.

Biles and Cothren (2001) reported increased fruit retention, earlier maturity and higher yield along with reduced square abortion in response to MC treatment, although yield increase was inconsistent. Increased lint yield and fiber length was reported for all strategies of MC application over control. However, lower lint percent was reported in MC treated plants, while there was no effect on uniformity index and strength compared to control.

While MC inhibits plant growth by reducing or blocking GA synthesis (Halmann, 1990), cyclanilide (1-(2, 4-dichlorophenylaminocarbamoyl) - cyclopropane carboxylic acid), acts as an inhibitor of auxin transport by acting as a polar auxin transport inhibitor. Both 2-naphthalen-1-ylcarbamoyl (NPA) and 2,3,5-triiodobenzoic acid (TIBA) inhibit auxin transport, however, cyclanilide's effect on protonated IAA efflux and influx from Zucchini

hypocotyls was different from that of NPA and TIBA, suggesting a mode of action different from other auxin inhibitors (Burton et al., 2008). Cyclanilide is combined with other chemicals for specific growth regulation purposes. It is combined with Ethephon as a harvest aid chemical to induce abscission and boll opening (Finish^{TM}). Cyclanilide has been reported to induce loss of apical dominance and promote growth of lateral branches in red kidney beans (Pedersen et al., 2006). It may also increase fruiting at more distant, lateral positions in cotton (Thomas et al., 2006). Alternatively, it is combined with MC for controlling excessive vegetative growth. Mepiquat chloride + cyclanilide (MCC) is a relatively new PGR labeled for plant growth suppression in cotton. The mixture of MC and cyclanilide, acts synergistically by enhancing the efficacy of MC in reducing plant height and vegetative growth (Thomas et al., 2006). However, information comparing the field performance of MCC and MC is limited. This study was conducted to compare the effect of these two PGRs on growth characteristics and lint yield.

Materials and Methods

Field experiments were conducted for two growing seasons during summer of 2006 and 2007 at Central Crops Research Station at Clayton, NC and at Upper Coastal Plains Research Station at Rocky Mount, NC respectively. The soil type was Wagram loamy sand (Fine-loamy, kaolinitic, thermic typic kandiudults) at Clayton and Goldsboro fine sandy loam (Fine-loamy, siliceous, subactive, thermic, aquic paleudult) at Rocky Mount. Rainfall totals for both environments are shown in Figure 2.1. Cotton cultivar Fibermax FM 960 BR was planted on 17 May 2006 and on 14 May 2007 with a two row vacuum planter at a 97 cm row

spacing, with four rows per plot. All measurements were taken from central two rows. The experiment design was a randomized complete block design with four replications. Mepiquat chloride was applied at rate of 0.036 kg a.i. ha^{-1} and MCC was applied at the rate of 0.0048 kg and 0.0193 kg a.i. ha^{-1} for cyclanilide and MC, respectively. Application of MC and MCC was performed at 68 and 63 DAP in 2006 and 2007, respectively (early anthesis) with a CO_2 pressurized backpack sprayer with Teejet XR110-02 Flatfan nozzles calibrated at 103 kpa (15 psi) to deliver 140 Lha^{-1}. Pest management and production practices other than those for specific treatments were administered uniformly to the entire test and were standard for the region.

On day of treatment, quarter sized, (24.26 mm diameter) or larger main stem leaves and one located two nodes below were tagged with jeweler tags. These leaves will be designated as leaves T and T + 2, respectively. Twenty plants were tagged per plot. Tagged leaves were sampled at 3, 7, 10, 15, 23, and 39 days after treatment (DAT). The harvested leaves placed in plastic bags and kept on ice during transportation to laboratory for analysis. Leaf area of each leaf was measured using a LI-COR LI- 3100 area meter (LICOR Inc. Lincoln, NE). Three leaf disks of 0.3 cm^2 from each leaf were taken with a punch and were placed in 3 ml dimethylformamide (DMF) in the dark for 2 days at 4^0C. Total chlorophyll and Chl a/b ratio was determined from the DMF extract spectrophotometrically (Moran, 1982). Concentration of anthocyanin was determined by placing 3 leaf discs in 3 ml acidified methanol with 10 ml concentrated HCl/L for 2 days. Light absorbance of methanol extracts was determined at 530 nm and 658 nm. Anthocyanin concentration was calculated

using the following formula of Mancinelli et al. (1998):

$$Ant = A_{530} - 0.25\ (A_{658}).$$

After taking samples for pigment analysis, the leaves were placed in hot air oven at 60^0 C for 72 h. Specific leaf weight was obtained by dividing the leaf area by dry weight. Leaves from two replications were harvested separately for measurement of leaf thickness samples in 2007. Middle regions of leaves, excluding the vascular tissue, were cut with a sharp blade to get six thin section samples per leaf. Leaf thickness was determined in millimeters using a dissecting microscope fitted with a graduated ocular scope.

The plots were harvested using a two row spindle picker on 20 Oct. 2006 and 10 Oct. 2007. Seed cotton yield was determined at the time of harvest. A 200 g sample of seed cotton was taken at that time to determine the lint percentage, which was used to calculate lint yield. A sample of ginned lint was analyzed at Cotton Inc. by high-volume instrument (HVI) to determine fiber properties.

All data was analyzed using Proc Mixed in SAS. Years, plants, leaves and sampling dates were considered as random effects. Specific error terms were used to determine significance of treatments. Means were separated using Fisher's protected LSD at $p \leq 0.05$.

Results and Discussion

Leaf Growth Characteristics

The PGR treatments had a significant effect on leaf area, chlorophyll, fresh weight and dry weight. There were no concomitant effects on anthocyanin concentration, specific

leaf weight, and leaf thickness. Mepiquat chloride treated plants had the highest average leaf area of 77 cm^2 while plants treated with MCC had a reduced leaf area of 70 cm^2 (Table 2.2). The UTC was intermediate with a leaf area of 75 cm^2 and was not different from either growth regulator treatment.

Similarly, MC had greatest fresh leaf weight which larger than the MCC treated leaves (Table 2.2). The untreated control (UTC) was intermediate in fresh weight but was not different from either MC or MCC. The same trend was found for dry weight with MCC greater than MC and not different from the UTC. Chlorophyll, on the other hand, was 5.0 and 5.7% greater in the MC and MCC treatments than the UTC, respectively (Table 2.2).

It has been documented that MC reduces leaf area, especially under optimal growing conditions (Fernandez et al., 1992). Mepiquat chloride inhibits cell expansion and reduces leaf area by inhibiting biosynthesis of gibberellic acid while auxins are involved in inducing the enzymes that metabolize GA 20 into active form (Burton et al., 2008). Thus, the synergistic effect of cyclanilide on MC efficacy seems responsible for enhanced inhibition of leaf expansion and related characteristics compared to MC alone. These observations occurred despite the dry conditions experienced throughout the experiments (Figure 2.1). In 2006, only 1.5 and 4.8 cm of rainfall was received during June and July thus resulting in less than optimum growth. Kerby (1985) found that MC operated optimally in the San Joaquin Valley of California only when either the season is extremely short or final control plant height exceeded 110 cm.

The leaves at position T were smaller and of lower fresh and dry weight than those

found at position T+2 (Figure 2.2). Leaves higher on the plant were only approximately half the size and weight of those at position T+2 when measured by these parameters. Wells (1988) found a similar trend in the size of main stem leaves tagged at different days after plant emergence. Leaves emerging at 62 days after planting (DAP) had more than twice the area of leaves emerging at 89 DAP. The leaves tagged at 62 DAP emerged prior to the onset of anthesis and developed when competition for assimilates from the reproductive organs was low, thus promoting larger leaves.

There were year effects on leaf characteristics as is commonly found. Leaf area, fresh and dry weight, and chlorophyll all were greater in 2007 than observed in 2006 (Figure 2.3). These differences may be attributable to the greater rainfall observed in 2007 during that months of June and July when the tagged leaves were developing (Figure 2.1).

Lint Yield and Quality

Fiber yield and properties were unaffected by growth regulator treatment (Table 2.3). There was, however, a year effect on micronaire and fiber strength. Jones and Wells (1998) found that micronaire was closely related to accumulated heat units throughout a season. They also found that micronaire was closely associated to boll mass. Meredith and Bridge (1973) reported similar relationships between micronaire and dry weight per boll in their data. Further, Yudhveer et al., (2010) found a close relationship between irrigation level and boll weight. Water replacement levels ranging from 50 to 100% of evapotranspiration resulted in boll weights ranging from 2.73 to 3.33 g $boll^{-1}$. Considering the different rainfall

patterns observed between years in the present study it is reasonable that boll weights and micronaire would be quite different.

Moisture deficit during both growing environments caused inhibition of cotton growth. However, one interesting find is the reduction of leaf area, Chl, fresh weight and dry weight in response to MCC compared to MC and control (Table 2.2 and Figure 2.4). Presence of cyclanilide, which is an auxin transport inhibitor, when applied together with MC, acts as a synergist for vegetative growth suppression. This clearly indicates that addition of cyclanilide increased the efficacy of MC for growth inhibition which was not observed in application of MC alone, even under conditions where response from MC is not expected.

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Table 2.1. ANOVA summary for MC and MC plus cyclanilide on leaf area (LA), anthocyanin (Ant), fresh wt. (FW), dry wt. (DW), specific leaf wt. (SLW) and leaf thickness (LT).

Variable	LA	Chl	Ant	FW	DW	SLW	LT
				p > F			
Year (Yr)	0.2638	0.0143	0.2812	0.2362	0.0409	0.0009	-
Treatment (Trt)	0.0226	0.0326	0.3015	0.0268	0.0455	0.8167	0.1117
Yr*trt	0.9111	0.0937	0.5450	0.7400	0.7269	0.8108	-
Harvest date (Hd)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0064
Yr*Hd	0.9544	<0.0001	<0.0001	0.9768	0.0284	<0.0001	-
Yr*Trt*Hd	0.4879	0.4632	0.8228	0.4876	0.1282	0.0422	-
Trt*Hd	0.4224	0.7177	0.4321	0.5081	0.6363	0.5062	0.1436
Leaf position (Lp)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Yr*Lp	0.8654	<0.0001	0.0358	0.8021	0.0266	0.9569	-
Trt*Lp	0.0389	0.4371	0.3644	0.0113	0.0548	0.6971	0.4819
Yr*Trt*Lp	0.3230	0.6586	0.5256	0.4916	0.2833	0.2139	-
Hd*Lp	0.0060	<0.0001	0.1700	0.0012	0.0018	<0.0001	0.313
Yr*Hd*Lp	0.2729	0.0415	0.1052	0.1334	0.5460	0.0416	-
Trt*Hd*Lp	0.9913	0.4763	0.8437	0.9959	0.9364	0.7913	0.2672
Yr*Trt*Hd*Lp	0.5725	0.6406	0.7232	0.4566	0.3293	0.6074	-

Table 2.2. Effect of Mepiquat chloride (MC) and MC plus cyclanilide (MCC) on area, chlorophyll, fresh weight, and dry weight of cotton leaves.

Variable	Leaf Area	Chl	Fresh Wt.	Dry Wt.
	cm^2	mg cm^2	g $leaf^{-1}$	g $leaf^{-1}$
UTC	75.0 ab†	14.0 b	2.0 ab	0.60 ab
MC	76.9 a	14.8 a	2.1 a	0.63 a
MCC	70.3 b	14.7 a	1.9 b	0.57 b

†- Within columns, values followed by the same letter are not different at $p \leq 0.05$.

Table 2.3. Analysis of variance summary for main and interaction effects of mepiquat chloride and mepiquat plus cyclanilide on cotton yield and fiber properties.

Variable	Yield	Lint%	Micronaire	UHM	Uniformity Index	Strength
	p > F					
Year	0.2845	0.4484	0.0079	0.5337	0.1535	0.0010
Treatment	0.9725	0.1479	0.9720	0.8608	0.9783	0.6414
Yr*trt	0.1281	0.3773	0.5371	0.8915	0.9893	0.6802

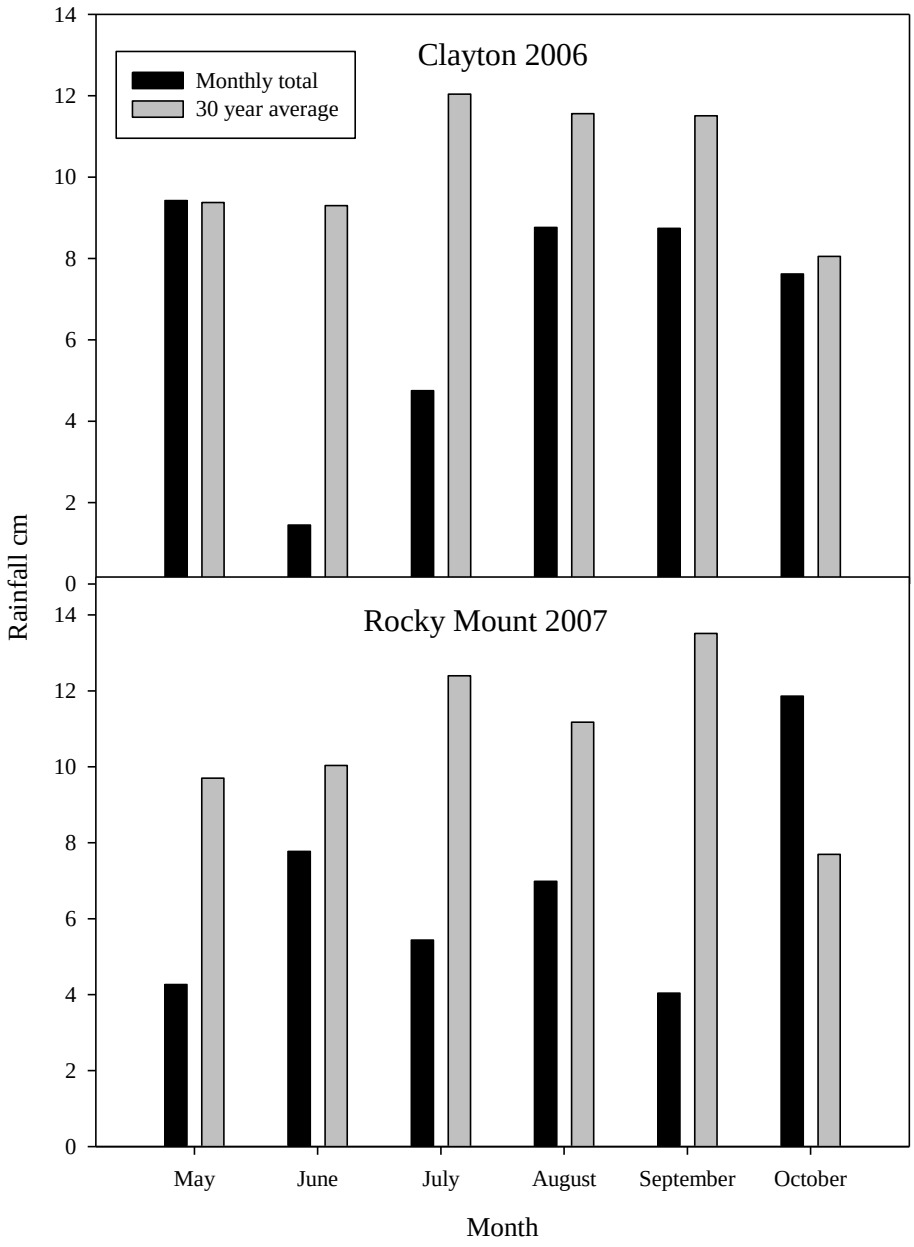

Figure 2.1. Monthly and 30-year average rainfall observed at Clayton, NC in 2006 and in Rocky Mount, NC in 2007.

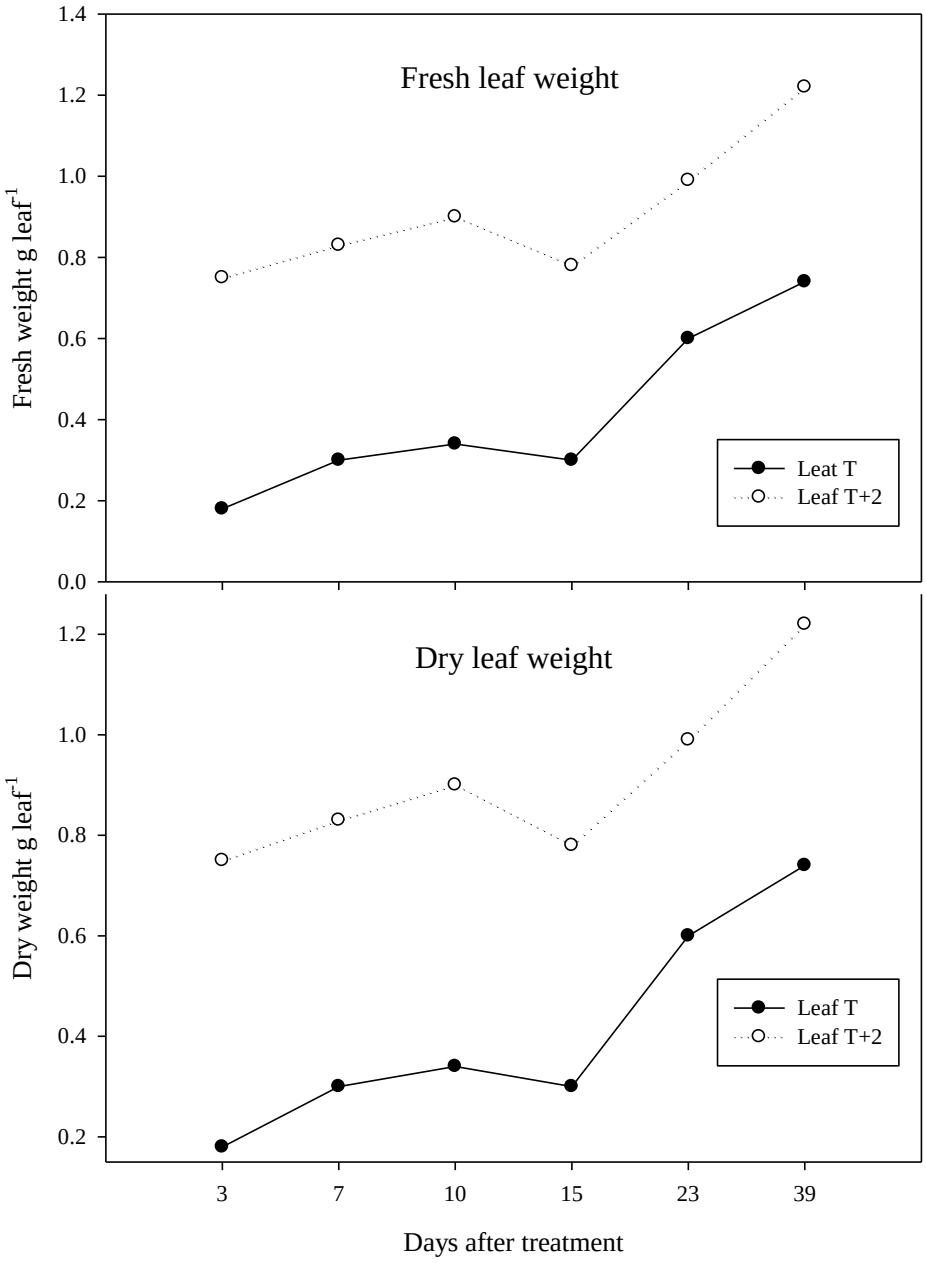

Figure 2.2. Leaf fresh and dry weight for sympodial leaves tagged at time of treatment (T) and two nodes below the tagged leaf (T + 2) at various days after treatment. Values are averaged across growth regulator treatment and environments.

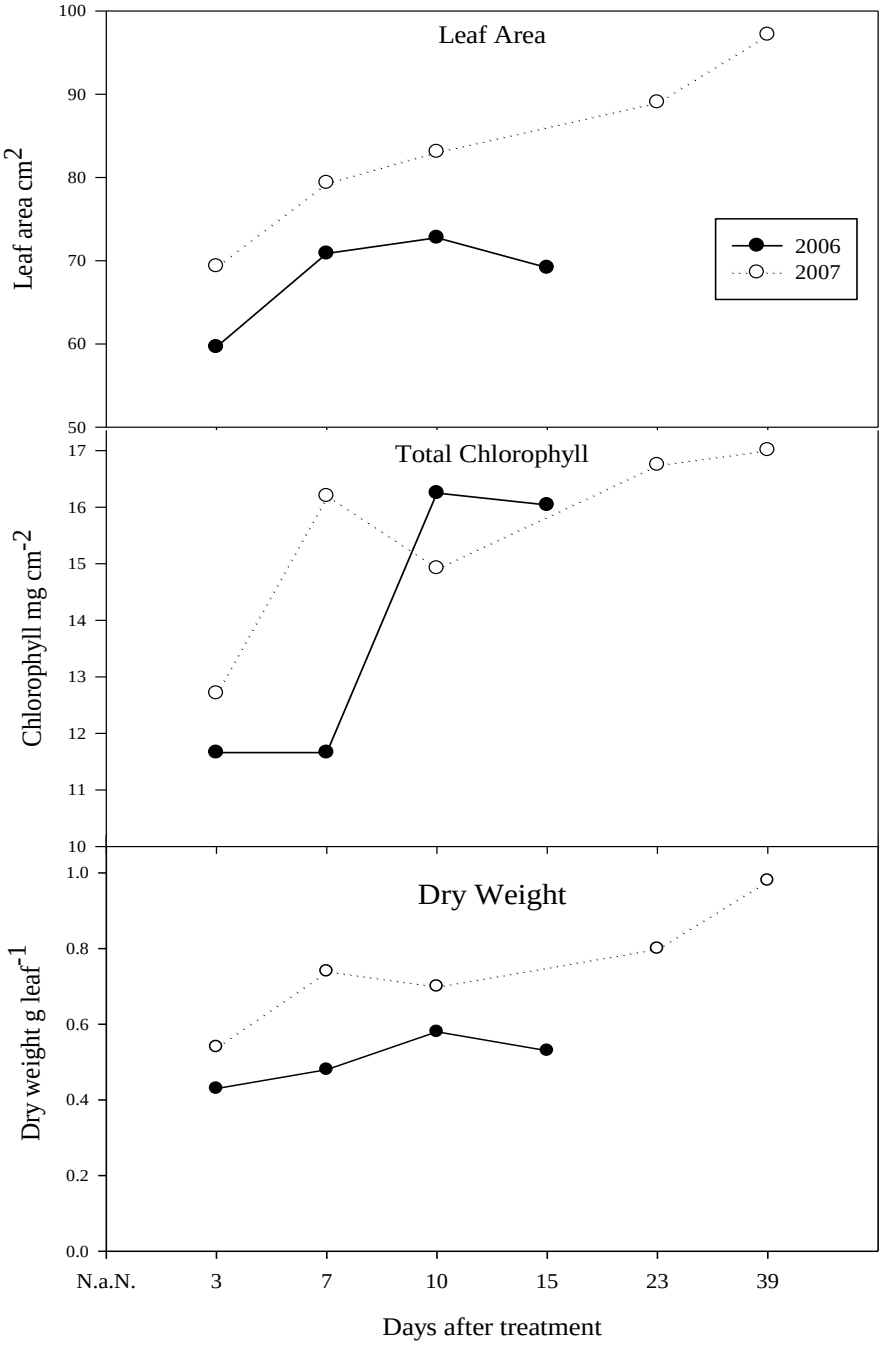

Figure 2.3. Leaf area, total chlorophyll and leaf dry weight in different environments at various days after treatment. Values are averaged across growth regulator treatment and leaf position.

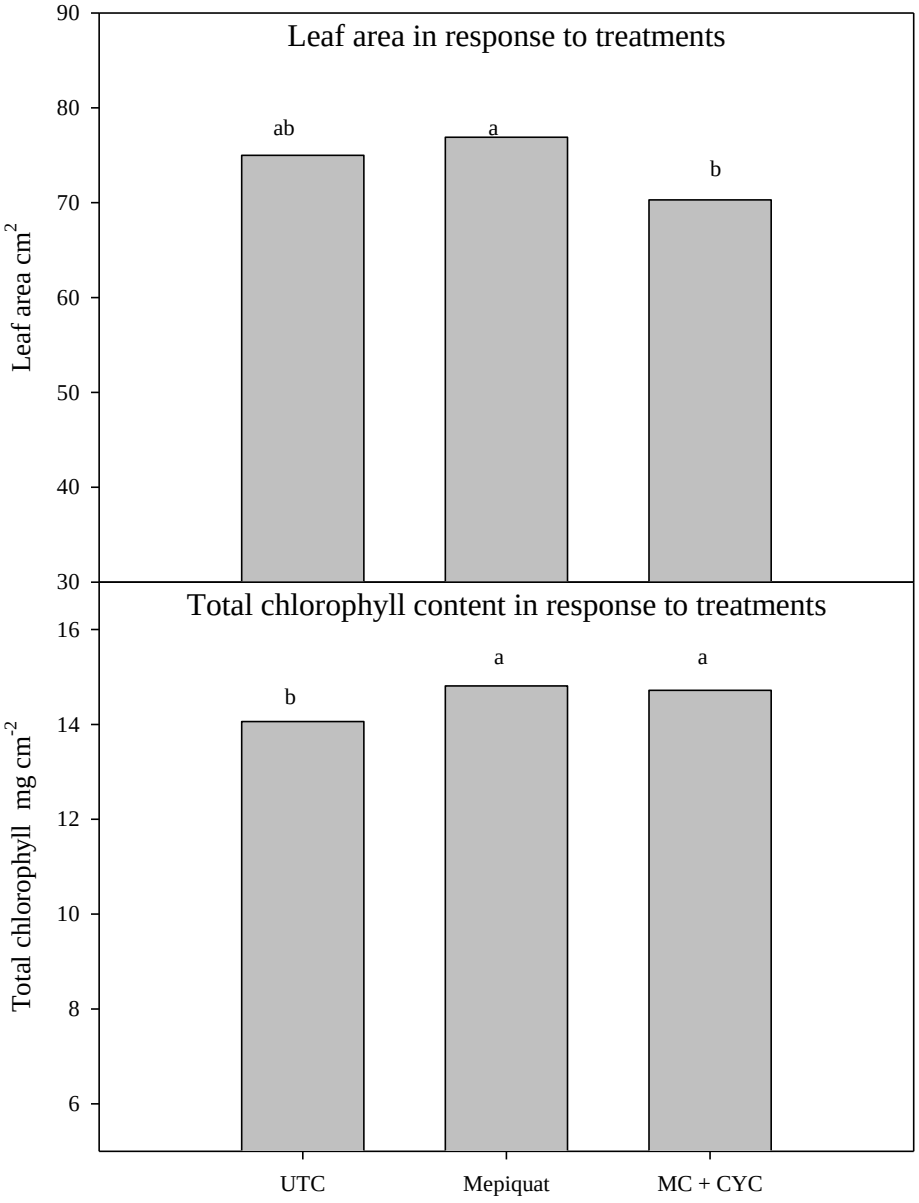

Figure 2.4. Leaf area and total chlorophyll content in response to MC and MCC treatments. Values are averaged across environments, leaf position and date of measurement.

Compensation in Cotton (*Gossypium hirsutum*) for Lost Fruiting Sites: When Does Early Fruit Loss Reduce Yield?

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Abstract

Cotton (*Gossypium hirsutum* L.) yield is dependent on the number of bolls produced and retained by the plant. Many biotic and abiotic stresses can induce the cotton plant to shed reproductive structures such as squares and young bolls. This study was conducted to determine the effect of simulated early fruit removal on lint yield, boll distribution, fiber properties and leaf pigmentation of cotton. The experiment was conducted for three years on Goldsboro loamy sand and Norfolk fine sandy loam soil (fine-loamy, siliceous, thermic, Typic Paleudult). Treatments consisted of early fruit removal and untreated check. The first three weeks of fruiting forms were removed by hand from treatment plots. At harvest, six plants per plot were sampled for box mapping. Fruit removal caused a 4% decrease in lint yield over control that was not significant. For nodes 3-5, the control plants had 5.3% of total bolls compared to 0.83% for removal plots (significant at $p \leq 0.1$). For nodal positions 6-10, the control had 44% bolls compared removal plots. Conversely, the removal treatment had more bolls than the control at higher nodal positions. Yield of the removal treatment was equivalent to the control due to the addition of these higher boll positions.

Abbreviations:

Ant, Anthocyanin; Chl, chlorophyll; DAT, Days after treatment; DAP, Days after planting; HNR, Height to node ratio; PPFD, Photosynthetic photon flux density; SLW, Specific leaf weight. LOS, Level of significance; MIC, Micronaire; UHM, Upper half mean length; DMF, dimethylformamide.

Introduction

Naturally, cotton plant sheds around 60% of young bolls and squares because of over production of fruiting structures (Oosterhuis and Jernstedt, 1999). The number of fruiting sites produced on a plant is more than the plant can supply with sufficient assimilates to support maturation (Wullschleger and Oosterhuis, 1990). Drought, insects and mechanical shock are some of the stress factors which can cause the cotton plant to shed squares and young bolls (Guinn, 1982).

Simultaneous vegetative and reproductive growth ameliorates the negative impact of fruit and square abscission due to biotic or abiotic stresses (Pettigrew et al., 1992). Pettigrew et al. (1992) reported increased vegetative growth, increased late season fruiting, delayed reproductive development, and no change in fiber quality in response to square removal. Increased late season fruiting did not contribute to yield as late formed bolls never matured by the end of growing season. They reported no change in lint yield in one year and 7% reduction in yield due to fruit removal by Ethephon.

Jones et al. (1996 a) documented that removing the first three weeks of flowers did not have any influence on lint yield, however later removals (4th week and later) reduced yield and caused significant delay in maturity while redistributing bolls on upper and outer sympodial positions on the plant. They also reported increased boll weight in response to flower removal with the limited boll number of individual bolls benefiting from a greater allocation of dry matter. Early season removal did not affect any fiber properties while late season removal (4th week and later) increased micronaire. Removal of active sinks (i.e.

developing bolls) reduced the reproductive sink strength resulting in increased vegetative growth compared to control Jones et al. (1996 b). In addition, delayed maturity in response to early fruit removal exposed the late formed bolls to frost at end of the season before they could fully mature. They reported that retaining all the bolls produced during 4 to 5 weeks period of early fruit development is not indispensible for higher yield and the plants compensate for lost fruit by producing and retaining more bolls on distal or outer positions on the lower sympodial nodes and on higher first positions on nodes 11-15. Later flower removals (4 week and later) resulted in lowered yields because it resulted in an earlier cessation of reproductive growth. However, the level of compensation for lost fruiting structures depends on time of growing season, stress levels and the previous management history of the crop (Jones et al., 1996 a). Wells (2001) reported 8% reduction in lint yield in response to fruit removal over control in three years; however these differences were significant only in one year, while individual boll weight was higher in response to removal. It was also reported that fruit removal changed the boll distribution pattern on the plant, with more bolls retained on lower outer and upper first positions.

Bednarz and Roberts (2001) reported reduction in weight of first position bolls in response to square removals of varying length and severity. In removal treatments, more lint yield was obtained from outer position bolls lower in the canopy than first position bolls higher in the canopy. First position bolls above node 12 never weighed more than the first position bolls below node 12 even after the lower bolls were removed. Upper and outer position bolls are normally dropped from the plant thus they do not contribute to yield. Cook

and Kennedy (2000) reported increased vegetative growth in response to early bud loss. This phenomenon therefore caused shading of young bolls less than 14 days old deep in the canopy leading to reduced boll retention lower in the canopy. Removal of forty percent of the squares at 10-14 days after pinhead stage resulted in 12% increase in plant height over the control. The plants compensated for lost lower position bolls by retaining outer and upper position bolls. However, yield differences were observed due to differences in boll numbers per plant. Increased boll number on the upper and outer positions in removal treatments contributed mainly to yield compensation. Also heavier bolls on second positions compensated for yield lost due to removal of first position bolls. Little or no effect of early season insect damage on lint yield and maturity has been reported by Wilson et al. (2003), however, recovery from this damage by reproductive growth later in the season is largely dependent on growing conditions, nutrient supply, water availability and plant population.

Sadras (1995) published an extensive review of fruit removal effects on cotton compensation and concluded that fruit loss can increase rate of photosynthesis in cotton, as it extends the duration of leaf expansion resulting in increased light interception, carbon assimilation and higher growth and yield. Early season fruit loss has been reported to alter the crop canopy structure and rate of photosynthesis, and increase radiation use efficiency (Sadras, 1996), but the effects of fruit removal on individual leaf light characteristics have not been investigated. The study contained herein examines the effect of early season fruit removal on leaf physiology, plant growth, and lint yield.

Materials and Methods

Field experiments were conducted for three growing seasons. The locations were at Clayton, NC in 2006 and in Beulaville, NC in 2007 and 2008. The soil type was Goldsboro loamy sand at Clayton and Norfolk fine sandy loam soil (fine-loamy, siliceous, thermic, typic paleudult) at Beulaville. Rainfall totals for both environments are shown in Figure 3.1. Cotton cultivar FM 960 BR was planted on 17 May 2006, 3 May 2007 and on 6 May, 2008 with a two row vacuum planter at 97-cm row spacing, with four rows per plot. All observations were taken from central two rows only. The experiment was laid out as a randomized complete block design with four replications. The treatments consisted of removing all fruits at three weeks after blooming (70, 81 and 76 days after planting in 2006, 2007 and 2008, respectively) with an untreated control. Pest management and production practices other than those for specific treatments were administered uniformly to the entire test and were standard for the region.

Prior to harvest, a sample of six plants per plot was taken for box mapping in two of the three growing seasons. Plant height, total nodes, first sympodial node, monopodial boll number and weight, sympodial boll number, position and weight were determined from these six plants for each plot.

The plots were harvested using a two row John Deere Spindle picker on 20 Oct. 2006, 12 Oct. 2007 and 16 Oct. 2008. Seed cotton yield was determined at the time of harvest. A 200 g sample of seed cotton was taken at that time to determine lint percent, which was used to calculate final lint yield per hectare. A fiber sample was analyzed at Cotton Inc. by high

volume instrumentation to determine fiber properties.

All data was analyzed using Proc Mixed in SAS. Years, leaves and sampling dates were considered as random effects. Specific error terms were used to determine significance of treatments. Means were separated using Fisher's protected LSD at $p \leq 0.05$.

Results and Discussion

Lint Yield

Lint yield did not differ due to fruit removal (Table 3.1 and 3.2). Fruit removal caused 4% decrease in lint yield over control. Although the interaction of Environment x Treatment was not significant, there was a difference among years ($p = 0.068$) (Table 3.1). In three environments, the fiber yields were 875, 1,215, and 912 Kg ha^{-1} for 2006, 2007, and 2008, respectively (Table 3.3). Jones et al. (1996 a) also found no effect of fruit removal during the first three weeks of anthesis. Early removals moved boll initiation to more distant positions out the sympodial branches and to higher main stem nodes (Jones et al., 1996 a).Wells (2001) reported 8% reduction in lint yield in response to early fruit removal; however, the reduction was significant in one year only.

Other than uniformity index, no fiber properties were influenced by fruit removal (Table 3.1 and 3.2). Conversely, environment affected all fiber properties except uniformity index, with p-values of 0.0278, <0.0001, 0.0006, and <0.0001 for lint percentage, micronaire, length, and fiber strength, respectively.

Boll Weight and Distribution

The number of bolls found at nodes 3-5 differed in response to removal at the 0.1

level of probability (Table 3.4). The control had 0.36 bolls/ plant while removal had 0.05 bolls/plant. Bolls at nodes 6-10 were ($p = 0.0003$) reduced in the fruit removal as compared with the control, with the control having 3.5 bolls/plant compared to 1.8 bolls/plant. Number of bolls at upper nodes did not differ, although removal treatment tended to have slightly higher number of bolls compared to control. The control had 16% more total bolls per plant compared to fruit removal.

Seed cotton weight by nodes: Seed cotton weight by node sections in response to fruit removal differed only for nodes 6 -10, where control plants had 11.35 g seedcotton weight per plant compared to 5.52 g for removal plants. All other node sections and total boll weight per plant did not differ between treatments (Table 3.5). Bednarz and Roberts (2001) found similar results wherein boll weight in response to removal was reported to be same while the number of bolls at first position was reduced and those bolls were redistributed to outer positions on the lower nodes in response to early fruit removal.

Average weight of single boll by position differed only for nodes 3-5, where control had average boll weight of 2.42 g per boll compared to 0.45 g for removal treatment (Data not shown). Removing previously set and growing bolls for main stem and first positions for the respective nodes led to this decline and the small weight per boll was from the contribution of later set outer set position bolls, which did not grow well due to competition from upper position bolls, reduced sink strength due to smaller size and longer distance from upper canopy well lit leaves photosynthesizing at higher rates than lower canopy shaded leaves. Average boll weight for other node positions and overall for the entire plant did not

differ between treatments. Bednarz and Roberts (2001) found similar results wherein reduction in boll weight at first position was reported. They also reported that first position bolls above node 12 were always lighter in weight even after bolls below node 12 were removed. The plant compensates for loss of fruiting structures by retaining more bolls at outer positions on the lower nodes instead of producing and retaining more first position bolls higher on the plant. These outer position bolls are normally lost if the plant attains decent boll retention, but are retained if the first position bolls lower in the canopy are lost. Similar findings were reported by Cook and Kennedy (2000).

Fruit removal had a significant effect on percent of bolls found at each nodal zone. For nodes 3-5, the control plants had 5.3% of total bolls compared to 0.8% for removal plots, though these differences were statistically similar (Data not shown). For nodes 6-10, control had 43.8% bolls compared to 27% on same nodes for removal plots. Conversely, on nodes 11-15, control had only 33.4% of the total bolls while removal had almost 50% of their total bolls on these nodes. Percentages of bolls on higher nodes were similar.

Removal plants had a higher percentage of vegetative monopodial bolls, 12.8%, compared to 7.8% of control. Thus in case of plants with fruit load removed, the plants recovered by producing and retaining a higher percentage of their bolls on higher up positions on the plant and also by producing and retaining more and heavier vegetative bolls. This increased number of heavier vegetative bolls might be responsible for a part of yield stability in removal treatments despite loss of earlier set fruit, in addition to production and retention of more bolls higher up on the plant. Similar results regarding redistribution of boll

position and weight have been reported by Pettigrew et al. (1992), Jones et al. (1996 b), Wells (2001), and Cook and Kennedy (2000). Number of vegetative monopodial bolls per plant did not differ for treatments. Average weight per vegetative boll was also statistically similar but removal plants had 86% higher weight per vegetative boll compared to control.

Pettigrew et al. (1992) reported no difference in lint yield in response to fruit removal treatments in one year while 7% reduction was reported in response to fruit removal by Ethephon in another year of study. No changes in fiber properties were reported in response to any fruit removal treatments. Jones et al. (1996 a) reported that removing fruiting structures three weeks after bloom did not influence lint yield and fiber properties but delayed crop maturity while later flower removal caused a reduction in lint yield with increase micronaire of the fiber.

Fruit removal did not have any effect on plant height, first reproductive node, total nodes per plant and height to node ratio (Data not shown). Pettigrew et al. (1992) reported similar results wherein fruit removal did not affect plant height in one year of the study. However, the first fruiting node was higher in fruit removal plots by two nodes, as the removal treatment consisted of removing lower main stem bolls formed at the time of treatment.

Conclusions

The failure to induce yield losses through removal of the first three weeks of anthesis appears to be attributable to a shift in boll production to both higher nodal positions and to monopodial branches. This shift, plus favorable environments that allowed later boll production, combined to allow sufficient growth to overcome the early losses.

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Table 3.1. Analysis of variance summary for yield and fiber properties in response to early fruit removal and non-removal (control) treatments in cotton.

Factor	Yield	Lint percentage	Micronaire	Length	Uniformity Index	Strength
	---------- p > F ----------					
Year	0.0678	0.0278	<0.0001	0.0006	0.1391	<0.0001
Treatment (TRT)	0.5782	0.0550	0.1899	0.817	0.0300	0.1273
Year x TRT	0.1310	0.6005	0.9195	0.0445	0.0265	0.2715

Table 3.2. Yield and fiber property responses to fruit removal during the first three weeks of anthesis and non-removal (control) treatments in cotton. Values are averaged over years.

Treatment	Yield	Lint percentage	Micronaire	Length (UHM)	Uniformity Index	Strength
	Kg ha^{-1}	%		cm	%	g tex^{-1}
Control	1035†	41	4.70	1.08	81.5 b	30.2
Removal	990	40	4.49	1.10	82.7 a	31.1

† Means within a column followed by the different letters are different at $p \leq 0.05$.

Table 3.3. Fiber yield in responses to fruit removal during the first three weeks of anthesis and non-removal (control) treatment over three years in North Carolina.

Treatment	Year		
	2006	2007	2008
	----------Kg ha^{-1}----------		
Control	830	1320	920
Removal	920	1110	900

† Means within a column followed by the different letters are different at $p \leq 0.05$.

Table 3.4. Analysis of variance summary for the number of bolls found in varying nodal zones and total bolls per plant in response to early fruit removal.

	Bolls/plant				
Source	nodes 3-5†	nodes 6-10	nodes 11-15	nodes 16-20	Total
	----------	----------	p > F	----------	----------
Year	0.0292	0.1307	0.0310	0.0508	0.0422
Treatment (TRT)	0.0970	0.0003	0.3363	0.5667	0.4417
Year x TRT	0.0970	0.5063	0.9522	0.5667	0.7733

†Node number is counted from the plant base.

Table 3.5. Mean seed cotton weight per plant for node sections and total per plant obtained from box mapping.

Source	Seedcotton node 3-5†	Seedcotton node 6-10	Seedcotton node 11-15	Seedcotton node 16-20	Total seedcotton
	----------g/plant----------				
Control	1.35a‡	11.35 a§	9.78	2.25	27.21
Removal	0.15b	5.52 b	12.12	3.21	23.73

† Node number is counted from the plant base.

‡ Means within column followed by the different letters are different at $p \leq 0.1$.

§ Means within column followed by the different letters are different at $p \leq 0.05$

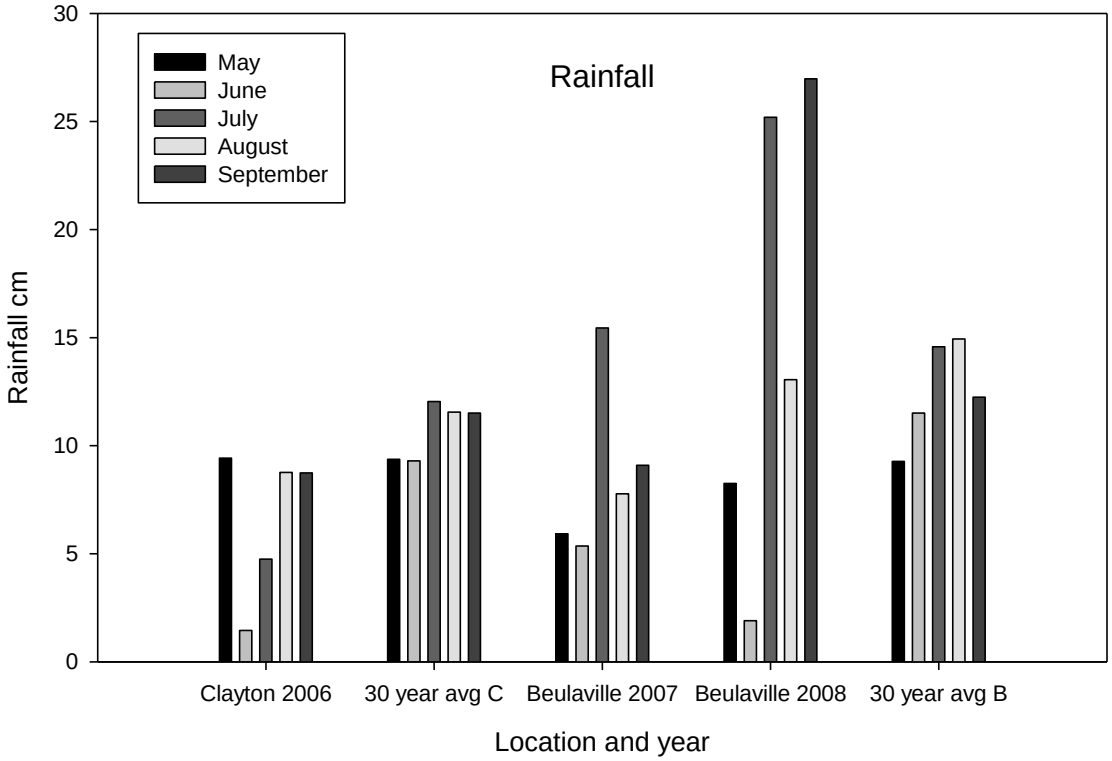

Figure 3.1. Seasonal rainfall (cm) with monthly totals for growing seasons of 2006 at Clayton (C) and for 2007 and 2008 at Beulaville (B), with 30 years average for both locations.

Changes in Cotton (*Gossypium hirsutum*) Leaf Pigmentation after Abnormal Exposure to Sunlight

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Abstract

Leaves are adapted to respond differentially to changing light conditions and intensity. Cotton leaves are normally green during the growing season. However, mechanical shock or wind sometimes turns the leaves over causing them to invert exposing the abaxial side to direct sunlight resulting in development of red pigmentation in otherwise green leaves. Based on this observation, this study was conducted to determine the effect of exposure of abaxial surface to direct sunlight on leaf pigmentation. Field experiments were conducted in 2007 and 2008 on Norfolk Loamy Sand. At 92 DAP, fully opened quarter seized (24.26 mm diameter) or larger main stem leaf (T) and the second leaf (T+2) below that were clamped in an opaque plastic rectangular frame fixed to a bamboo staff supporting the frame. The frame, with the leaf clamped in it, was turned at 180^0 to completely invert the leaf from its normal position. Inverting the leaves caused a visual reddening of the abaxial surface of the leaf blade while there was no effect on leaf area, specific leaf weight (SLW), fresh weight or dry weight of the leaves. The leaf at T+2 had higher leaf area, fresh weight, and dry weight while SLW was reduced compared to leaf at position T. Inverting the leaves increased the anthocyanin concentration while causing a reduction in chlorophyll content over the same period. Leaf at position T+2 had higher anthocyanin and lower chlorophyll content. Inverting exposed the underside abaxial side of the leaves to direct sunlight which normally is protected from exposure. This abnormal exposure to direct sunlight caused the leaves to actively synthesize more anthocyanin. Abnormal exposure of lower leaf surface to direct sunlight caused reduction in chlorophyll content and increase in anthocyanin content

which is synthesized in response to light for its role in photoprotection and quenching of reactive oxygen species. In this case increased anthocyanin could not apparently protect the chlorophyll from damage due to excessive sunlight.

Abbreviations:

Ant, Anthocyanin; Chl, chlorophyll; DAT, Days after treatment; DAP, Days after planting; SLW, Specific leaf weight. DMF, dimethylformamide; PAR, Photosynthetically active radiation; ROS, Reactive oxygen species.

Introduction

Cotton (*Gossypium hirsutum* L.) leaves have a dark green pigmentation during normal growing conditions. However, some diseases, stresses and the cessation of new growth at the end of the reproductive cycle cause the leaves to lose green color and become reddish to bronze in color due to anthocyanin production (Wells, 2001). Sunlight, bacterial infections (Kangatharalingam et al., 2002), cold night temperature (Neill and Gould, 2003) along with maturity and senescence (Hoch et al., 2003) are some of the factors that cause the development of red color in leaves due to biosynthesis of anthocyanins.

Anthocyanins are abundant in juvenile and senescing leaves and their concentration increases in response to exposure to ultra-violet radiation, high intensity PAR, drought, and nutrient deficiency (Merzlyak et al., 2008; Steyn et al., 2002).Young and immature leaves have been documented to have higher anthocyanin content than mature leaves (Hatier and Gould, 2009). At this stage, their role is to protect the young leaves and its developing photosynthetic machinery from damage caused by excessive solar radiation and oxidative stress (Hoch et al., 2001). Anthocyanins are synthesized in the cytosol and are predominantly stored in vacuoles of epidermal cells of cotton leaves (Kangatharalingam et al., 2002). The anthocyanins are low molecular weight photo-protectors which quench the excessive reactive oxygen species in young leaves and acts as an antioxidant to prevent photobleacing of chlorophyll. As the leaf matures and more chlorophyll is produced in the leaves, higher amounts of solar radiation can be utilized for photosynthesis thereby reducing the need of anthocyanin as photo-protector. This change leads to decline in anthocyanin concentration in

fully expanded leaves.

Approaching senescence, chlorophyll breaks down into basic building blocks including nitrogen and once again renders the leaves prone to damage by photoinhibition. This stress leads to increased synthesis of anthocyanin which protects the leaf physiological machinery during the dismantling process to successfully recycle the metabolites into storage forms away from leaves. Presence of a strong sink such as developing fruit increases the resorption of these metabolites (Hoch et al., 2003). Buildup of anthocyanin concentration during senescence follows chlorophyll breakdown in the leaves even before any color change or pigmentation is visible (Hoch et al., 2001).

Cotton is inherently a perennial plant which is agronomically managed as an annual. The perennial growth habit complicates the problems for growers at the end of growing season by virtue of increased difficulty of defoliating in preparation for mechanical harvest and regrowth of terminal and basal leaves which can cause trash in lint and discoloration and staining of lint during harvest. At the time of defoliation, it is often observed that greener leaves are harder to defoliate compared to leaves which have developed some red pigmentation. Guthrie (1983) reported reddened cotton foliage due to increased anthocyanin and decreased chlorophyll concentration in response to two applications of methomyl (s-methyl-N-{methylcarbamoyl)oxy}thioacetamide), which resulted in premature defoliation of the reddened foliage.

Red pigmentation or anthocyanin synthesis at end of the growing season was mediated by low night temperature and high light intensity in five species (Merzlyak et al.,

2008). In maize seedlings grown under low temperature regime of $18/11^0$ C day/night, anthocyanins absorbed 43% of light energy compared to just 1.4% in plants grown under high temperature of $23/30^0$ C (Pietrini and Massacci, 1998). Chlorophyll breakdown and anthocyanin synthesis is normal process in the leaves at end of the growing season, when high PAR intensity and occasional low night temperatures are experienced. Chlorophyll and anthocyanin levels can be used to determine decrease in photosynthetic rates in cotton leaves at senescence (Wells 2001). However, during peak growth season before boll opening or even cutout, it is commonly observed that parts of cotton leaves turned over or inverted by wind or mechanical force are exposed to direct sunlight on their abaxial surface, which normally never receives direct sunlight. That part of the leaf receiving direct sunlight turns red while rest of the leaf remains green. Kangatharalingam et al. (2002), while investigating the role of anthocyanins in imparting resistance to bacterial leaf blight in cotton, reported the development of anthocyanins on abaxial surface of leaves after exposure to light. It is not known whether abnormal exposure of the abaxial surface to sunlight would increase anthocyanin levels prior to later stages of crop maturity. The experiment contained herein was conducted to determine the effect of exposure of abaxial surface to direct sunlight on leaf pigmentation.

Materials and Methods

Field experiments were conducted in 2007 and 2008 at Central Crops Research Station, Clayton NC on Norfolk Loamy Sand. Cotton variety DP 147 was planted on 29 Apr. 2007 and 14 May 2008 with a two row vacuum planter at 97 cm row spacing. Each plot

consisted of four rows. Treatments were imposed on the two interior rows. The experiment was laid out as a randomized complete block design with four replicates.

At 92 DAP, fully opened quarter seized (24.26 mm diameter) or larger main stem leaf (T) and the second leaf (T+2) below that were clamped in an opaque plastic rectangular frame fixed to a bamboo staff supporting the frame. The frame, with the leaf clamped in it, was turned at 180^0 to completely invert the leaf from its normal position, with the abaxial side facing upwards and the adaxial side facing the ground (Figure 4.1). Four plants per plot were set up in this manner, with third and fifth leaf from the plant apex enclosed. The control treatments were enclosed in the frames with the abaxial side facing the ground and the adaxial side facing upwards (normal leaf orientation). The frames were set up in way as to minimize mechanical pressure or damage to the leaves.

At 92, 98, 105, and 111 DAP, enclosed leaves (inverted and control) were harvested from one plant per plot. The leaves were sealed in plastic bags and placed on ice for transportation to laboratory for analysis. Leaf area was measured on LI-COR, LI 3100 area meter (LICOR Inc. Lincoln, NE). For pigment analysis, three leaf disks of 0.3 cm^2 from each leaf were placed in 3 ml dimethylformamide (DMF) in the dark for 2 days at 4^0C. Total chlorophyll and ratio of Chl a/b was determined from the DMF extract spectrophoto-metrically (Moran, 1982). Concentration of Anthocyanin was determined by placing three leaf discs (0.3 cm^2) in 3 ml acidified methanol with 10 ml concentrated HCl/L for 2 days at 4^0C. Light absorbance of the DMF and methanol extracts were determined at 530 nm (Anthocyanin) and 658 nm (Chl). Anthocyanin concentration was calculated using the

following formula of Mancinelli et al. (1998):

$$Ant = A_{530} - 0.25\ (A_{658}).$$

The remaining leaves were placed in a drying oven at 60^0 C for 3 days for obtaining dry weight. Specific leaf weight was obtained by dividing the leaf area by dry weight.

All data was analyzed using Proc Mixed in SAS. Years, leaves and sampling dates were considered as random effects. Specific error terms were used to determine significance of treatments. Means were separated using Fisher's protected LSD at $p \leq 0.05$.

Results and Discussion

Inverting the leaves caused a visual reddening of the abaxial surface of the leaf blade (Figure 4.1A). The region of the leaf that the plastic frame covered showed a lack of reddening (Figure 4.1B). Inverting the leaves did not have any effect on leaf area, specific leaf weight (SLW), fresh weight or dry weight of the leaves (Table 4.1). The leaf at T+2 had more leaf area, fresh weight, and dry weight while SLW was reduced compared to leaf at position T. The larger leaf area is related to the smaller leaves size that was found at higher nodes. Wells (1988) found that leaves emerging at 62 days after planting (DAP) had more than twice the area of leaves emerging at 89 DAP. The reduction in SLW for larger leaf could be an indication of the negative relationship that exists between leaf area and SLW. Wiebold and Kenworthy (1985) found negative correlations between SLW and leaf area in maturity group IV soybean cultivar terminal trifoliolate leaves at position 8 and 10 from the plant apex (-0.46* and -0.69**, respectively).

Inverting the leaves increased the anthocyanin concentration while causing a reduction in chlorophyll content over the same period (Figure 4.1 and 4.4). Leaf at position T+2 had higher anthocyanin and lower chlorophyll content (Figure 4.2 and 4.3). Inverting exposed the underside abaxial side of the leaves to direct sunlight which normally is protected from exposure. This abnormal exposure to direct sunlight caused the leaves to actively synthesize more anthocyanin. Wells (2001) reported an increase in anthocyanin synthesis and decline in chlorophyll concentration in cotton leaves between 100 and 110 DAP. It has been suggested that anthocyanin plays the role of a sunscreen (Gould, 2004). By absorbing high-energy quanta, anthocyanins protect chloroplasts from the photo-inhibitory and photo-oxidative effects of strong light. Neill and Gould (2003) reported that anthocyanins offer protection by two processes, by acting as a mask for filtering green light and by scavenging reactive oxygen species (ROS), thereby reducing the losses from photoinhibition after leaves are exposed to strong light. They also proposed that higher incidence of anthocyanins in stress environment is the last line of defense against ROS and photoinhibition after all other mechanisms of protections have been exhausted. Hoch et al.(2003) theorized that anthocyanins facilitate foliar nutrient resorption during senescence by protecting photosynthetic tissues from excess light. Using wild type and anthocyanin-deficient mutants of three deciduous woody species they found wild type plants maintained higher photochemical efficiencies than mutants and were able to recover more easily from the effects of a high light, low temperature environment than could the mutants. Based on these reports it is possible that the anthocyanin increase in the inverted leaves is induced as a

photo-protectant from light directed at tissue that is normally unexposed. The difference in anthocyanin production due to leaf position suggests that there is a leaf age component to the response. Younger leaves may not have the capacity for anthocyanin production. Anecdotally, this is observed in the appearance of redness in only older field-grown plants during the season.

Conclusions

Abnormal exposure of lower leaf surface, which normally never receives direct sunlight, caused reduction in chlorophyll content and increase in anthocyanin content. Anthocyanin was synthesized in response to light exposure possibly for its role in photoprotection and quenching of reactive oxygen species. Albeit a response of individual leaves exposed to an abnormal situation, the reddening of cotton approaching cutout may play a photoprotection role during this period of transition from one cycle of growth into the next.

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Table 4.1. Analysis of variance summary for Leaf area, Chlorophyll, Anthocyanin (Ant), Fresh, Dry and Specific Leaf Weight in response to year, treatment, harvest date and leaf position.

Source	Leaf area	Ant	Total Chl	Fresh Weight	Dry Weight	SLW
	---------- p > F ----------					
Year	0.0002	0.7781	0.0611	0.0001	<0.0001	0.1324
Treatment (Trt)	0.7123	0.0015	0.0031	0.8153	0.8365	0.7420
Year x Trt	0.5403	0.2701	0.5737	0.8920	0.9598	0.9238
Date	0.7754	0.0744	0.7126	0.6713	0.0292	0.0006
Year x Date	0.3333	0.3834	0.3857	0.2861	0.0331	0.0269
Date x Trt	0.7490	0.3876	0.6838	0.8014	0.3702	0.3827
Year x Trt x Date	0.8043	0.0777	0.7801	0.2583	0.2390	0.3336
Leaf Position (LP)	<0.0001	0.0282	<0.0001	<0.0001	0.0172	<0.0001
Year x LP	0.0363	0.0019	0.2340	0.0592	0.2942	0.0256
Trt x LP	0.1498	<0.0001	<0.0001	0.2136	0.2029	0.6582
Year x LP x Trt	0.2683	0.0810	0.7892	0.2574	0.5811	0.8733
Date x LP	0.7553	0.7484	0.0236	0.2556	0.0572	0.1000
Year x date x LP	0.4035	0.4419	0.8524	0.5556	0.9490	0.4122
Date x LP x Trt	0.9751	0.0230	0.3793	0.9776	0.5224	0.6605
Year x Date x Trt x LP	0.8738	0.0213	0.7850	0.5763	0.5784	0.6329

Table 4.2. Treatment means for Leaf area, Chlorophyll, Anthocyanin, Fresh, Dry and Specific Leaf Weight (SLW). Values are averaged for two years.

Factor	Leaf area	Anthocyanin	Total chlorophyll	Fresh Weight	Dry Weight	SLW
	cm^2	cm^{-2}	$g\ m^{-2}$	g	g	$g\ m^{-2}$
Control	94.85	0.21 B	14.78 A	2.74	0.73	0.0073
Inverted	95.96	0.26 A	13.59 B	2.79	0.71	0.0072
Harvest Date 1	100.33 A†	0.23	14.17	2.89	0.88 A	0.0078 A
Harvest Date 2	97.65 AB	0.24	14.52	2.82	0.75 B	0.0062 B
Harvest Date 3	95.40 AB	0.22	14.05	2.76	0.63 B	0.0069 B
Harvest Date 4	88.90 B	0.26	14.19	2.58	0.71B	0.0080 A
Leaf T	79.57 B	0.23 B	14.86 A	2.32 B	0.68 B	0.0080 A
Leaf T+2	111.69 A	0.25 A	13.59 B	3.21 A	0.76 A	0.0065 B

† Means within a column followed by the different letters are different at $p \leq 0.05$.

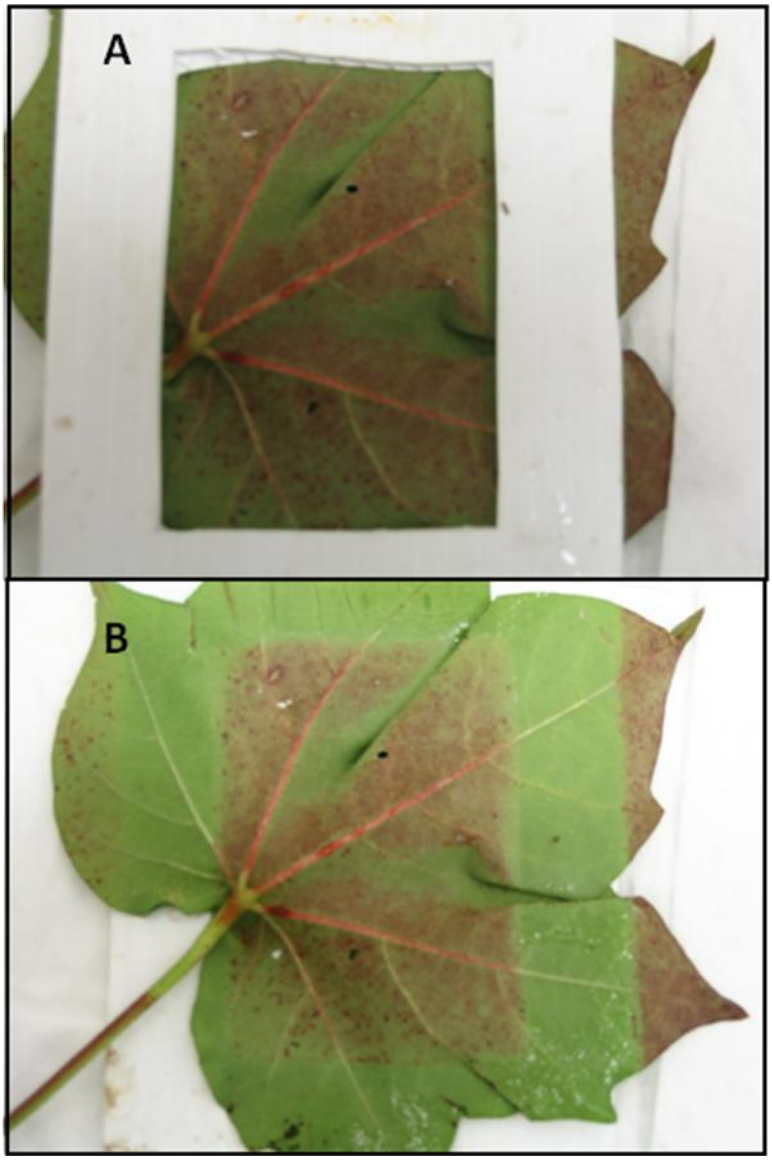

Figure 4.1. Effect of direct sunlight on inverted cotton leaves mounted in plastic frames. The exposed parts of the leaves have developed red color due to anthocyanin synthesis while the part covered by the frame is normal green.

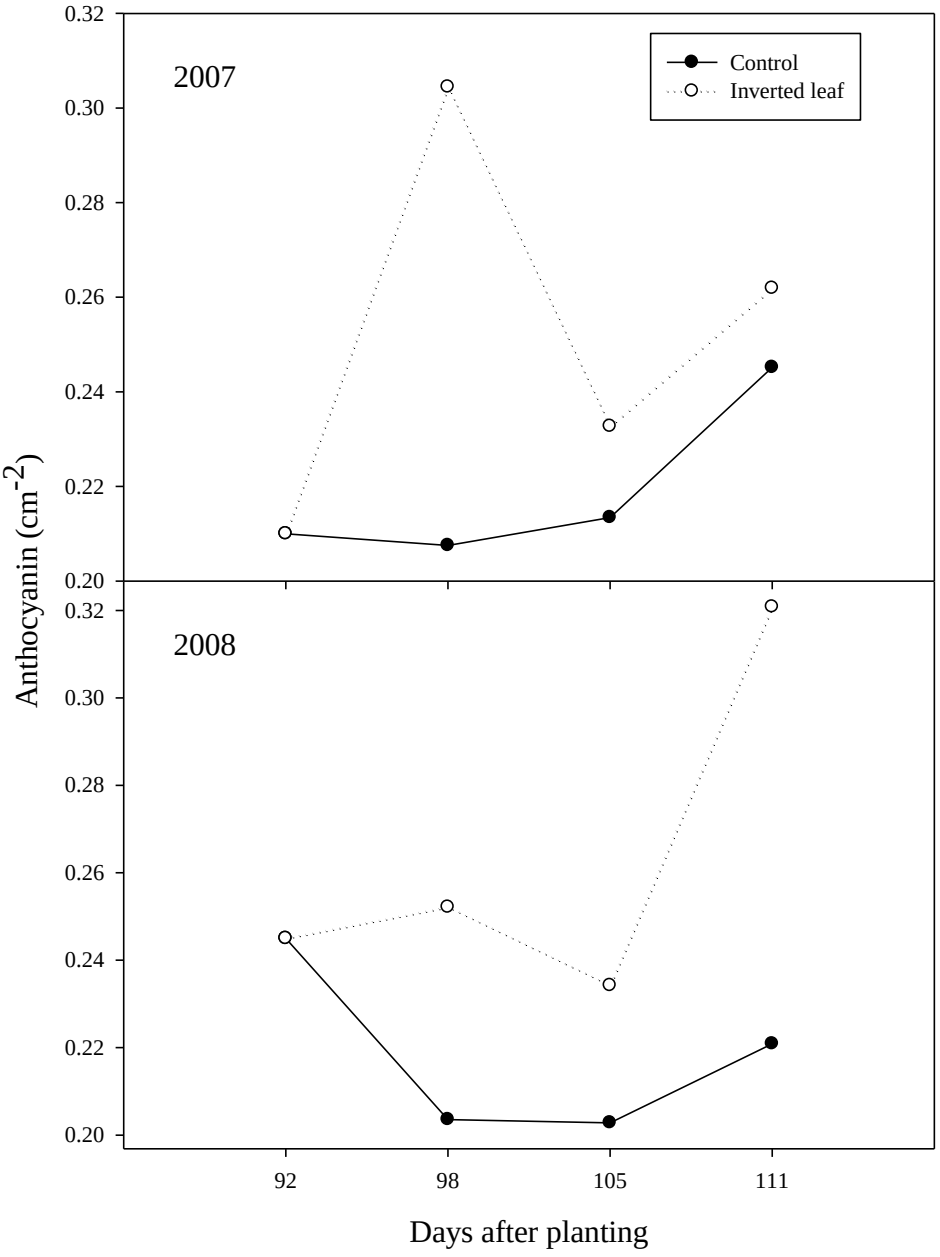

Figure 4.2. Level of anthocyanin in control and inverted leaves for two years at 92, 98, 105, and 111 DAP. Values are averaged over leaf position T and T+2.

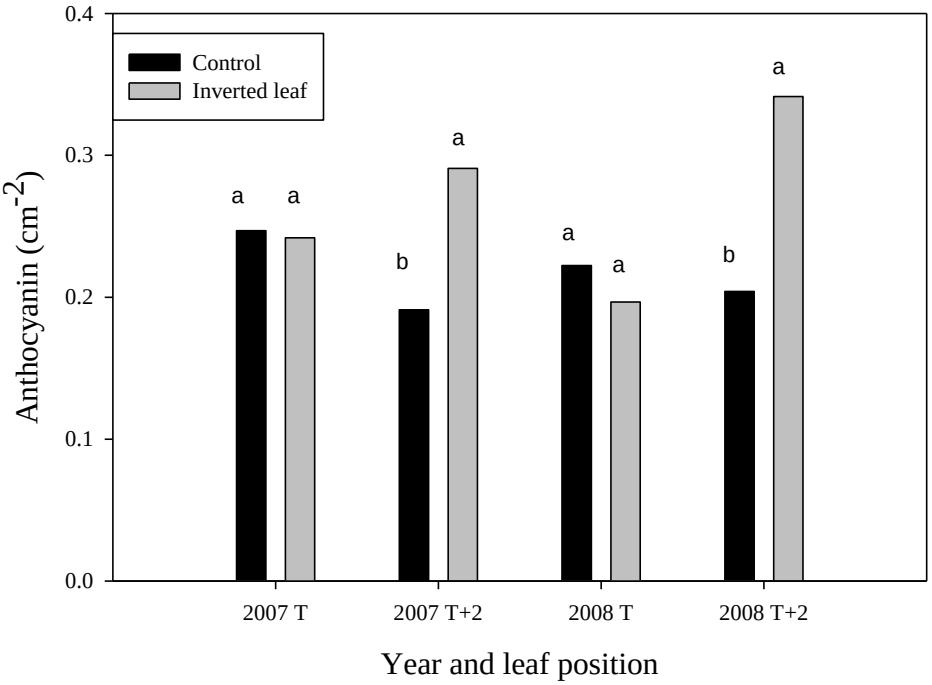

Figure 4.3. Level of anthocyanin in control and inverted leaves over two years at position T and T+2. Values are averaged over harvesting dates.

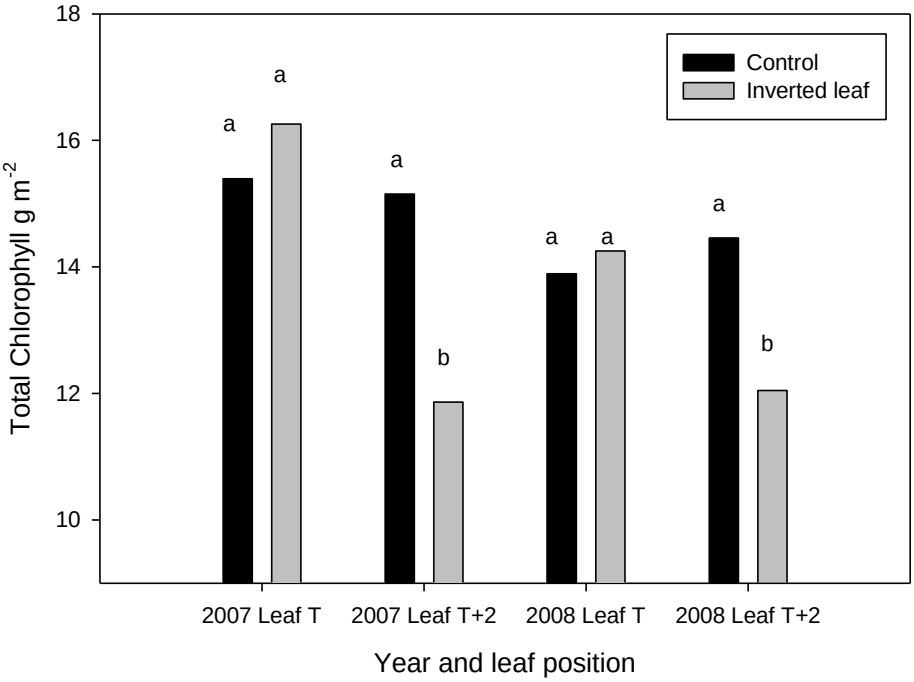

Figure 4.4. Level of total chlorophyll in control and inverted leaves over two years at position T and T+2. Values are averaged over harvesting dates.

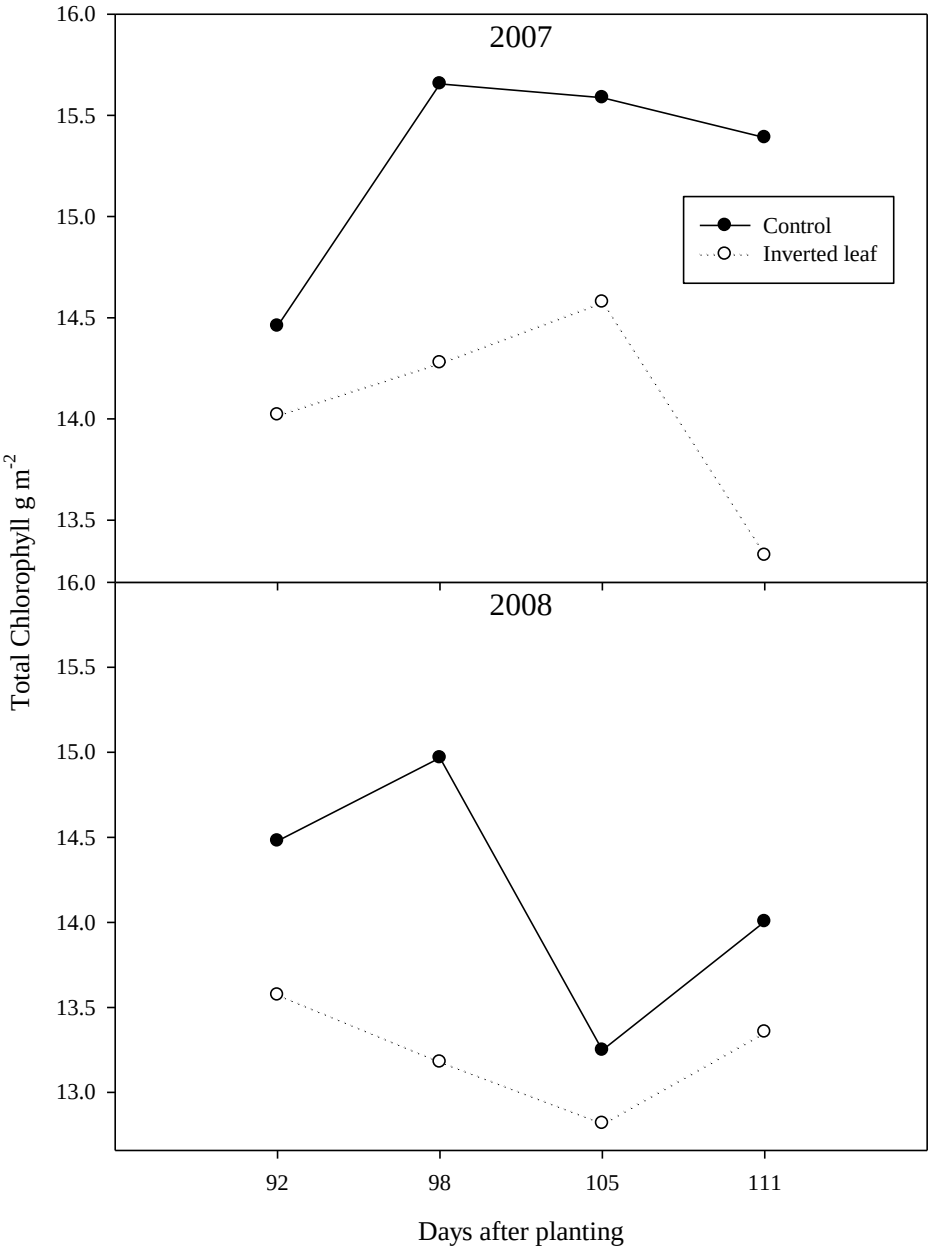

Figure 4.5. Level of total chlorophyll in control and inverted leaves for two years at 92, 98, 105, and 111 DAP. Values are averaged over leaf position T and T+2.

Appendices

Table A.1.1: Mean number of bolls per plant by node sections in response to row spacing, leaf morphology and population. Data pooled over locations and years.

Source	Number of bolls per plant for nodes					Total bolls/plant
	3-5	6-10	11-15	16-20	21-25	
Row 38 cm	0.10 b†	2.66 a	2.53	0.41	0.011	6.82
Row 97 cm	0.17 a	2.84 a	2.34	0.44	0.002	6.94
Leaf Normal	0.12	2.86	2.51	0.54 a	0.011	7.14
Leaf Okra	0.15	2.64	2.36	0.31 b	0.002	6.63
Pop. 7 plants/m^2	0.18 a	3.38 a	3.14 a	0.67 a	0.017	9.63 a
Pop. 12 plants/m^2	0.15 a	2.67 b	2.41 b	0.42 b	0.000	6.50 b
Pop. 18 plants/m^2	0.08 b	2.21 c	1.75 c	0.20 c	0.003	4.52 c

† Means within a column followed by the different letters are different at $p \leq 0.05$.

Table A.1.2: Average seedcotton weight per plant by node section in response to row spacing, leaf morphology and population. Data pooled over locations and years.

Source	Average boll weight (g) per plant for nodes					Total boll wt./plant
	3-5	6-10	11-15	16-20	21-25	
Row 38 cm	0.42 b†	12.60	11.89	1.47	0.03	31.51
Row 97 cm	0.76 a	13.65	11.64	1.68	0.00	33.12
Leaf Normal	0.59	13.75	11.96	1.93 a	0.024	33.34
Leaf Okra	0.59	12.49	11.57	1.22 b	0.008	31.29
Pop. 7 plants/m^2	0.90 a	16.81 a	15.84 a	2.53 a	0.048	46.96 a
Pop. 12 plants/m^2	0.56 b	11.87 b	11.24 b	1.59 b	0.00	29.00 b
Pop. 18 plants/m^2	0.30 b	10.69 b	8.20 c	0.59 c	0.00	20.98 c

† Means within a column followed by the different letters are different at $p \leq 0.05$.

Table A.1.3: Average seed cotton weight per boll by node section and overall average for whole plant in response to row spacing, leaf morphology and population. Data pooled over locations and years.

	Weight per boll (g) for nodes					Avg. wt/boll
	3-5	6-10	11-15	16-20	21-25	
Row 38 cm	1.53	4.72 b†	4.50	1.53	0.08 a	4.53 b
Row 97 cm	1.63	4.93 a	4.66	1.98	0.00 b	4.75 a
Leaf Normal	1.54	4.77	4.55	1.59	0.036	4.61
Leaf Okra	1.62	4.88	4.62	1.93	0.048	4.68
Pop. 7 plants/m^2	1.93 a	5.04 a	4.97 a	2.39 a	0.12	4.86 a
Pop. 12 plants/m^2	1.91 a	4.62 b	4.52 b	1.96 a	0.00	4.52 b
Pop. 18 plants/m^2	0.89 b	4.81 ab	4.26 b	0.93 b	0.00	4.55 b

† Means within a column followed by the different letters are different at $p \leq 0.05$.

Table A.1.4: Means for plant morphological characteristics in response to row spacing, leaf morphology and population. Data pooled over locations and years.

Source	Plant ht. (cm)	First rep node	First set node	Total nodes	Height /node ratio
Row 38 cm	82.46 b†	6.99 a	7.62 a	19.71 a	4.22 b
Row 97 cm	92.00 a	6.85 a	7.44 a	20.06 a	4.64 a
Leaf Normal	85.36 b	6.76 b	7.48	20.13 a	4.28 b
Leaf Okra	89.11 a	7.07 a	7.58	19.64 b	4.58 a
Pop. 7 plants/m^2	88.89 a	6.97	7.35 b	20.76 a	4.31 c
Pop. 12 plants/m^2	87.81 a	6.90	7.53 ab	19.91 b	4.43 b
Pop. 18 plants/m^2	85.00 b	6.88	7.72 a	18.99 c	4.54 a

† Means within a column followed by the different letters are different at $p \leq 0.05$.

Table A.1.5: Monopodial boll characteristics per plant in response to row spacing, leaf morphology and population. Data pooled over locations and years.

Source	Average vegetative bolls/plant	Avg. vegetative boll weight/plant	Avg. weight/ vegetative boll
Row 38 cm	1.09 a†	5.09	3.76
Row 97 cm	1.13 a	5.38	3.91
Leaf Normal	1.07	5.07	4.02
Leaf Okra	1.15	5.40	3.65
Pop. 7 plants/m^2	2.23 a	10.81 a	4.58 a
Pop. 12 plants/m^2	0.84 b	3.71 b	4.20 a
Pop. 18 plants/m^2	0.26 c	1.19 c	2.73 c

† Means within a column followed by the different letters are different at $p \leq 0.05$.

Table A.3.1: Plant characteristics for two years in response to early fruit removal.

Source	Plant height (cm)	First rep node	First set node	Total nodes	Height /node ratio
2007	106.31 b†	5.39 b	6.77 b	19.09 b	5.65
2008	141.41 a	7.83 a	8.87 a	24 a	5.91
Control	116.75	6.05	6.47 b	20.75	5.69
Removal	119.27	6.36	8.47 a	20.69	5.79

†Means within a column followed by the different letters are different at $p \leq 0.05$.

Table A.3.2: Number of bolls per plant in response to years and early fruit removal. Treatments values are averaged over years.

Source	Number of Bolls/plant node 3-5	Number of Bolls/plant node 6-10	Number of Bolls/plant node 11-15	Number of Bolls/plant node 16-20	Total bolls/plant
Year 1	0.31 a†	2.85a	2.54b	0.37b	6.62b
Year 2	0 b	2.21b	5.17a	2.25a	11.03a
Control	0.36 a	3.5a	3.05	0.94	8.69a
Removal	0.05 b	1.77 b	3.77	1.05	7.52b

† Means within a column followed by the different letters are different at $p \leq 0.05$.

Table A.3.3: Average weight of bolls per plant in response to years and early fruit removal. Treatments values are averaged over years.

Source	Avg. boll wt./plant node 3-5	Avg. boll wt./plant node 6-10	Avg. boll wt./plant node 11-15	Avg. boll wt./plant node 16-20	Total boll wt./plant
Year 1	1.13a†	9.34a	8.21b	0.94b	21.30b
Year 2	0b	6.63b	16.43a	6.3a	33.8a
Control	1.35a	11.35a	9.78b	2.25b	27.21a
Removal	0.15b	5.52b	12.12a	3.21a	23.73b

† Means within a column followed by the different letters are different at $p \leq 0.05$.

Table A.3.4: Weight per boll in response to years and early fruit removal. Treatments values are averaged over years.

Source	Weight/boll node 3-5	Weight/boll node 6-10	Weight/boll node 11-15	Weight/boll node 16-20	Avg. weight/boll
Year 1	2.15a†	3.22	3.24	1.86b	3.22
Year 2	0b	3.01	3.13	2.61a	2.99
Control	2.42a	3.21	3.26	2.25a	3.18
Removal	0.45b	3.09	3.14	1.99b	3.10

† Means within a column followed by the different letters are different at $p \leq 0.05$.