QUANTIFYING THE EFFECTS OF THE TIMING OF WATER DEFICIT STRESS AND WATER DEFICIT STRESS ALLEVIATION ON COTTON (*Gossypium hirsutum* L.) GROWTH AND YIELD UNDER RAIN-SHELTERED CONTROLLED CONDITIONS

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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August 2015

Major Subject: Agronomy

ABSTRACT

Water deficit is a major limitation for cotton yield in drought-prone Texas croplands. Where underground or surface water is available and cost affordable, water is applied to crops using a variety of irrigation techniques to mitigate the yield-limiting effects of water deficits. However, dwindling water resources and increased costs can restrict the use of this practice considerably.

Most of the work on the effects of timing of water deficits on cotton has focused on yield under variable field growing conditions. A better understanding of the responses of growth and yield would be achieved by quantifying these effects under controlled environmental conditions, where soil variability can be eliminated and water supply accurately controlled.

Two studies were conducted in 2014 in the Drought Tolerance Laboratory (Texas AgriLife Research and Extension Center in Corpus Christi, TX) to i) quantify the effects of the timing of water deficits on growth and yield of cotton, and ii) quantify the effects of water deficit stress alleviation at different phenological stages on growth and yield of moderately water-stressed cotton. This facility consists of two joined modified greenhouses where computerized systems control the irrigation regime and collect and process plant water use data automatically.

Both studies used cultivar PHY375WRF, which is an early-medium maturity variety with an indeterminate growth habit. Plants were grown in 13.5-L (3.6-gallon)

pots filled with fritted clay soil. The experiments were laid out as complete randomized designs with 4 treatments and 4 replications.

Data collected shows that water deficits from 1st bloom to mid bloom and from mid bloom to 1st cracked boll had severe effects cotton's dry biomass production and partitioning, primarily through its decreasing effects on fruit retention, which led to lower economic yield and lower water use efficiency. Supplemental irrigation increased whole-plant transpiration irrespective of phenological timing, but increased total dry biomass of moderately water-stressed cotton only when applied from match head to 1st bloom and from 1st bloom to mid bloom. But these effects did not impact significantly yield or water use efficiency.

DEDICATION

To my father Luiz Henrique Carvalho, mother Vera Lucia Da Ros Carvalho, and brother Rafael Da Ros Carvalho. I can always rely on their love, support, and encouragement to follow my dreams.

To Dr. J. Tom Cothren (*in memoriam*). I hold him in the highest regard and deepest appreciation. It was an honor to have met him. He will always be a role model for me.

ACKNOWLEDGEMENTS

I would like to thank the following people who helped, directly or indirectly, to make this work possible:

Dr. Carlos J. Fernandez, committee chair, for giving me the opportunity to join Texas A&M University for my graduate studies. For that, I will be forever grateful. Additionally, I would like to thank him for his mentorship, support, guidance, and patience. I learned so much from you.

Dr. J. Tom Cothren (*in memoriam*), Dr. Gaylon D. Morgan, and Dr. Nithya Rajan, committee members, for their guidance and support throughout the course of this research.

Murilo and Andrea Maeda, for the friendship, help, and support. I don't have enough words to thank you; any of this would be possible without you.

Dr. Juan A. Landivar, for indicating me to Dr. Fernandez and encouraging me to pursue my Master's degree.

All AgriLife Center at Corpus Christi personnel. I feel truly blessed for being able to work with such great people; all of you made my time in Corpus a one of a kind experience. I would like to specially thank Mr. J. Carlos Correa and Mr. Pete Flores for their assistance and friendship. It was a pleasure to work with you every day. I would like to acknowledge the technical assistance of Ms. Alexandra Gamboa, Mr. Julio Correa, and Ms. Megan Dupnik.

Dr. Ederaldo José Chiavegato, Dr. Antonio Roberto Pereira, Dr. Marcos Silveira Bernardes, and Dr. Ricardo Ferraz de Oliveira for encouraging me to continue my studies in graduate school and for inspiring me to become a scientist.

Clayton Lewis, Dr. Katie Lewis, Landon Crotwell, Steven Garcia, Cristina Barrera, and Dustin Kelly, for the great times we shared.

Finally, thanks to my family. Your love and support were fundamental for helping me complete this degree.

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CHAPTER I

INTRODUCTION

Although water is the most abundant molecule on Earth's surface, its availability is the factor that most strongly restricts terrestrial plant production on a global scale (Lambers et al., 2008). In drought-prone crop production regions, soil water deficit is the most dominant yield-limiting environmental factor. Water deficits impose so many restrictions to agricultural production that over a period of 40 years, 40.8% of the insurance indemnities distributed for crop losses in the US are due to drought (Boyer, 1982).

Where underground or surface water is available and cost affordable, water is applied to crops using a variety of irrigation techniques to mitigate the yield-limiting effects of water deficits. However, irrigation costs and limited water supplies constrain this practice throughout crop production regions (Loka et al., 2010).

Most of the crop production areas in the world are under rain-fed conditions and, therefore, exposed to soil water deficits at some point during the growing season. Irrigated crops may also be exposed to water deficits as dwindling irrigation water resources and/or higher pumping costs prompt the use of deficit irrigation practices. Crop production could be significantly increased and become more stable with improved crop management practices, including irrigation, that take into account crop water status and its sensitivity to both water deficit stress and water deficit stress alleviation, as this would reduce the negative impacts of water deficits on yield and quality.

The main thrust of this research project is to be directed to cotton (*Gossypium hirsutum* L.) since this crop is one of the most commonly planted in Texas. Cotton is the leading cash row crop in the State of Texas with about 6 million acres planted annually producing about 4.5 million bales and generating a statewide economic impact of $5.2 billion (Anonymous, 2007a,b).

Water deficit stress

According to Larcher (2003), in biological systems stress is considered a significant deviation from the optimal condition of life. This author also argues that stress induces changes and responses at all functional levels of the organism, which are initially reversible, but may become permanent. Therefore, in plant systems, stresses can be measured in relation to plant survival, crop yield, biomass accumulation, or CO_2 uptake (Taiz and Zeiger, 2010).

Based on this definition, water deficit stress is the condition plants are under when the stress factor is lack of available water for uptake. A further specification can be integrated in this definition according to the scale of the study. On a microscopic level, water deficit can be defined as the water content on a vegetative cell or tissue that is below the maximum content when the cell or tissue is fully hydrated (Taiz and Zeiger, 2010). On the other hand, on a macroscopic level, water deficits can be defined as the difference between potential evapotranspiration and actual evapotranspiration, that is, the amount of water that is not being transferred to the atmosphere due to restrictions on soil water availability (Pereira et al., 2007).

For the purposes of the studies presented in the thesis, the stress definition is referenced to the macroscopic scale, with responses measured at the individual plant level.

The effect of water deficit stress on plants

The effect of drought on plants is complex, and they respond to it with many protective adaptations (Henckel, 1964). Under field conditions, these responses can be synergistically or antagonistically modified by the superimposition of other stresses, and the way that plants cope with drought normally involve a mixture of strategies (Chaves et al., 2002). In general, the exposure of plants to soil water deficits results in the sequential inhibition of expansive growth, transpiration, and photosynthesis (Bielorai and Hopmans, 1975). Under these conditions, plants conserve water by limiting leaf area growth and/or closing stomata (McCree and Fernández, 1989).

Jones (2014) divided the effects of water deficits on plant growth and development on 2 categories: short and long term responses. According to his classification, short-term responses are related to decrease in stomatal conductance and photosynthesis, and alterations on membrane permeability and ion transport. The author further divided long-term responses into 4 sub-categories: biochemical and physiological, growth, morphological, and reproductive. Examples of biochemical and physiological long-term responses in his classification are osmotic adaptation, increased wax production, desiccation tolerance, specific mRNA and protein synthesis, proline and betaine accumulation, and decreased photosynthetic enzyme activity. Long-term growth responses are general growth inhibition, decreased cell division and expansion,

inhibition of germination, increased root-to-shoot ratio, and alterations on root growth. Long-term morphological responses are related to increased production of trichomes, decreased stomatal index, and induction of dormancy and terminal buds. Lastly, long-term reproductive responses are increased flower abscission and decreased pollen viability and seed set.

In the studies presented in this thesis greater emphasis was placed on the responses of transpiration, growth and dry biomass production, and water use efficiency (WUE).

Transpiration consists on the water vapor loss from vegetative tissue to the atmosphere through the stomata. The vaporization of water occurs on the mesophyll cells, and then water vapor diffuses through intercellular spaces to the stomata, and from the stomata to the atmosphere following a concentration gradient (Kramer, 1969). Therefore, transpiration, like evaporation, depends on the energy intercepted by the leaves and on vapor pressure gradient (Allen et al., 1998). Transpiration is a fundamental process for plants. Reasons include: CO_2 that will be utilized on photosynthesis can diffuse inside the plants only when the stomata are open, it creates the physical force driving water uptake, and water vaporization provides a great degree of cooling to plants. Forty-four kilojoules of energy is required to convert one mole of liquid water into vapor (Campbell and Norman, 1998).

Water deficits exert a direct effect on stomatal aperture (Slatyer, 1967). When plants are exposed to drying soils, abscisic acid (ABA) is produced on the roots, and then travels through the xylem to the shoot (Blum, 2011), where it triggers changes on

ion fluxes in guard cells, thus promoting stomatal closing (Raghavendra et al., 2010). Therefore, under water deficit conditions, reductions on transpiration are also attributed to stomata closure.

Loomis and Connor (1992) explain that under water deficit conditions plants can adjust their leaf area or reduce the amount of transpiration per unit leaf area. According to them, in the first case, the alterations are achieved after days or weeks, due to reduced expansive growth or greater senescence, while in the later case, stomatal closure can reduce transpiration rates in a manner of minutes or hours. However, regardless the scenario, reduced transpiration is generally related to reduced growth. Passioura (1994) argues that the modulation of the leaf area might enable plants to open the stomata again, and because of that, may be more influential than adjustments in stomata conductance.

In terms of growth, water deficits impact directly cell enlargement. Water creates turgor pressure inside the cells, thus promoting expansive growth (Hsiao, 1973). Therefore, water is responsible for creating a physical force that drives growth. Consequently, plant growth decreases when under water deficit conditions, and so does dry matter production.

Water use efficiency (WUE) is generally referred to the ratio of the amount of carbon gained per water lost. It can be expressed in many ways. Most of the times, the way it is expressed reflects the scale of the measurements. Yoo et al. (2009) argue that WUE may be expressed into two main forms: instantaneous WUE and integrated WUE. Instantaneous WUE refers to simultaneous measurements of net CO_2 assimilation (μmol CO_2 m^{-2} s^{-1}) and transpiration (mmol H_2O m^{-2} s^{-1}) at the leaf level, while the integrated

WUE is related to the carbon dry matter gained per unit water lost (g kg^{-1}) measured over a long period of time. Loka et al. (2010) explain that WUE is an interesting parameter for drought studies because high WUE results in increased biomass per unit of water. In the studies presented in this thesis WUE will be calculated by combining transpiration and whole-plant dry biomass values, and will be partitioned into three components: WUE_{total}, $WUE_{economic}$, and WUE_{lint}.

The effect of water deficit stress on cotton

In order to be high yielding, the cotton plant must develop a vegetative framework big enough to allow the development and growth of fruit. Under water deficit conditions, the plants face restrictions on its vegetative and reproductive development, which, ultimately, leads to lower yields. According to Jordan (1986), water deficits induced by low available soil water and/or high evaporative demand reduce the total number of potential fruiting points as a result of a general reduction in shoot growth. Krieg and Sung (1986) show that in terms of source-sink relations, the effect of water deficit stress is towards reduced photosynthetic activity due to reductions on leaf area and photosynthetic rate, and not so much on translocation. These results show that the major effect of water deficit stress was on source activity rather than sink activity.

The WUE of cotton plants increased as plants were exposed to progressively increasing soil water deficits until these become very severe (Fernández et al., 1992). The effect of water stress on yield, however, depends on its timing, intensity, and duration (Jones and Rawson, 1979). Knowing the impact of soil water availability on initiation, retainment, and maturation of harvestable bolls is of most importance for

optimizing water management decisions in cotton crops (Hake and Grimes, 2010). Therefore, minimizing the negative impacts of water deficits on yield and lint quality becomes an essential goal in cotton production.

Due to its perennial nature (Cothren, 1994), it is possible to argue that cotton plants may show a different response in terms of growth, yield and yield components, depending on which of its growth stage the water deficit stress (WDS) or water deficit stress alleviation (WDSA) are imposed.

Quantification of the effects of WDS and WDSA in different phenological stages would be useful to further understand important aspects of cotton water relations and help improve the management of cotton grown in dryland and deficit irrigated conditions. Most of the work on the effects of timing of water deficits on cotton has focused on yield under variable field growing conditions. A better understanding of the responses of growth, yield, and fiber quality would be achieved by quantifying these effects under controlled environmental conditions where soil variability can be eliminated and water supply accurately controlled.

The objectives of my research were to:

1. Quantify the effects of the timing of water deficits on growth and yield of cotton;
2. Quantify the effects of water deficit stress alleviation at different phenological stages on growth and yield of moderately water-stressed cotton.

CHAPTER II

MATERIALS AND METHODS

General experimental procedures

Two studies were carried out in 2014 at the Drought Tolerance Laboratory in the Texas A&M AgriLife Research and Extension Center at Corpus Christi. They were focused on evaluating the effects of timing of either water deficit or supplemental irrigation on the water economy of individual cotton plants, including their growth and WUE.

The Drought Tolerance Laboratory at the Texas AgriLife Research and Extension Center in Corpus Christi consists of two joined modified greenhouses structures converted to rain shelters equipped with computerized systems for controlling irrigation regimes in sets of individual plants and continuously monitoring whole-plant water transpiration using a lysimetric method (Figure II1). The studies presented in this thesis were conducted in one of the four test benches available in the laboratory. Each of these benches is equipped with 32 electronic load-cells (S-type tension model RSC-100 25555, HBM Inc., Marlborough, MA) connected to a data-logger (model CR1000, Campbell Scientific Inc., Logan, UT) via two relay multiplexers (model AM16/32B, Campbell Scientific Inc., Logan, UT). The data-logger was programmed to collect pot weight data at 10-minute intervals using the software LoggerNet version 4 (Campbell Scientific Inc., Logan, UT). Individual potted test plants were continuously suspended from the load-cell during the duration of the studies (Figure II.1).

Figure II.1. View of the test benches and potted test plants used in the water economy studies. Image courtesy: C.J. Fernandez (2014).

Each bench is divided in four timer-controlled irrigation zones. Test plants were irrigated individually with a modified Hoagland's nutrient solution (Table II.1). Zone's watering regime are controlled by timers (model SST400I, RainBird Corporation, Azusa, CA). To prevent disrupting the diurnal data collected from the load-cells, all irrigations were scheduled to be applied at night, when transpiration values are negligible.

Table II.1. Composition of the modified Hoagland's solution used in the water economy studies. Source: Fernandez, C. J. (1989).

Tank	Component	Concentration (g/L)
1	$NH_4H_2PO_4$	46
2	KNO_3	121
3	$Ca(NO_3)_2$. 4H2O	189
4	$MgSO_4$. $7H_2O$	99
5	H_3BO_3	0.62
	$MnCl_2$. $4H_2O$	0.4
	$ZnSO_4$. $7H_2O$	0.046
	$CuSO_4$. $5H_2O$	0.02
	Na_2MoO_4 . $2H_2O$	0.02
	NaCl	1.17
6	Na_2-EDTA	6.7
	$FeSO_4$. $7H_2O$	5
7	KOH	4

Production of test plants and specifications of experimental treatments for the two cotton water economy studies

Cultivar Phytogen 375 (PHY 375), which is an early-medium maturity variety with an indeterminate growth habit, was planted on April 2nd. Seeds were germinated between moistened paper towel sheets for planting. When germinated seeds had radicles about 1.5 cm long, they were planted at four seeds per pot.

The pots used in the studies had a volume of 13.5-L (3.6-gallon). All pots were equally filled with 10.8 L of dry fritted clay. Fritted clay was chosen as the soil medium due to its large volumetric holding capacity, which is about 0.46 L L^{-1} (VanBavel et al. 1978). Two air-conditioning filter strips (4 cm wide and 30 cm long) were placed in the bottom of the pots to allow drainage while preventing soil losses.

In each pot, the soil surface was leveled and covered with finely perforated aluminum foil (60 uniformly distributed needle-size perforations). The aluminum foil was used with the double purpose of minimizing soil water loss due to evaporation and allowing a uniform distribution of irrigation water across the soil surface. Two diagonal cuts were made in the aluminum foil to expose a central soil area for planting the seeds. The soil in the pot was irrigated in excess before planting. A bamboo stick was inserted at the center of the pot for plant support when they grew about 0.3 cm high. The plants were thinned to one-per-pot when they had their third true leaf fully expanded, 18 days after emergence (DAE) on April 25th. At this time the pots were hung from the load-cells for measurement of their weights at 10-minute intervals.

The experimental treatments in both water economy studies were designed to evaluate the effects of one-time exposure to either water deficits or supplemental irrigation of well-watered plants or moderately water stressed plants, respectively, during critical phenological stages of development. Treatment specifications are shown in Tables II.2 and II.3.

Table II.2. Treatment specifications for the water economy study evaluating the effects of one-time exposure to water deficits at different phenological stages.

Treatment	Irrigation/stress schedule
1	Control (fully irrigated throughout the study) - 2.4 L/day
2	Stressed from match head (MH) to 1st bloom (1B) – 1 L/day
3	Stressed from 1st bloom (1B) to mid bloom (MB) – 1 L/day
4	Stressed from mid bloom (MB) to 1st cracked boll (CB) -1 L/day

Table II.3. Treatment specifications for the water economy study evaluating the effects of one-time supplemental irrigation at different phenological stages.

Treatment	Irrigation/stress schedule
1	Control (moderately stressed throughout the study) – 1.0 L/day
2	Fully irrigated from match head (MH) to 1st bloom (1B) – 2.4 L/day
3	Fully irrigated from 1st bloom (1B) to mid bloom (MB) – 2.4 L/day
4	Fully irrigated from MB to 1st cracked boll (CB) - 2.4L/day

The experimental treatments in both water economy studies were initiated on May 7th, when plants reached the MH phenological stage. All pots were irrigated with 0.8 L/day until the initiation of the treatments. Upon termination of irrigation treatment, plants were returned to their respective control's irrigation level.

Both studies were laid out as complete randomized designs with 4 replications. Each replication of each treatment had three plants individually potted. Of these three plants, one was hung permanently from an electronic load-cell for continuous measurement of pot weight, and these data were used to calculate hourly and daily whole-plant transpiration. The other 2 plants were spares, to be used as substitute in case of treatment disruptions, such as irrigation failure.

Leaf measurements (length of main-stem leaves' mid rib, and number of leaves in each sympodial branch) were obtained at each of the critical phenological stages (MH, 1B, MB, and CB) to estimate plant leaf area (PLA). PLA at CB was used to estimate plant leaf dry mass (PLM). The equations used for these calculations were obtained in a preliminary study conducted to develop a non-destructive PLA and PLM measurement method. This section is covered in detail in Appendix A.

Both studies were terminated at full maturity on August 14th, when plants were harvested for measuring final dry biomass partition among plant parts and yield components. No harvest aid agrochemicals, such as defoliants and boll openers were applied prior to harvest. Irrigation was stopped once the majority of the bolls were mature (open). Plants were mapped before harvest for collecting data on boll retention.

Calculation of whole-plant transpiration and transpiration per unit leaf area

Prior to initiating the cotton water economy studies, all load-cells were calibrated to secure the accuracy of weighing data. The data calibration procedure consisted of obtaining regression equations of known weights (in grams) on load-cell outputs (in mV). Load-cell calibration data were obtained at 10-second intervals with the bench data-logger. Load-cell outputs were logged for 3 minutes while loaded with each of 3 known weights (5.02, 9.89, and 13.62 kg), which were selected to cover the weight range to be measured during the studies. Only the second minute of data was used for calculating the calibration equations. Because of the linearity of the load-cell response, linear regression models were applied (Figure II.2). The 32 linear regression equations for the load-cells used in the studies are shown in Table II.4.

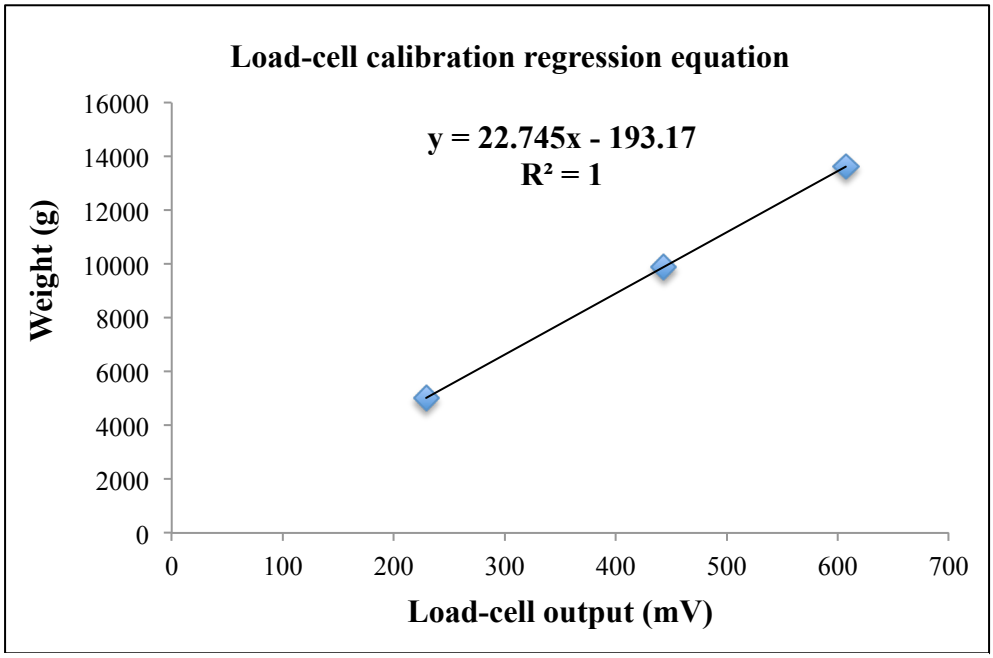

Figure II.2. Example of a calibration equation for a load-cell.

The data collection system is depicted by the diagram in Figure II.3. The load cells are connected to relay multiplexers which are connected to data-loggers. All the programming of the data-loggers is done using the software LoggerNet version 4.

Table II.4. Load-cells calibration information.

Load cell #	mx	b	R^2
	Slope	Intercept	
1	22.745	-193.17	1
2	22.806	171.76	1
3	22.812	17.332	1
4	22.675	189.22	0.99999
5	22.872	-178.77	1
6	22.84	-175.84	1
7	22.661	-286.45	0.99999
8	22.704	-53.073	1
9	22.758	-425.32	1
10	22.782	-223.38	1
11	22.764	-172.11	1
12	22.752	-305.8	1
13	22.791	-249.72	1
14	22.805	-243.91	0.99999
15	22.835	-546.36	1
16	22.752	-287.6	1
17	22.984	-707.37	1
18	22.858	-254.03	1
19	22.752	-243.84	1
20	22.736	-621.91	1
21	22.807	-420.13	1
22	22.637	-160.74	0.99999
23	22.659	-151.69	1
24	22.769	6250.7	1
25	22.798	-315.91	0.99999
26	22.804	-192.98	1
27	22.681	-180.86	1
28	22.637	-499.12	0.99999
29	22.809	-253.96	1
30	22.77	-498.33	1
31	23.119	-320.12	0.99999
32	22.891	-186.8	1

The data-loggers are connected to a dedicated desktop computer that retrieves and stores the data twice daily. The raw data are uploaded to a server for further processing and using a web-based program developed by C.J. Fernandez (unpublished). This program removes data spikes produced by draining excess irrigation (Figures II.4 and II.5), which otherwise would be introducing errors to the calculated hourly and daily whole-plant transpiration values.

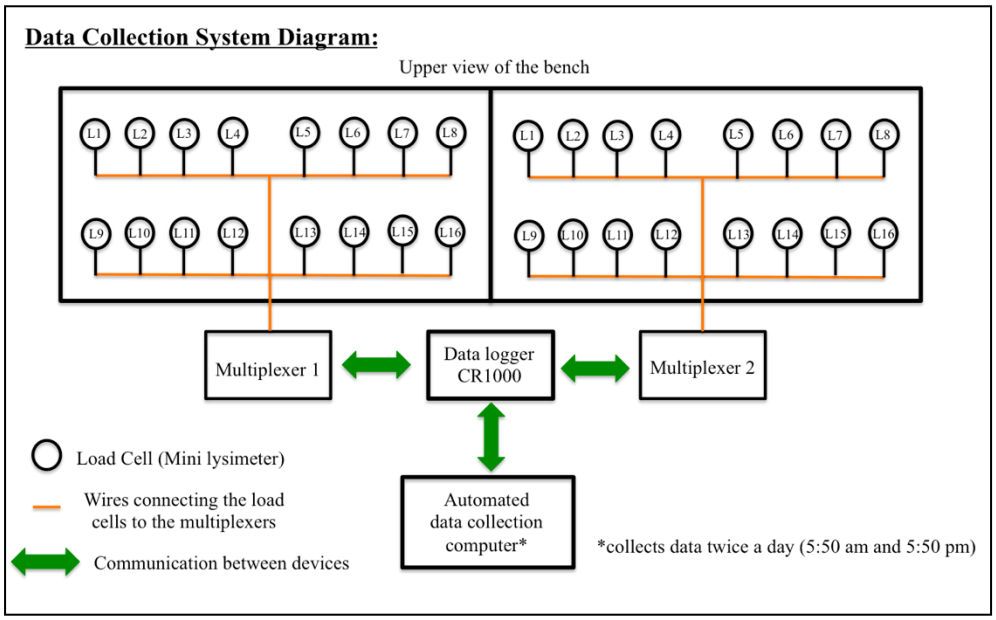

Figure II.3. Data collection system diagram. In this diagram just one bench is represented.

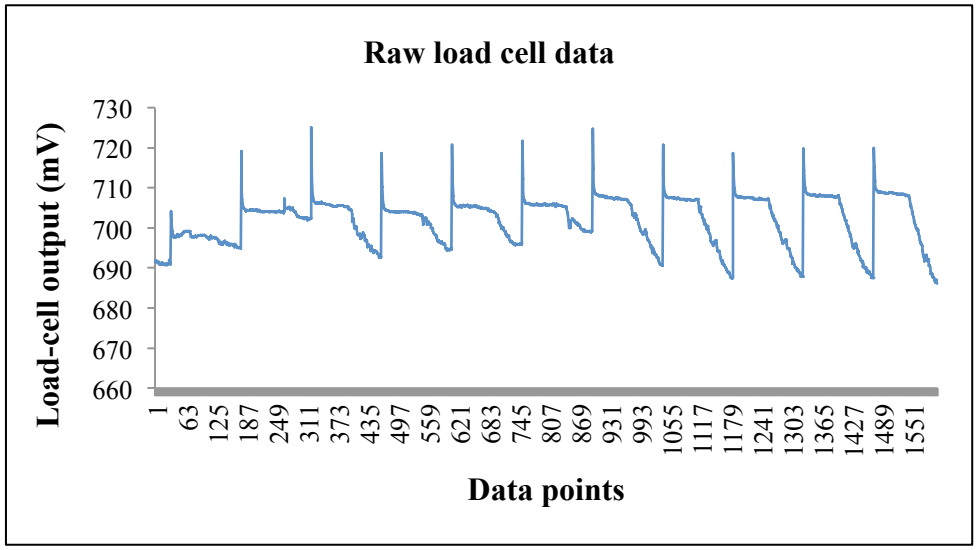

Figure II.4. Example of a raw piece of the load-cell output data. Spikes can be noted at every irrigation event.

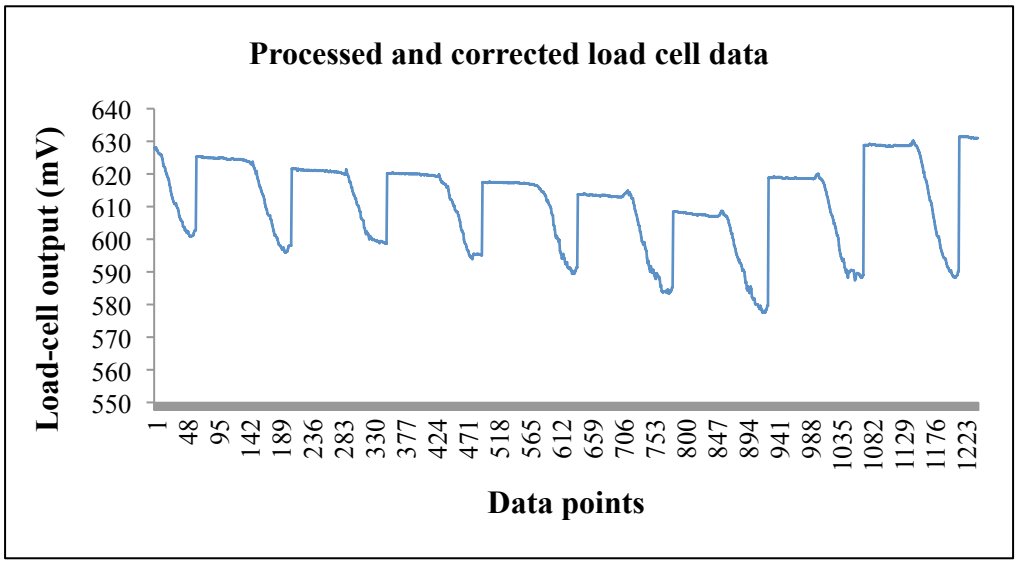

Figure II.5. Example of processed piece of the load-cell output data. Spikes were removed.

Daily whole-plant transpiration (DWPT) is calculated as the 24-hr sum of hourly whole-plant transpiration. The hourly whole-plant transpiration is calculated as the weight differences between consecutives hours by assuming that the change in weight between consecutive hours is almost all due to transpiration and minimally affected by change in plant biomass. Soil evaporation is also assumed negligible, since the top surface of pots is covered with reflective aluminum foil with only needle-made tiny holes. DWPT is then calculated with the following equation:

$$DWPT = \sum_{W_{ti}=1}^{n=24} (W_{ti+1} - W_{ti}) \div 1000 \quad (1)$$

where W_{ti} and W_{ti+1} are the weights (in grams) of the pot at consecutive hours, and the factor 1/1000 converts grams into liters (assuming 1 ml of water is equal to 1 g). Therefore, DWPT is expressed in L day^{-1}.

Daily transpiration per unit LA (L m^{-2}) was calculated by dividing daily whole-plant transpiration by plant leaf area estimated at MH (May 7th), 1B (May 30th), MB (June 20th), and CB (July 11th).

Biomass apportionment measurements and water use efficiency estimates

Plant parts collected at harvest were placed in bags and dried for 72 hours at 73.8 ^{o}C (165 ^{o}F) using a P0M7-806F drier (Blue M., Garland, TX) and their dry biomass measured with an AC-12k scale (Denver Instrument Company, Bohemia, NY). The

seed-cotton fraction (lint plus seeds) of each plant was ginned to obtain their separated dry biomass.

Whole-plant dry biomass (WPB) was partitioned into vegetative components (leaves, stem, branches, burs, and roots) and two economic components, namely seed and lint (WPSL) and lint only (WPL). These data, along with the cumulative whole-plant transpiration (CWPT) were then used to calculate a harvest index (HI, in %), and three indexes for estimating WUE, one based on WPB (WUE_{total}), another based on WPSL ($WUE_{economic}$), and a third based on WPL (WUE_{lint}). These four indexes were calculated as follows:

$$HI = \frac{WPSL}{WPB} \, x \, 100 \tag{2}$$

$$WUE_{total} = \frac{WPB}{CWPT} \tag{3}$$

$$WUE_{economic} = \frac{WPSL}{CWPT} \tag{4}$$

$$WUE_{lint} = \frac{WPL}{CWPT} \tag{5}$$

where WPB, WPSL, and WPL are in units of g $plant^{-1}$. CWPT is expressed as L $plant^{-1}$. Therefore, HI is expressed as percentage, while the three WUE indexes are in g L^{-1}.

Experimental data processing

The data was summarized using Excel 2010 (Microsoft Inc., Redmond, WA) and analyzed using SAS version 9.2 (SAS Institute Inc., Cary, NC). To check for significance, the factors were tested by the analysis of variance (ANOVA). In case of finding significance, the means of the treatments will be subjected to Fisher's Least Significant Difference (LSD) procedure at the 5% probability level. The ANOVA tables for each of the analyses with their respective coefficients of variation (CV) are shown in Appendix C.

CHAPTER III

RESULTS AND DISCUSSION

Effects of timing of water deficit on cotton water economy, growth, and yield

The progressions of DWPT showed distinct patterns for each of the water deficit treatments during the span of the study (Figure III.1). DWPT in Trt. 1 (Control) and Trt. 4 increased until June 4 as plant leaf area increased, then leveled as plants began the production of fruits and the production of new leaves slowed down. Daily variation of DWPT was caused by variation in environmental conditions affecting evaporative demand. All water deficit treatments (Trts. 2, 3, and 4) showed clear declines in DWPT upon initiation of watering restrictions. Trt. 2, however, had a delayed response, which can be explained by delayed soil water depletion due to smaller plant size and lower evaporative demands during this earlier growth stage. The fritted clay soil medium has a very large volumetric water holding capacity (0.46 L L^{-1}), which also contributed to the slower onset of water stress. The decline in DWPT in treatments 3 and 4 was more sudden upon the initiation of water restriction. After the water stress was imposed, plants on treatment 3 decreased transpiration rates from 1.8 L day^{-1} to less than 1 L day^{-1}, while plants on treatment 4 decreased from 2 L day^{-1} to less than 1 L day^{-1}. Another important response to water deficits was the gradual increase of DWPT upon cancelling the water restriction and returning to full irrigation. This gradual increase in DWPT is mostly attributed to gradual increase in plant leaf area. With respect to that, plants under Trt. 2 appeared to respond more readily than the ones under Trts. 3 and 4.

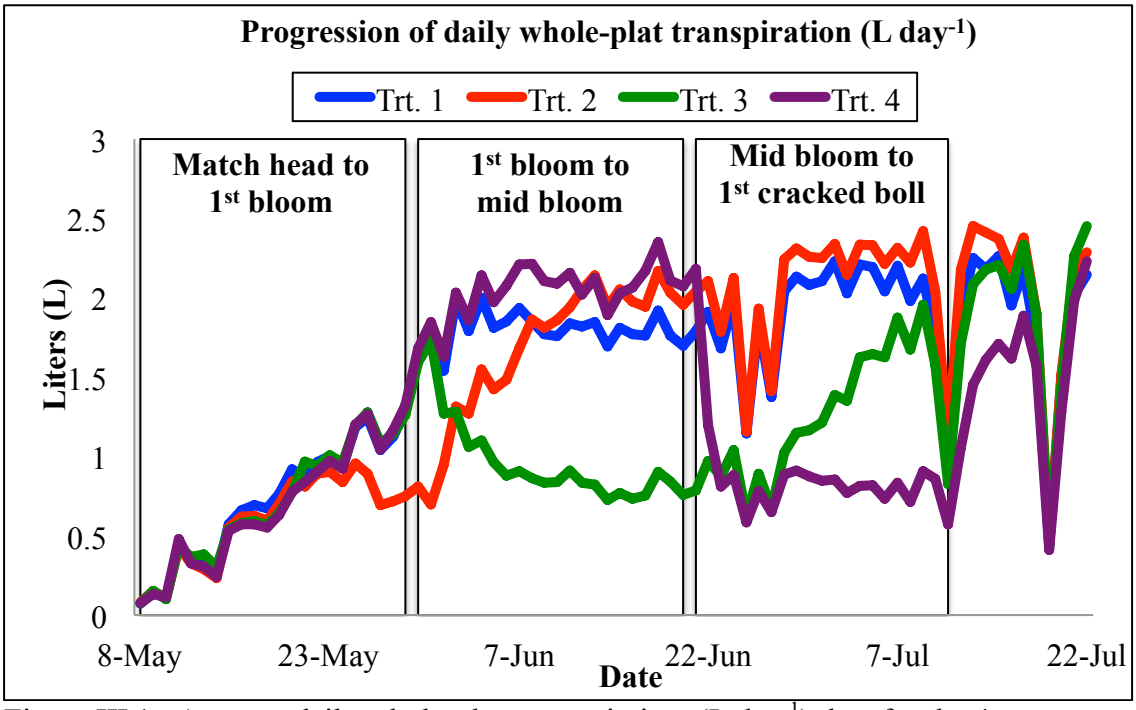

Figure III.1. Average daily whole-plant transpiraiton (L day^{-1}) data for the 4 treatments in the water deficit stress timing study during the season.

The distinct patterns of DWPT shown by the treatments were reflected on the cumulative whole-plant transpiration values (CWPT) at each treatment period (Table III.1). There were no significant differences in CWPT among treatments during the first stage from MH to 1B. The decline in DWPT in treatment 2 was delayed enough to prevent its CWPT to be significantly different from the control and the other treatments. This was not the case for Trts. 3 and 4, whose CWPT values were lower than the control. Because of faster recovery of DWPT upon resuming full irrigation, CWPT in Trt. 2 was not different from that of the control during the following growth stage from 1B to MB. Conversely, due to a slower recovery of DWPT, CWPT in Trt. 3 was lower than the

control in the subsequent growth stage from MB to 1CB. The total CWPT during the duration of the test was significantly higher in Trts. 1 and 2 by 38% and 22%, respectively.

Table III.1. Cumulative whole-plant transpiration (CWPT) per stage and across the season for the 4 treatments in the water deficit stress timing study.

Treatment	Cumulative whole-plant transpiration (L)			
	Match head to 1st bloom	1st bloom to mid bloom	Mid bloom to 1st cracked boll	Total
1 (Control)	15.6 a	39.6 b	39.9 a	115.6 a
2 (Stressed MH-1B)	13.0 a	36.9 b	43.1 a	115.3 a
3 (Stressed 1B-MB)	15.3 a	21.4 c	25.9 b	83.7 b
4 (Stressed MB-CB)	14.9 a	44.8 a	18.5 c	95.0 b

Means with different letters are significantly different at the 5% level.

Plant leaf area (PLA) was affected by the timing of the water deficits (Figure III.2 and Table III.2). PLA was not significantly different among treatments at MH stage, which was the test initiation time. The equality in PLA and the similar initial DWPT values (Figure III.1) are confirmation that all the test plants were equal at the beginning of the study. Water deficits decreased PLA in Trt. 2 and 3. There were no differences among treatments at CB stage, despite a sharp decline in PLA on plants in Trt. 4. Reductions in whole-plant leaf area due to water stress have been previously reported by Krieg and Sung (1986).

Table III.2. Plant leaf area (PLA) at 4 different stages for the 4 treatments in the water deficit stress timing study.

Treatment	Plant leaf area (m^2)			
	Match head	1st bloom	Mid bloom	1st cracked boll
1 (Control)	0.11 a	0.49 a	0.67 a	0.64 a
2 (Stressed MH-1B)	0.10 a	0.40 b	0.75 a	0.73 a
3 (Stressed 1B-MB)	0.10 a	0.55 a	0.51 b	0.67 a
4 (Stressed MB-CB)	0.09 a	0.50 a	0.76 a	0.53 a

Means with different letters are significantly different at the 5% level.

There were significant differences among treatments in terms of daily transpiration per leaf area at 1B, MB, and CB stages (Table III.3). As with initial DWPT and PLA, there were no differences among treatments in daily transpiration per unit leaf area at MH stage. Water deficits between MH and 1B (Trt. 2) decreased transpiration per unit leaf area at 1B stage. Similarly, water deficits between 1B and MB (Trt. 3) decreased transpiration per unit leaf area at MB stage. Also similarly, water deficits between MB and CB (Trt. 4) significantly decreased transpiration per unit leaf area at CB. Trt. 3 was also lower than the control at CB, which might indicate that stomata aperture was not fully recovered upon termination of water restriction during MB stage. These results agree with the findings of Loomis and Connor (1992) that under water deficit conditions plants adjust their leaf area or reduce the amount of transpiration per unit leaf area.

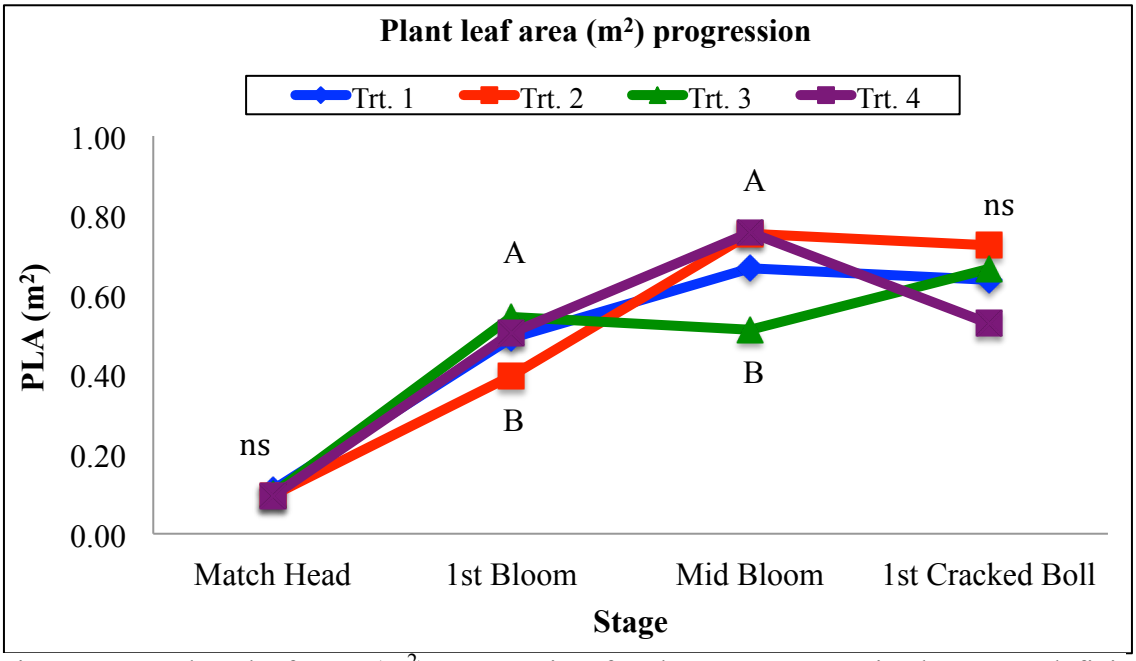

Figure III.2. Plant leaf area (m^2) progression for the 4 treatments in the water deficit stress timing study. Means with different letters are significantly different at the 5% level, ns = non-significant.

Table III.3. Daily transpiration per unit leaf area at 4 different stages for the 4 treatments in the water deficit stress timing study.

Treatment	Daily transpiration per leaf area (L m^{-2})			
	Match head	1st bloom	Mid bloom	1st cracked boll
1 (Control)	0.6 a	3.4 a	2.6 a	1.7 a
2 (Stressed MH-1B)	0.8 a	2.0 b	2.6 a	1.5 a
3 (Stressed 1B-MB)	0.7 a	2.9 a	1.5 b	1.2 b
4 (Stressed MB-CB)	0.8 a	3.3 a	2.8 a	1.1 b

Means with different letters are significantly different at the 5% level.

There were significant differences in terms of total dry biomass yield (Figure III.3) as well as in terms of its main components: vegetative and reproductive yield (Figures III.4 and III.5). Total dry biomass yield in Trts. 1 and 2 was higher than that of Trts. 3 and 4, and that of Trt. 3 was smaller than Trt. 4. Vegetative dry biomass yield in Trt.3 was about 40 g lower than that of the other treatments (Figure III.4), while reproductive yield in Trts. 1 and 2 was higher than that of treatments 3 and 4 in terms of both seedcotton per plant and lint per plant (Figure III.5). Studying plant influences in evaporative flux, Ritchie and Burnett (1971), found that cotton crops under rain-fed conditions greatly decreased growth and above ground dry biomass production due to prolonged periods without rain.

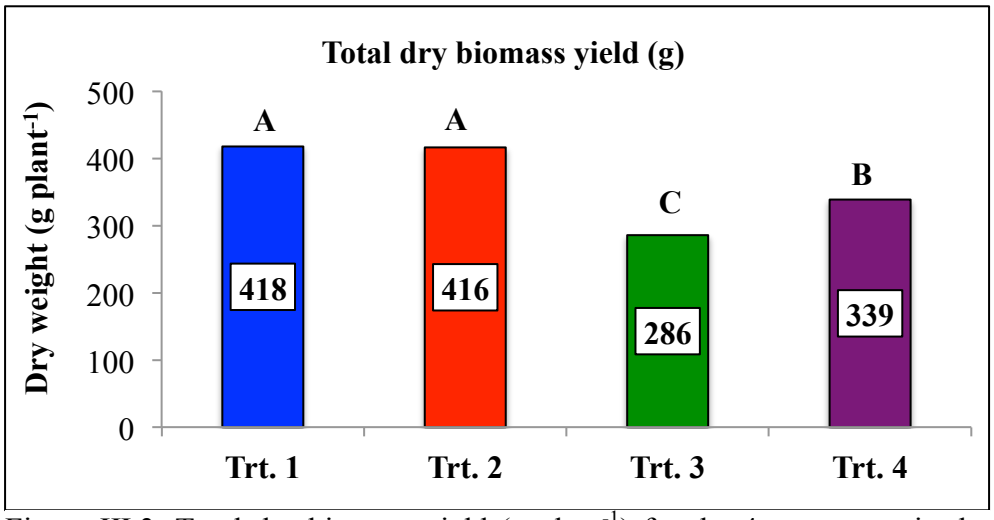

Figure III.3. Total dry biomass yield (g plant^{-1}) for the 4 treatments in the water deficit stress timing study. Means with different letters are significantly different at the 5% level.

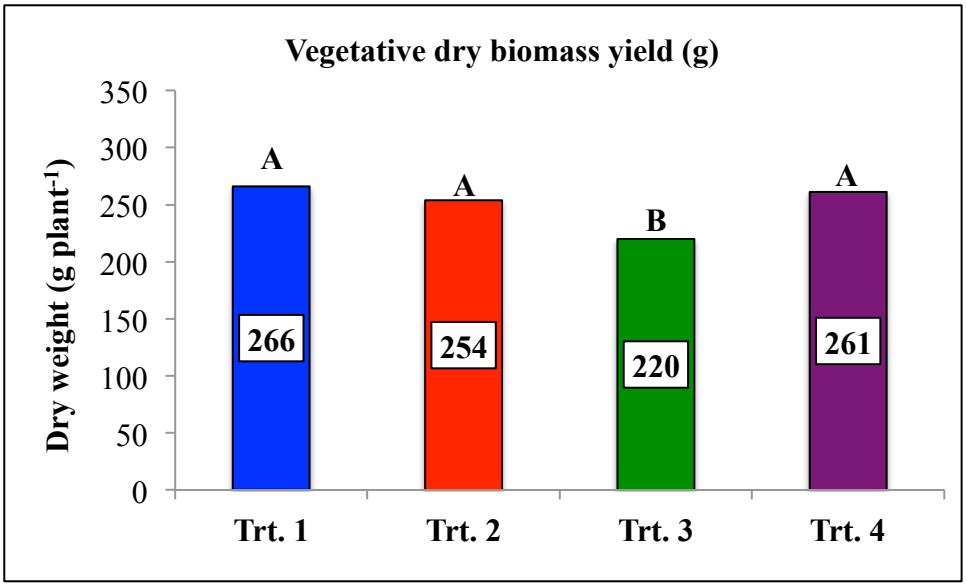

Figure III.4. Vegetative dry biomass yield (g plant^{-1}) for the 4 treatments in the water deficit stress timing study. Means with different letters are significantly different at the 5% level.

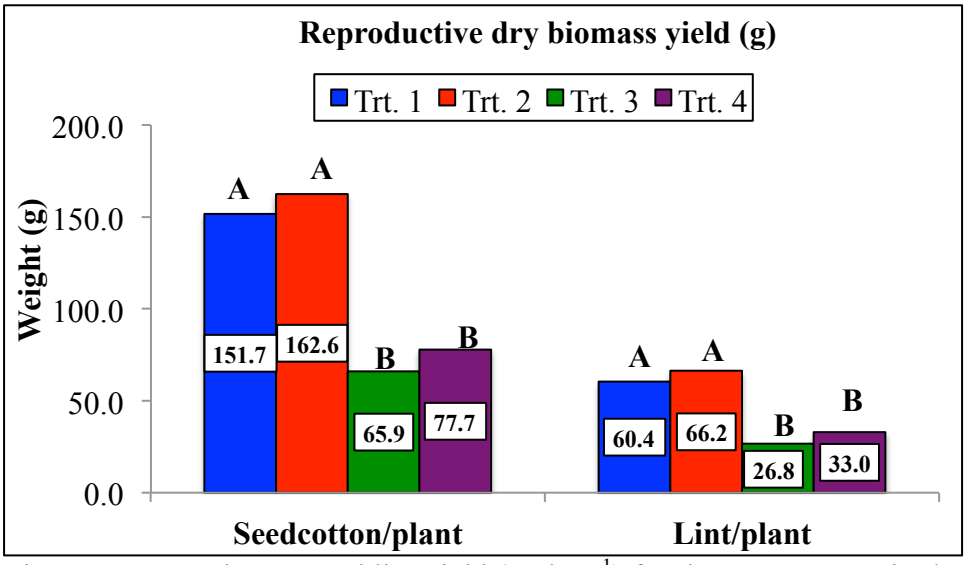

Figure III.5. Seedcotton and lint yield (g plant^{-1}) for the 4 treatments in the water deficit stress timing study. Means with different letters are significantly different at the 5% level.

Analyses of vegetative biomass partitioning data that was collected at harvest also showed significant differences among treatments (Table III.4). Main-stem dry biomass in Trt. 4 was higher than that of Trts. 2 and 3, but not different from the control. Branch dry biomass in Trt. 4 was higher than that of Trt. 2. Bur dry biomass was higher in Trts. 1 and 2 than in Trts. 3 and 4. Root dry biomass in Trt. 4 was higher than that of all other treatments. No significant differences were observed in leaf dry biomass between treatments. Pace et al. (2009) reported that cotton plants at the end of 13 days of water restrictions had lower dry weights of stems and leaves when compared to the control treatment.

No significant differences among treatments were observed regarding the total number of fruiting positions in plants (Table III.5). However, water deficit treatments imposed after 1B stage significantly decreased boll retention (Table III.5 and Figure III.6). Water deficits imposed from MH to 1B (Trt. 2) did not affect boll retention. This significant decrease in boll retention caused by water deficits imposed during fruiting after first bloom stage (1B) explained the lower reproductive yield in Trts. 3 and 4. As expected, this major effect of water deficits was also reflected on the significant differences in HI (Figure III.7). Rijks (1965) found that plants grown with low water supply (140 mm) had fewer fruiting forms, but managed to retain the majority of the bolls at the first position. Snowden et al. (2014) studied the effects of the timing of episodic drought and found that events during early flowering and peak bloom caused significant reductions in yields, fruit retention, and fiber quality.

Table III.4. Vegetative dry biomass yield components (g plant^{-1}) for the 4 treatments in the water deficit stress timing study.

Treatment	Dry biomass (g plant^{-1})				
	Main-stem	Branches	Burs	Roots	Leaves
1 (Control)	44.4 ab	58.1 ab	74.4 a	51.5 b	37.8 a
2 (Stressed MH-1B)	38.2 b	48.1 b	72.9 a	52.2 b	42.5 a
3 (Stressed 1B-MB)	41.5 b	58.1 ab	35.3 b	45.5 b	39.6 a
4 (Stressed MB-CB)	50.4 a	70.5 a	42.1 b	65.6 a	32.5 a

Means with different letters are significantly different at the 5% level.

Table III.5. Plant mapping analysis for the 4 treatments in the water deficit stress timing study.

Treatment	Number of reproductive structures			Retention
	Total	Number of bolls	Number of aborted structures	
1 (Control)	67 a	47 a	21 c	0.69 a
2 (Stressed MH-1B)	73 a	47 a	26 c	0.64 a
3 (Stressed 1B-MB)	79 a	21 b	58 a	0.27 b
4 (Stressed MB-CB)	67 a	25 b	42 b	0.37 b

Means with different letters are significantly different at the 5% level.

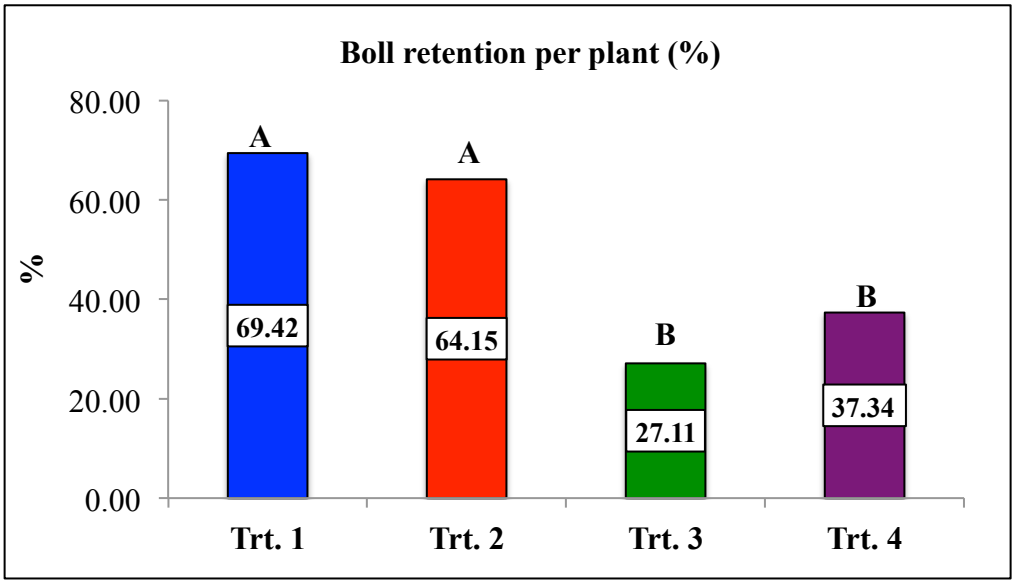

Figure III.6. Boll retention (%) for the 4 treatments in the water deficit stress timing study. Means with different letters are significantly different at the 5% level.

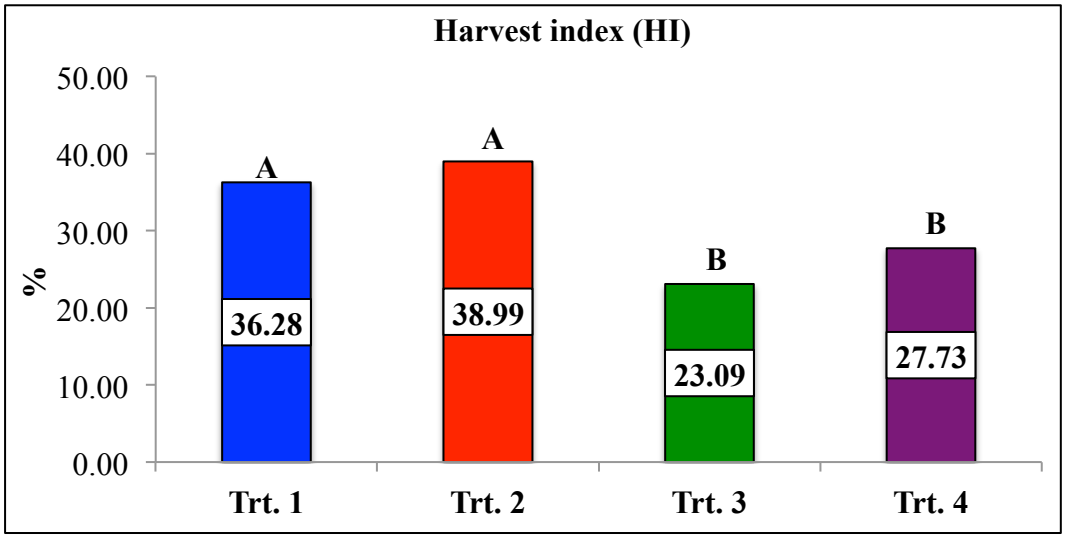

Figure III.7. Harvest index (%) for the 4 treatments in the water deficit stress timing study. Means with different letters are significantly different at the 5% level.

Since water deficit treatments affected both whole-plant transpiration and total biomass production in the same direction, no significant differences in WUE_{total} were observed among treatments (Figure III.8). Since water deficits imposed after 1B stage decreased boll retention and, therefore, reproductive yield, $WUE_{economic}$ and WUE_{lint}, however, were significantly lower in treatments 3 and 4 than in the control and treatment 2. Jordan (1986) reported that water use efficiency values for rained crops range between 0 and 0.45 kg of lint per m^3 of water, but according to Hearn (1979) most values fall in the range of 0.1 to 0.3 kg m^{-3}

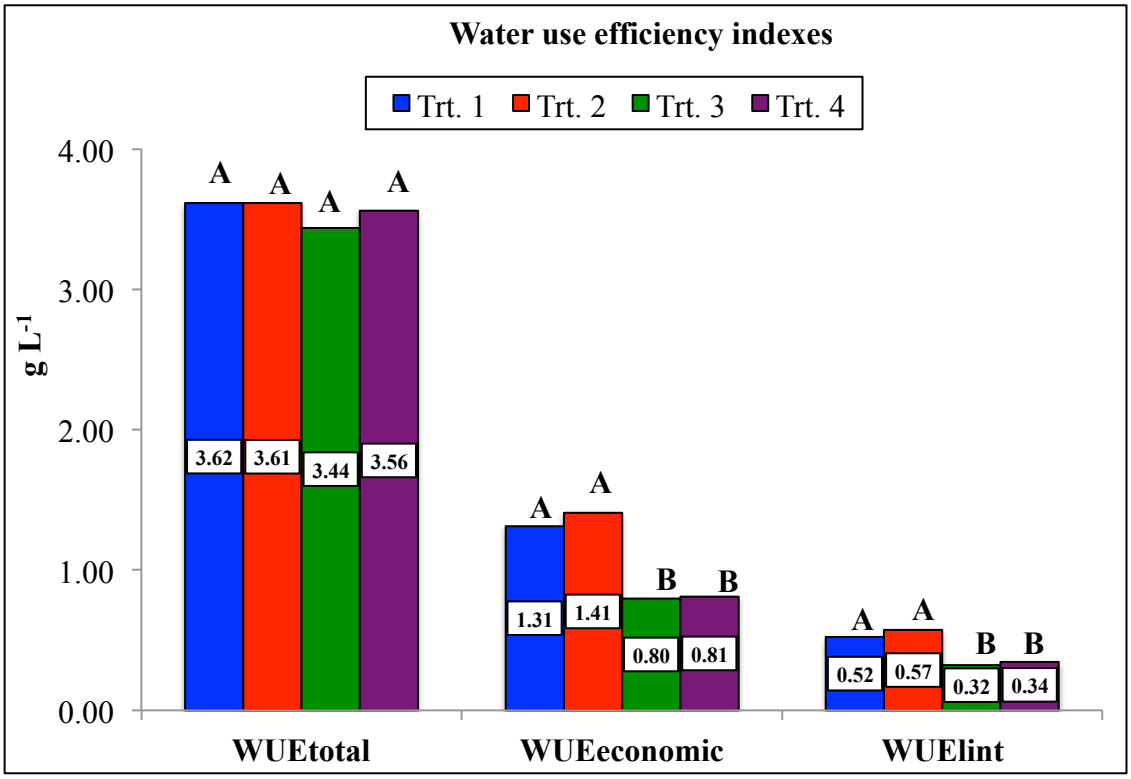

Figure III.8. Water use efficiency (g L^{-1}) indexes for the 4 treatments in the water deficit stress timing study. Means with different letters are significantly different at the 5% level.

Total biomass and its major components (vegetative and reproductive) declined concomitantly as cumulative whole-plant transpiration decreased (Figure III.9). All regression equations of biomass components on cumulative whole-plant transpiration during the test period showed high R^2 values. The highest concomitant decline, as shown by the slope values, corresponded to total biomass and this was caused by a high decline in seedcotton production. This reduction in seedcotton was likely related to the decline in seed biomass, as the difference in slope between seedcotton and lint indicates. Unlike

all other linear responses, the relationship between vegetative biomass production and cumulative whole-plant transpiration was best represented by a 2nd degree polynomial, which indicates that vegetative biomass decline did not accompany the decline in whole-plant transpiration until this decline was about 20% of the non-stressed maximum level of 120 L, suggesting the high sensitivity of fruiting structures loss due water deficits.

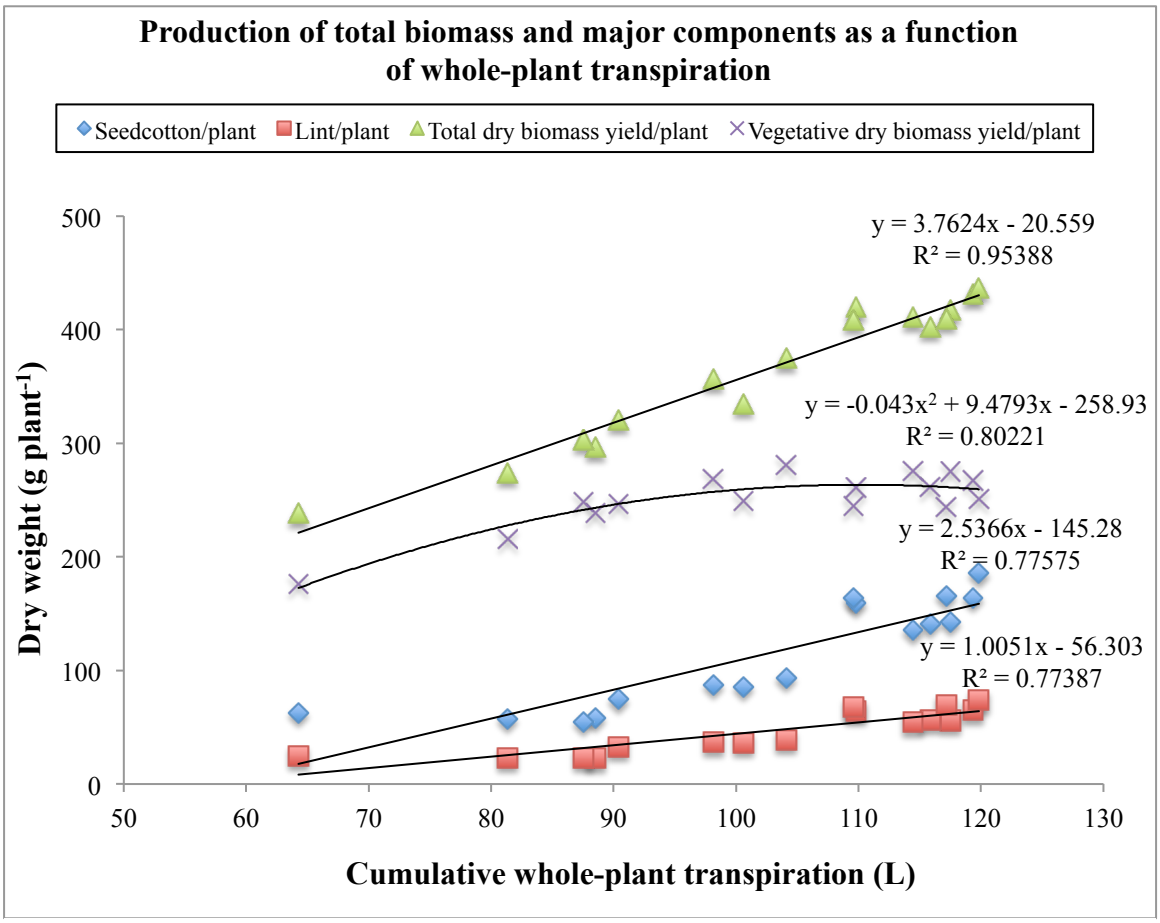

Figure III.9. Seedcotton, lint, total dry biomass yield, and vegetative dry biomass yield per plant as a function of cumulative transpiration during the duration of the water deficit stress timing study.

Effects of timing of supplemental irrigation on cotton water economy, growth, and yield

The progressions of daily whole-plant transpiration (DWPT) show distinct patterns for each of the irrigation supplemental treatments during the span of the study (Figure III.10). DWPT in all treatments continued to increase until May 22nd as plants continued to grow relatively unstressed due to small plant size and low atmospheric demand. After that date, Trts. 1, 3, and 4 began to show lower DWPT than Trt. 2, which was under the supplemental irrigation regime. Once Trt. 2 was over, its DWPT gradually decreased to near that of Trts. 1 and 4. From 1B to MB, Trt.3 showed increased DWPT as a result of supplemental irrigation, but once the plants returned to stress conditions, DWPT sharply declined. DWPT in Trt. 4 increased as water stress was alleviated from MB to CB. Once the treatment was finished, DWPT declined sharply as well. Daily variation of DWPT in all treatments was caused by variation in environmental conditions affecting evaporative demand.

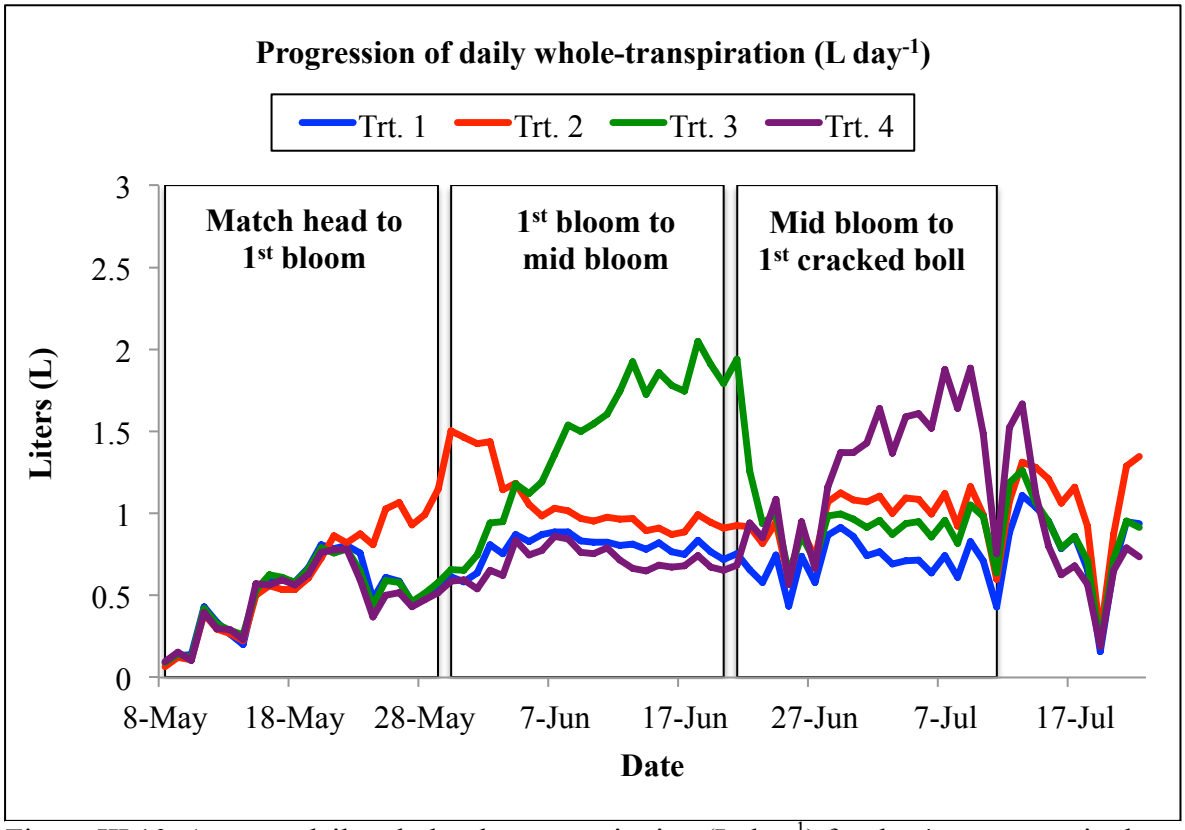

Figure III.10. Average daily whole-plant transpiration (L day^{-1}) for the 4 treatments in the timing of water deficit stress alleviation study during the season.

Supplemental irrigation treatments significantly increased CWPT at each phonological stage, and this effect was reflected on the total CWPT at the end of the test (Table III.6). CWPT in the control treatment was lower than that of treatments 2, 3, and 4, as expected.

Table III.6. Cumulative whole-plant transpiration (CWPT) per stage and across the season for the 4 treatments in the timing of water deficit stress alleviation study.

Treatment	Cumulative whole-plant transpiration (L)			
	Match head to 1st bloom	1st bloom to mid bloom	Mid bloom to 1st cracked boll	Total
1 (Control)	10.9 b	17.3 c	14.7 c	51.9 c
2 (Irrigated MH-1B)	13.5 a	23.5 b	20.3 b	69.0 ab
3 (Irrigated 1B-MB)	10.8 b	31.5 a	20.2 b	72.1 a
4 (Irrigated MB-CB)	10.2 b	15.5 c	26.4 a	61.5 b

Means with different letters are significantly different at the 5% level.

Plant leaf area (PLA) was similar in all treatments at the start of the test (Figure III.11 and Table III.7). Supplemental irrigation treatments significantly increased plant leaf area in treatments 2 and 3, but not in 4.

Table III.7. Plant leaf area (PLA) at 4 different stages for the 4 treatments in the timing of water deficit stress alleviation study.

Treatment	Plant leaf area (m^2)			
	Match head	1st bloom	Mid bloom	1st cracked boll
1 (Control)	0.09 a	0.34 b	0.37 c	0.38 a
2 (Irrigated MH-1B)	0.09 a	0.46 a	0.49 b	0.48 a
3 (Irrigated 1B-MB)	0.09 a	0.32 b	0.67 a	0.53 a
4 (Irrigated MB-CB)	0.09 a	0.29 b	0.33 c	0.49 a

Means with different letters are significantly different at the 5% level.

There were no significant differences in daily transpiration per unit leaf area at the start of the test (Table III.8). Trts. 2 and 3 affected daily transpiration per unit leaf area, while no differences were observed for Trt. 4. However, for Trt. 4, the day in which daily transpiration per unit leaf was calculated transpiration rates were depressed due to cloudy weather. That might explain the lack of significance for Trt. 4.

Table III.8. Daily transpiration per unit leaf area at 4 different stages for the 4 treatments in the timing of water deficit stress alleviation study.

Treatment	Daily transpiration per leaf area (L m^{-2})			
	Match head	1st bloom	Mid bloom	1st cracked boll
1 (Control)	1.0 a	1.8 b	1.9 b	1.2 a
2 (Irrigated MH-1B)	0.7 a	3.3 a	1.9 b	1.3 a
3 (Irrigated 1B-MB)	1.0 a	2.0 b	2.7 a	1.2 a
4 (Irrigated MB-CB)	1.1 a	1.6 b	1.5 b	1.5 a

Means with different letters are significantly different at the 5% level.

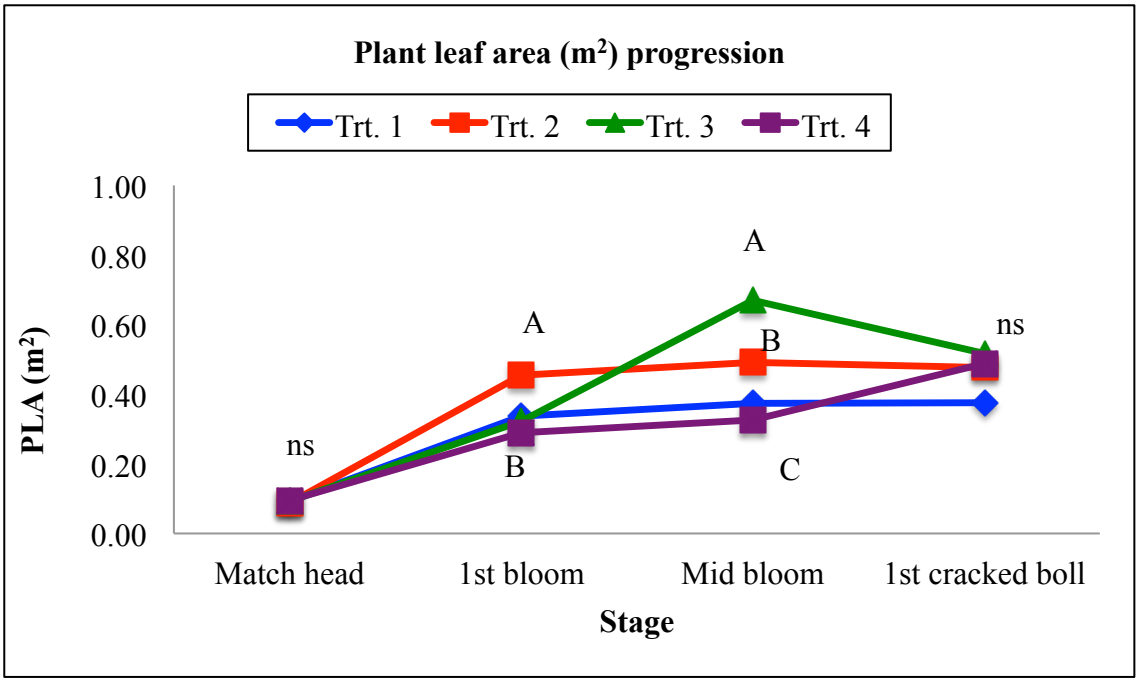

Figure III.11. Plant leaf area (m^2) progression for the 4 treatments in the timing of water deficit stress alleviation study. Means with different letters are significantly different at the 5% level, ns = non-significant.

There were significant differences in terms of total dry biomass yield (Figure III.12) and its vegetative yield component (Figure III.13), but no differences were observed in terms of reproductive yield between treatments (Figure III.14). Total dry biomass yield in Trts. 2 and 3 was higher than that of Trts. 1 and 4. Vegetative dry biomass yield was also higher in treatments 2 and 3 than in treatments 1 and 4 (Figure III.13).

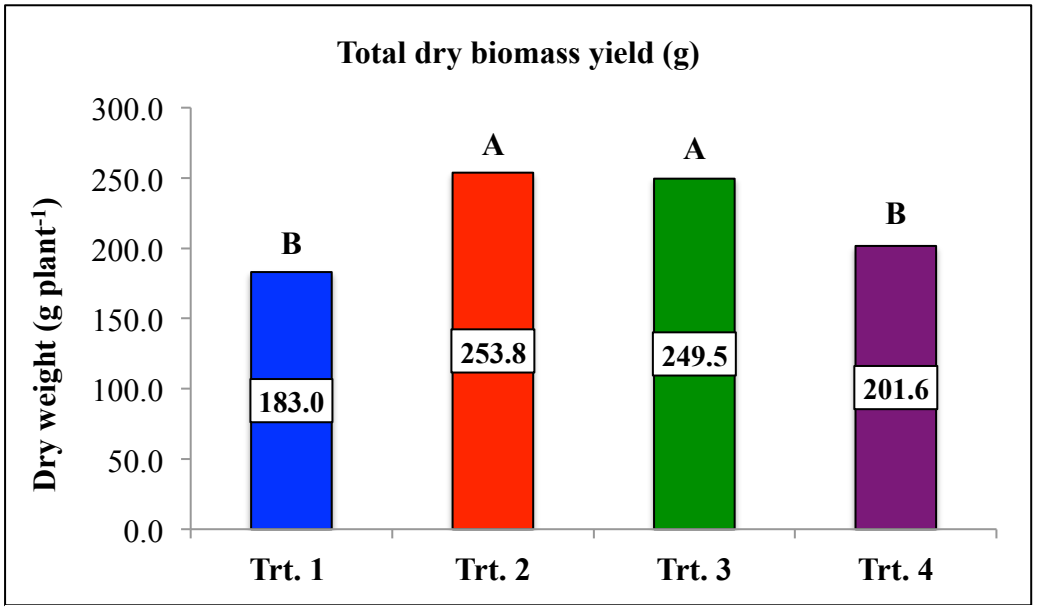

Figure III.12. Total dry biomass yield (g plant-1) for the 4 treatments in the timing of water deficit stress alleviation study. Means with different letters are significantly different at the 5% level.

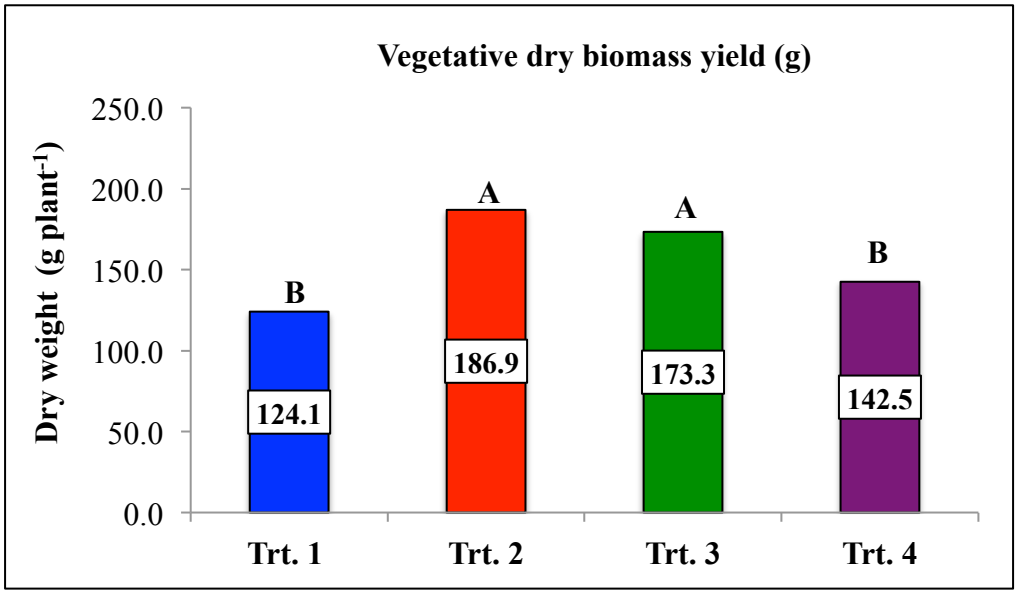

Figure III.13. Vegetative dry biomass yield (g plant^{-1}) for the 4 treatments in the timing of water deficit stress alleviation study. Means with different letters are significantly different at the 5% level.

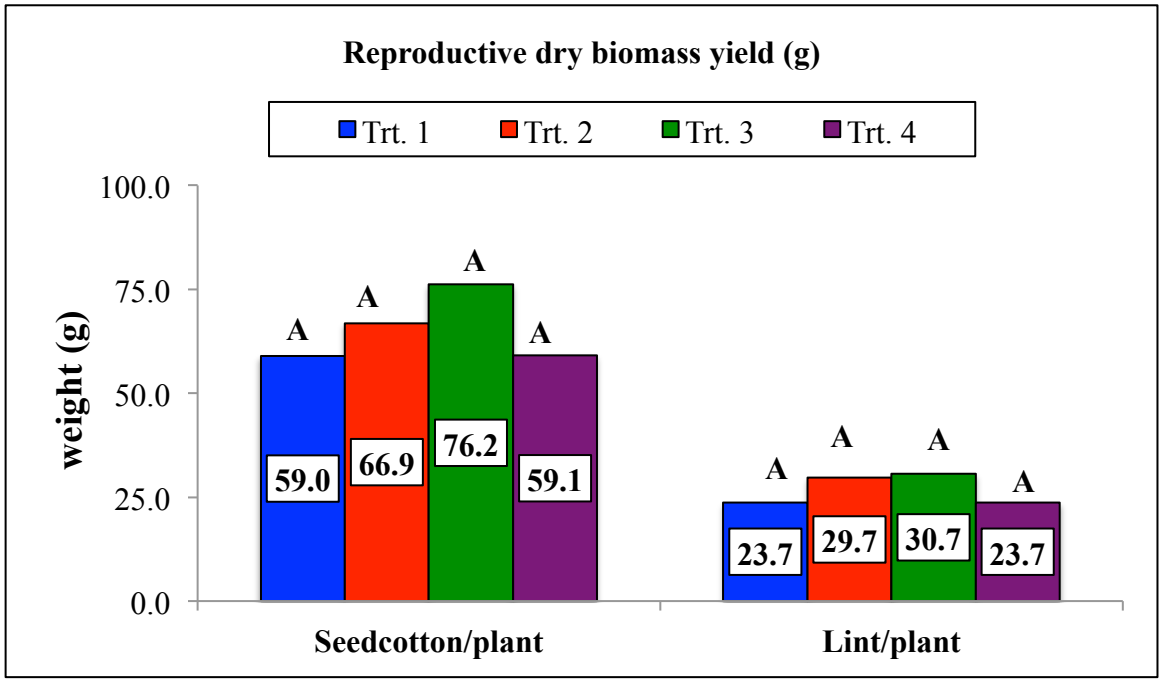

Figure III.14. Seedcotton and lint yield (g plant^{-1}) for the 4 treatments in the timing of water deficit stress alleviation study. Means with the same letter are not significantly different at the 5% level.

Analyses of vegetative biomass partitioning data that was collected at harvest also showed significant differences among treatments (Table III.9). Main-stem and branch dry biomass were higher in treatments 2 and 4 than in treatments 1 and 4. No differnces between bur dry biomass were observed among treatments. Root dry biomass was higher in treatment 2 than in treatments 1 and 4, but do not different from treatment 3. Leaf biomass was significantly higher in supplemental irrigation treatments 3 and 4, whereas treatment was not different from the control.

Table III.9. Vegetative dry biomass yield components (g plant^{-1}) for the 4 treatments in the timing of water deficit stress alleviation study.

Treatment	Dry biomass (g plant^{-1})				
	Main-stem	**Branches**	**Burs**	**Roots**	**Leaves**
1 (Control)	22.6 b	18.2 b	25.8 a	32.5 bc	25.0 b
2 (Irrigated MH-1B)	36.8 a	38.7 a	37.7 a	43.8 a	29.9 ab
3 (Irrigated 1B-MB)	33.4 a	33.9 a	36.0 a	37.7 ab	32.4 a
4 (Irrigated MB-CB)	24.0 b	22.3 b	33.3 a	28.2 c	31.8 a

Means with different letters are significantly different at the 5% level.

All supplemental irrigation treatments increased the total number of fruiting positions per plant, but there were no significant differences among treatments in the number of harvestable bolls per plant or boll retention (Table III.10). Therefore, supplemental irrigation treatments did not affect HI (Figure III.15). In studies on an alluvial clay soil in the coastal plain of Israel, Marani and Fuchs (1964) reported that a single irrigation (150 mm) at the beginning of flowering resulted in high yields, but amounts of water larger than that did not increased yield. Jordan (1983) argues that under field conditions, supplemental irrigations should provide water through all the extent of the rooting depth, which may greatly vary with soil type.

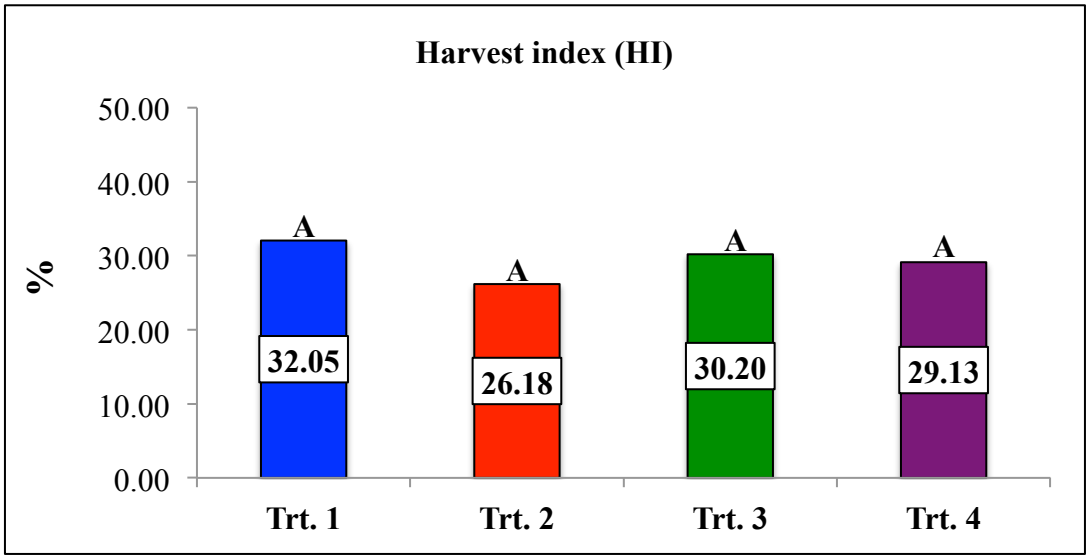

Figure III.15. Harvest index (%) for the 4 treatments in the timing of water deficit stress alleviation study. Means with the same letter are not significantly different at the 5% level.

Table III.10. Plant mapping analysis for the 4 treatments in the timing of water deficit stress alleviation study.

Treatment	Number of reproductive structures			Retention
	Total	Number of bolls	Number of aborted structures	
1 (Control)	34 b	16 a	18 a	0.47 a
2 (Irrigated MH-1B)	54 a	24 a	30 a	0.44 a
3 (Irrigated 1B-MB)	51 a	24 a	27 a	0.47 a
4 (Irrigated MB-CB)	45 a	21 a	25 a	0.48 a

Means with different letters are significantly different at the 5% level

The only significant effect of supplemental irrigation on WUE was a decrease of WUE_{total} in treatment 4, as a result of increased CWPT over that of the control, which

was not paralleled by an increase in biomass yield (Figure III.16). There were no differences in terms of $WUE_{economic}$ or WUE_{lint}.

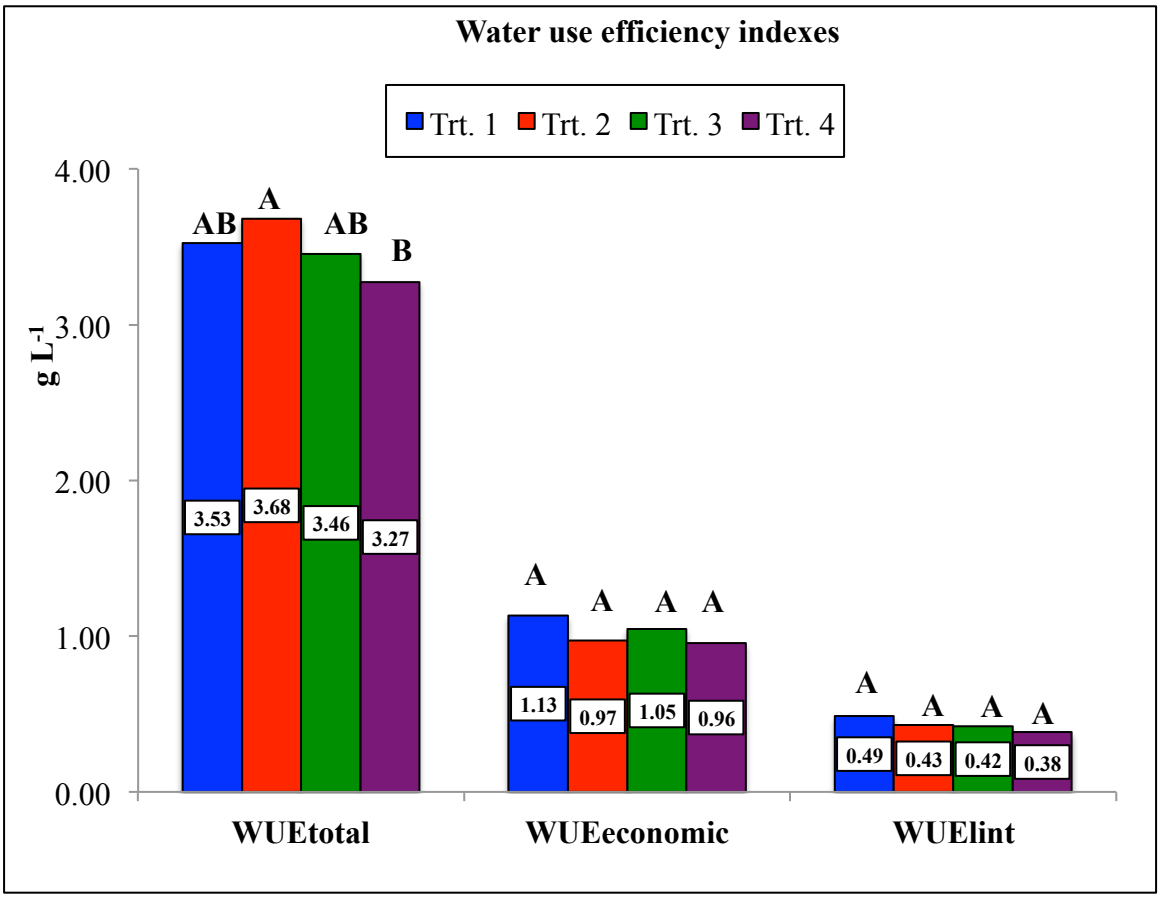

Figure III.16. Water use efficiency (g L^{-1}) indexes for the 4 treatments in the timing of water deficit stress alleviation study. Means with different letters are significantly different at the 5% level.

As seen on Figure III.10, treatment 2 plants increased transpiration rates from MH to 1B. That was also shown on the cumulative values. This increase in transpiration impacted significantly the vegetative framework of the plants. Compared to the control, the plants were able to maintain a greater PLA until MB. This greater PLA is also reflected in terms of total and vegetative dry biomass yield, which were greater than the control. Jordan (1986) argues that the production of potential fruiting points must be closely allied with vegetative growth. In that sense, the supplemental treatments were effective. Particularly, main-stem, branches, and roots were most important for increasing the dry biomass produced by the plants, since there were no differences in terms of seed cotton and lint per plant. Since the parameters HI, boll retention and WUE were not different than the control; it is possible to argue that the supplemental irrigation from MH to 1B provided water to support plant growth, but not enough to support significant differences in reproductive yield per plant, since boll retention decreased after plants went back to stress.

A similar analysis can be made for treatment 3. The increase in transpiration due to the supplemental irrigation from 1B to MB increased cumulative transpiration values and promoted vegetative growth, but failed to significantly impact yield. Together with main-stem and branches, leaf dry biomass was important for increasing the amount of total vegetative dry biomass produced. Based on the data it is possible to argue that the same comments made for treatment 2 are valid for treatment 3.

Total biomass and its major components (vegetative and reproductive) increased concomitantly as cumulative whole-plant transpiration increased, as shown by the linear

equation in Figure III.17. The regression equations of total biomass and vegetative biomass on cumulative whole-plant transpiration during the test period showed high R^2 values. Lower R^2 values were produced by the equations involving reproductive biomass components.

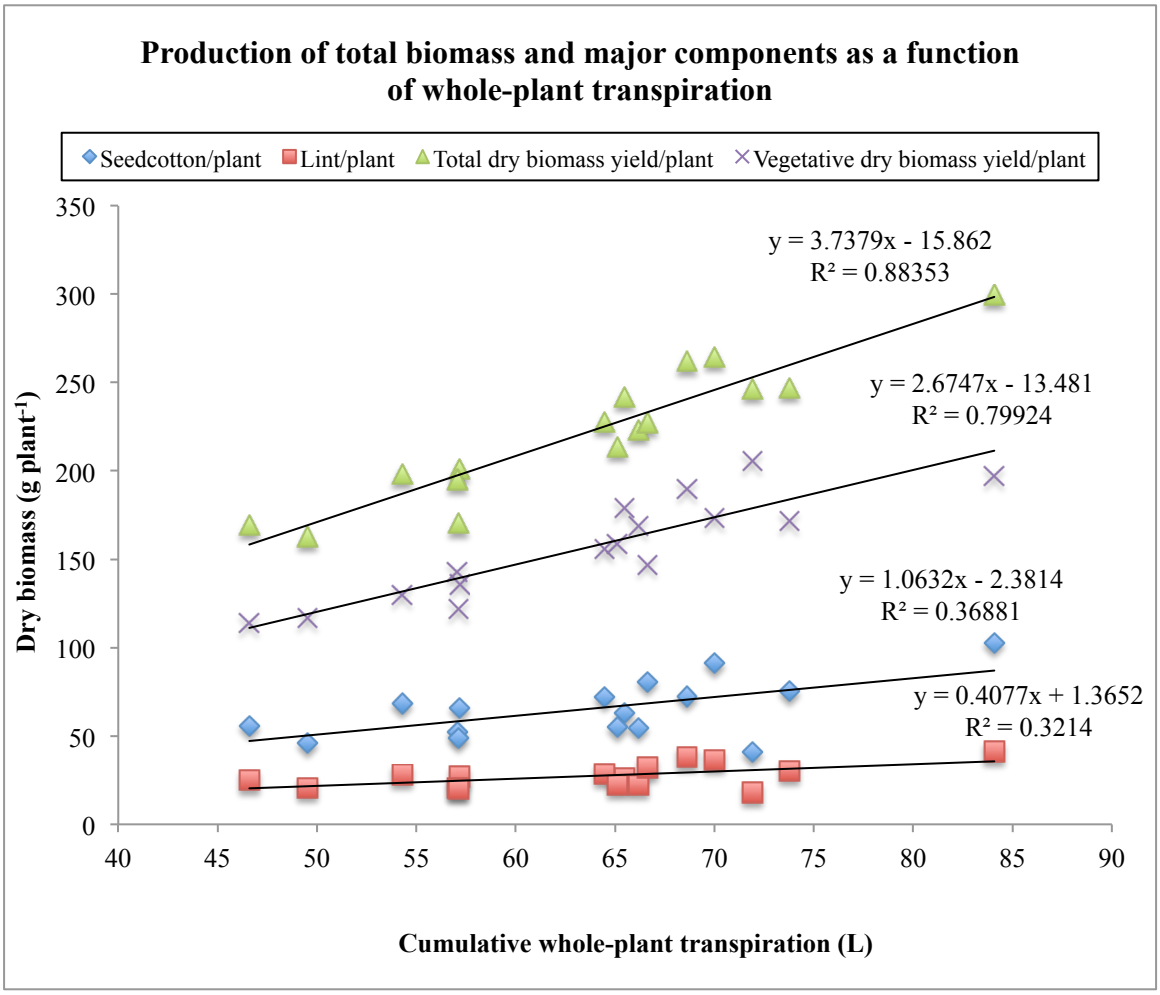

Figure III.17. Seedcotton, lint, total dry biomass yield, and vegetative dry biomass yield per plant as a function of cumulative transpiration during the duration of the timing of water deficit stress alleviation study.

CHAPTER IV

CONCLUSIONS

The timing of the water deficit stress decreased transpiration rates substantially. As a consequence, significant alterations in the vegetative/reproductive ratio of the plants were observed. More specifically, when well-watered cotton plants experience water deficits from 1st bloom to mid bloom and from mid bloom to 1st cracked boll, the effects on dry biomass production and partitioning are severe. Stress during these phenological stages decreases fruit retention significantly, which leads to lower economic and lower water use efficiency. The regression analysis indicates that vegetative biomass decline did not accompanied the decline in whole-plant transpiration until this decline was about 20% of the non-stressed maximum level of 120 L, suggesting the high sensitivity of fruiting structures loss due water deficits.

Supplemental irrigation treatments increased whole-plant transpiration in all the phenological stages they were applied. However, total dry biomass was only increased when water was applied from match head to 1st bloom and from 1st bloom to mid-bloom. These increases in total dry biomass didn't affect significantly economic yield or water use efficiency. The linear regressions indicate that the vegetative dry matter component has a steeper slope than the other components. Therefore, vegetative dry matter production dominates when water-stressed cotton plants receive short supplemental irrigations, and very little of the transpired water is used for the production of reproductive components.

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APPENDIX A

PROCEDURES IN PRELIMINARY STUDY FOR DEVELOPING A NON-DESTRUCTIVE LEAF AREA AND LEAF DRY BIOMASS MEASUREMENT METHOD

Introduction

As discussed in Chapter II, daily whole-plant transpiration (DWPT) is calculated as the 24-hr sum of the pot weight differences between consecutive hours. Since DWPT is dependent on plant leaf area (PLA) (Blad, 1983), this section focuses on PLA and plant leaf dry mass (PLM) measurements and estimates in experimental individual plants.

There are several destructive and non-destructive methods to measure or estimate leaf area of individual plants. In general, destructive methods involve detaching leaves from the plant for the measurements, while in non-destructive procedures leaves are preserved in the plants. In the case of experimental plants that are continuously monitored for calculating DWPT, destructive methods are not recommended, since it is imperative that the integrity of experimental plants is conserved. Non-destructive methods may involve measurements of leaf dimensions that can be input in empirical equations to estimate leaf area. Several combinations of measurements and models relating length and width to area have been developed for several plant species (Pandey and Singh, 2011).

In the case of cotton plants, Constable and Rawson (1980), using data from two glasshouse studies, developed a quadratic equation to depict the relationship between length and area for cotton leaves:

$$Y = 1.0526L^2 - 1.96L;\ r^2 = 0.98;\ n = 120 \quad (1)$$

Where Y is the leaf area (cm^2) and L is the leaf length (cm). This result shows that the relationship between length and area is very strong. Therefore, length can be used as an accurate predictor of area. Indeed, Maeda (2012) used this equation to calculate leaf expansion of different cotton genotypes grown under water deficit stress conditions and found significant differences between them. However, this equation was developed for a cultivar that is no longer available (Deltapine 16). Fernandez et al. (1996) also developed a regression equation for estimating the area of cotton leaves based on the product of leaf's length and width times 0.635. This equation was developed for the cultivar Stoneville 825. Because of differences in leaf shape, such empirical regression equations need to be determined for different species and cultivars with crop species by combining, for example, leaf area scanning methods and leaf area dimension measurements.

Therefore, in order to accurately calculate the PLA responses of the cultivar used in this dissertation and to avoid any possible bias, it was decided to develop a unique model for it. The PLA procedures were based on the relationships between the main-stem leaf (MSL) and the fruiting branch leaves (FBL) at the same node position. Two methodologies were tested.

The first approach was based on the ratio between main-stem (MS) leaves and fruiting branch (FB) leaves. Constable and Oosterhuis (2010) reported that fruiting branch leaves are smaller than the corresponding main-stem (MS) leaf at the same position by a factor of about 0.55, 0.4, and 0.3 for the first three positions on a fruiting branch (FB), respectively. Therefore, by measuring or estimating the area of the MS leaf and using these ratios it is possible to estimate leaf area in a per node basis. Then the sum of the areas of all nodes would provide an estimate of PLA. The second approach was based on regression equations using the area of the MS leaf as a predictor of the area of the leaves in the FB.

PLM estimates were based on the relationship of the PLA and its dry weight. A regression equation was developed to use PLA as a predictor of PLM.

This section describes procedures developed to estimate PLA and PLM of experimental cotton plants utilized in the studies conducted in the Drought Tolerance Laboratory at the Texas A&M AgriLife Research Center in Corpus Christi.

Materials and methods

Cultivar Phytogen 375 (PHY 375) was planted on February 17th, 2014, in six 13.5 L (3. 578 gallon) pots filled with fritted clay soil in the Drought Tolerance Laboratory at the Texas A&M AgriLife Research and Extension Center at Corpus Christi.

The plants were arranged in a complete randomized design, with each plant being considered one replication. Plants were fully irrigated until they were processed. From emergence (February 27th) to first square (April 21st), irrigation regime was 1 L/day.

From First Square to First Bloom (May 12nd), irrigation regime was changed to 3 L/day. Nutrients were provided according to the modified Hoagland solution discussed in Chapter II. Other management practices such as pest control were performed as needed.

Plants were processed when they reached phenological stage B1 (First Bloom). First, the pots were moved to an air-conditioned office during the morning to avoid loss of turgor pressure by the leaves, which could potentially affect the measurements. There, MS and FB leaves were clipped and then scanned with the LI-3100C Area Meter (LI-COR Inc., Lincoln, NE). Measurements of midrib length were taken only on the MS leaves using a ruler. After that, leaves were placed in a bag and dried for 72 hours at 73.8 ^{o}C (165 ^{o}F) using a P0M7-806F drier (Blue M., Garland, TX). In order to obtain the dry weight data the samples were weighted using a high precision Sartorius scale (Brinkmann Instruments Inc., Westbury, NY).

First, three types of functions were tested in order to develop a regression equation relating the length (cm) of the MS leaf midrib to its area: power, linear, and polynomial. The intercept was set to zero on both linear and polynomial functions. The reasoning behind it relies on the assumption that if the length is zero, then the area should be zero. That adjustment wasn't necessary for the power model, since zero powered to any number is always zero. Therefore, the power function naturally fits that premise. The significance of the slopes (β) of the lines were subjected to the analysis of variance (ANOVA) in order to test their significance (shown in Appendix B).

Considering the ratio approach, the values for the cultivar PHY 375 were calculated by dividing the area of the FB leaves by the area of the corresponding MSL at

the same node position. Then, it was calculated the 95% confidence interval (95% CI) for the means in order to check if the ratios obtained in this study are in agreement with the values reported by Constable and Oosterhuis (2010). A t-test was also performed in order to check for significant differences between the ratios.

The regression approach consisted of developing equations where the area of MS leaf is the predictor of the areas of the FB leaves.

For each plant, PLA was calculated as the sum of the area of all scanned individual leaves, while PLM was calculated as the sum of the dry mass of all leaves. The linear model was used to determine the relationship between PLA and PLM. In order to test its significance the ANOVA for the slope (β) was performed (shown in Appendix B).

The data was summarized using Excel 2010 (Microsoft Inc., Redmond, WA) and analyzed using JMP Pro 11 (SAS Institute Inc., Cary, NC).

Results and discussion

The results indicate that all models are significant at the 1% probability level (Table A1). The linear model showed the lowest R^2 value (0.75) and its graph (Figure A1) shows that for values of midrib length smaller than 10 cm the predicted area values are overestimated, while above 10 cm the values are underestimated. The polynomial model (Figure A2) showed a better fit to the data and a higher R^2 value than the linear model. The power model (Figure A3) showed a better fit when compared to the linear model, but similar to the polynomial. Due to its higher R^2 value, the model chosen for calculating the area of the MS leaves in this thesis was the power model.

Table A1. ANOVA results for significance of the regression models.

Model	Regression Equation	Regression parameters	
		R^2	P-value for β
Power	$y = 0.7357x^{2.1144}$	0.96	<0.01**
Linear	$y = 13.819x$	0.75	<0.01**
Polynomial	$y = 1.0097x^2 - 0.165x$	0.91	<0.01**

** = Significant at the 1% probability level; n = 90 observations.

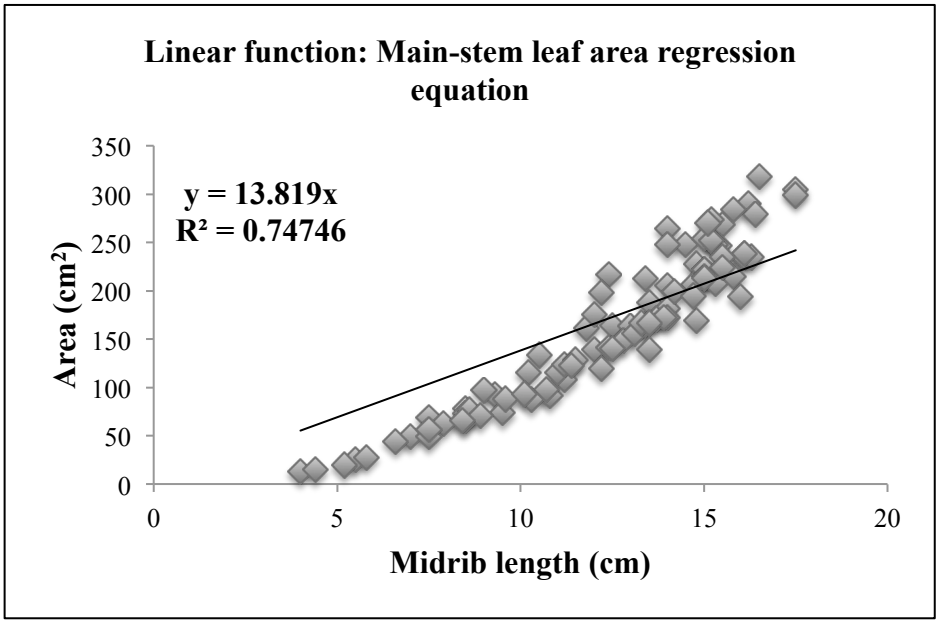

Figure A1. Linear regression model of midrib length (cm) on area (cm^2) with the intercept set on zero for main-stem leaves; n = 90.

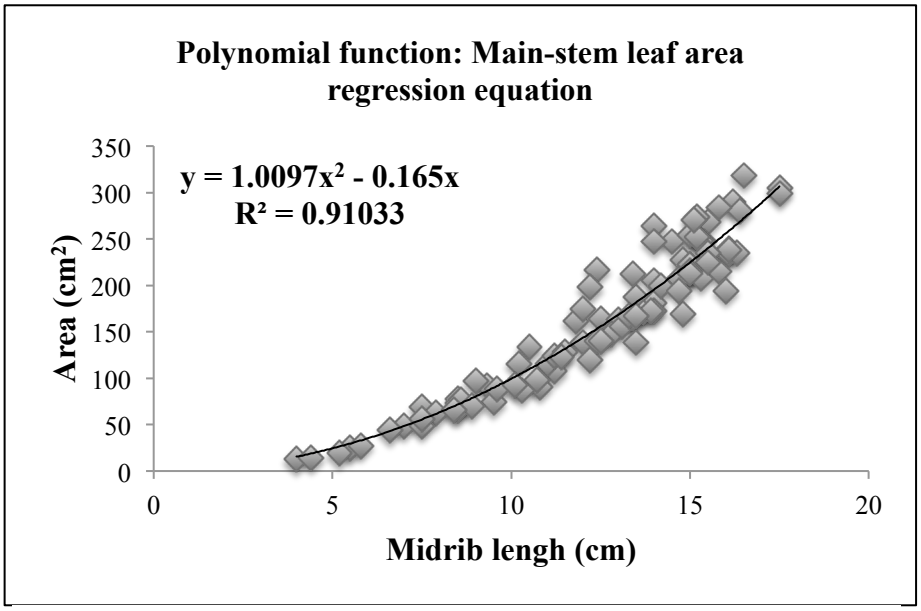

Figure A2. Second order polynomial regression model of midrib length (cm) on area (cm^2) with the intercept set on zero for main-stem leaves; n = 90.

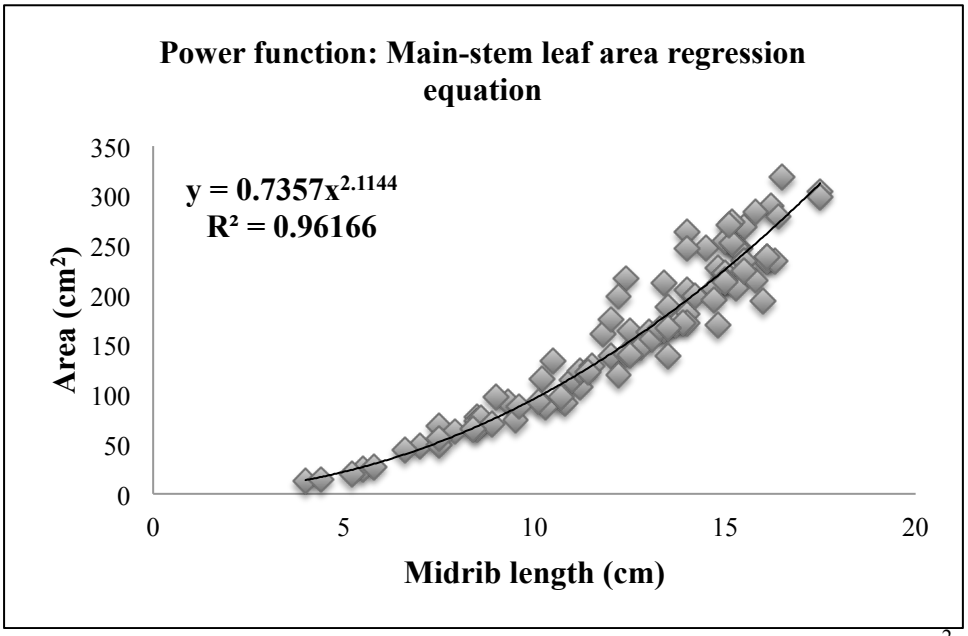

Figure A3. Power regression model of midrib length (cm) on area (cm^2) for main-stem leaves; n = 90.

Since the values reported by Constable and Oosterhuis are within the 95% CI of the means (Table A2), it is possible to conclude that they are not significantly different. Therefore, the ratios obtained for PHY375 are in agreement with what was previously reported. Additionally, based on the CI values it can be observed that some ratios are significantly different from each other. The ratios between the MS leaf and the 1st position leaf on the FB are significantly different from the ratios for the 3rd, 4th, and 5th position leaves (Table A3). That suggests a size pattern for the leaves in the fruiting branches, where the first 2 are about the same size and bigger than the rest.

Table A2. Confidence interval (CI) for the ratio between the fruiting branch leaves (FBL) and their corresponding main-stem leaf (MSL), and comparison between the ratio means and values reported in the literature.

Ratio type	Constable and Oosterhuis (2010)	Data Collected in this study			
		Mean	Lower 95% CI	Upper 95% CI	n
MSL:1st FBL	0.55	0.558461^{ns}	0.465006	0.651916	60
MSL:2nd FBL	0.4	0.466635^{ns}	0.384626	0.548645	42
MSL:3rd FBL	0.3	0.336048^{ns}	0.266203	0.405893	32
MSL:4th FBL	-	0.227003	0.141019	0.312988	19
MSL:5th FBL	-	0.13024	0.012895	0.247585	7

^{ns} = non-significant at the 5% probability level; n = number of observations.

Table A3. T-test results for significance between ratio means.

Ratio type	Mean
MSL:1st FBL	0.558461 a
MSL:2nd FBL	0.466635 ab
MSL:3rd FBL	0.336048 bc
MSL:4th FBL	0.227003 c
MSL:5th FBL	0.13024 c

Means with different letters are significantly different at the 5% probability level.

The regression approach, using the area of the main-stem leaf to predict the area of the FB leaves, was not effective. As can be seen on figures A4, A5, A6, A7, and A8 the relationship between the variables was very weak. In the case of the 1st position leaf graph, some FB leaves were bigger or about the same size of their corresponding MS leaf. Even though the R^2 for this relationship was decent (r^2 = 0.49715), the data suggests that the 1st position leaf on the FB is not always smaller than its corresponding MS leaf, which might explain why the regression was not able to depict the relationship with greater precision.

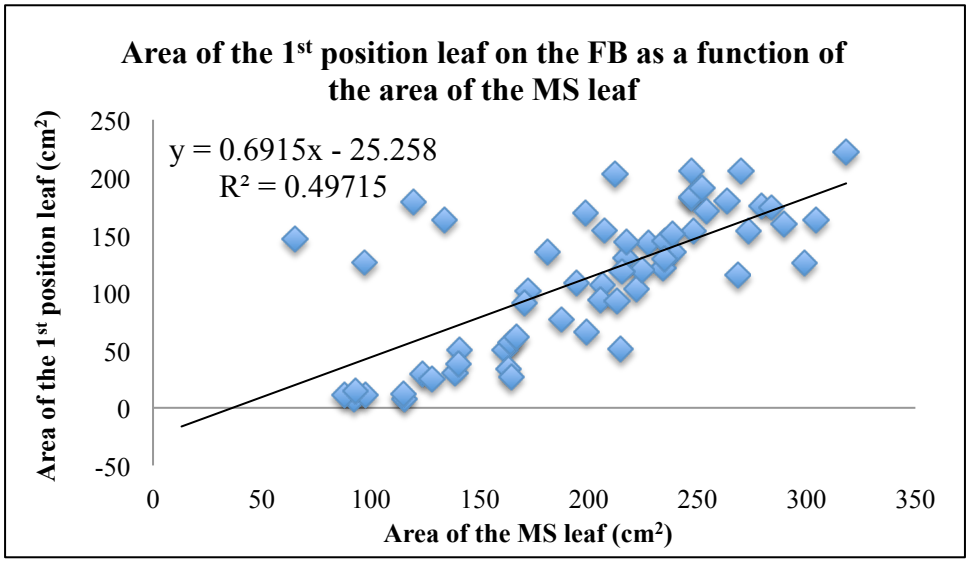

Figure A4. Regression equation: area of the main-stem leaf as a predictor of the area of the 1st position leaf on the fruiting branch; n = 60.

In the case of the other FB leaves, the regressions were poor. It is possible to hypothesize that the relationship is weak because the points on the FB leaves in the y-axis are clustering around the 200-300 cm^2 MS leaves on the x-axis. That can possibly indicate that for the conditions of this study, the MS leaves reached a limit in terms of area, above which they no longer grow, while the FB leaves are still growing. Therefore, a relationship can't be established due this lack of synchrony, since one of the components is no longer increasing while the other still is.

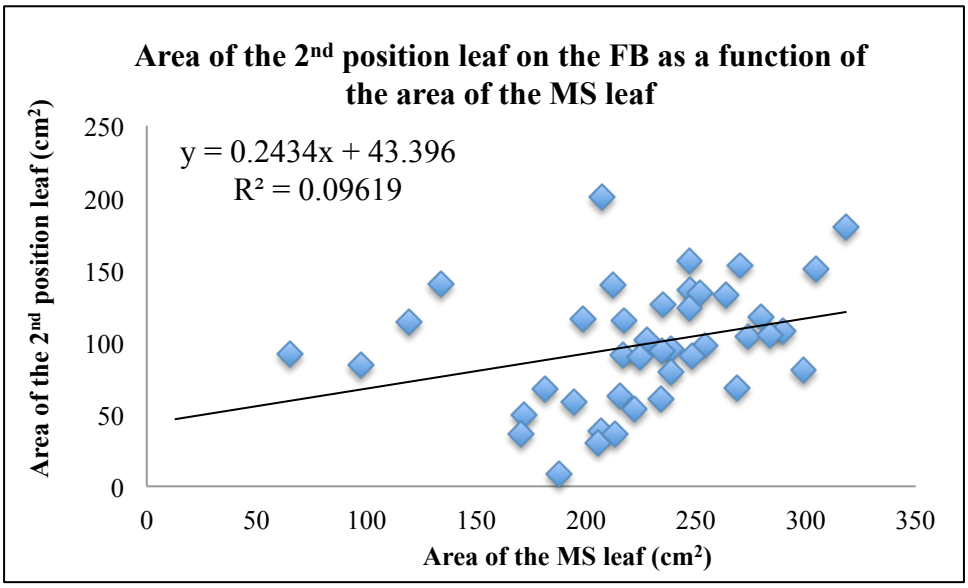

Figure A5. Regression equation: area of the main-stem leaf as a predictor of the area of the 2nd position leaf on the fruiting branch; n = 42.

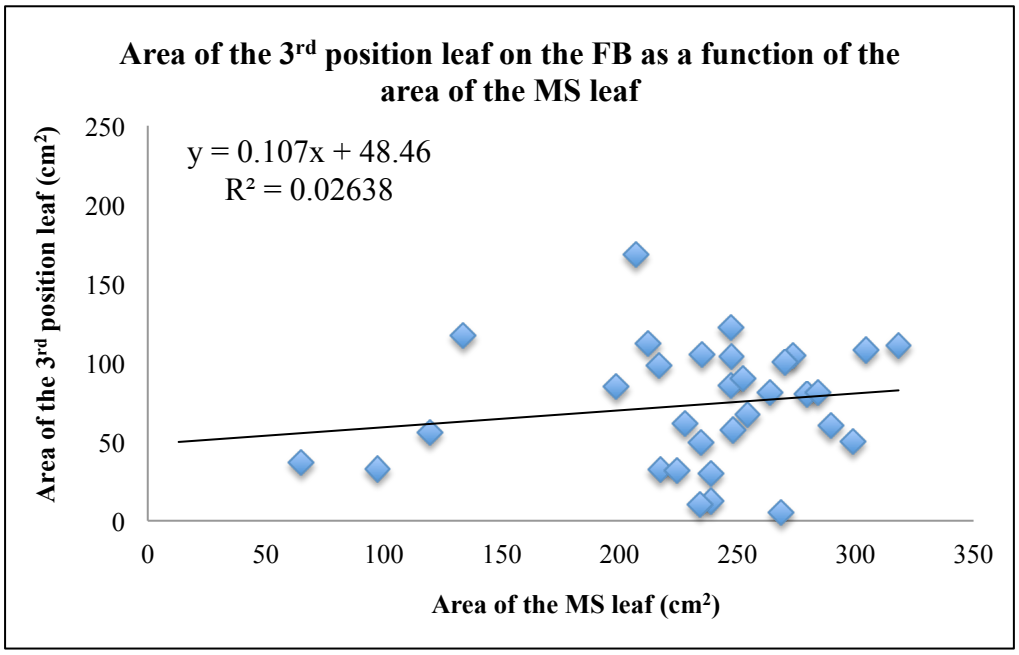

Figure A6. Regression equation: area of the main-stem leaf as a predictor of the area of the 3rd position leaf on the fruiting branch; n = 32.

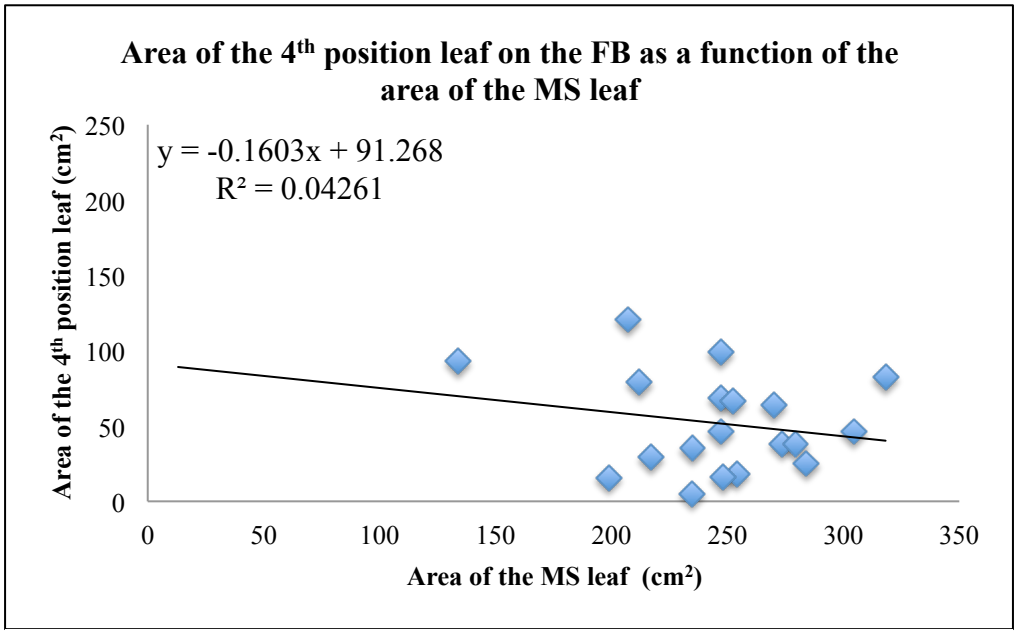

Figure A7. Regression equation: area of the main-stem leaf as a predictor of the area of the 4th position leaf on the fruiting branch; n = 19.

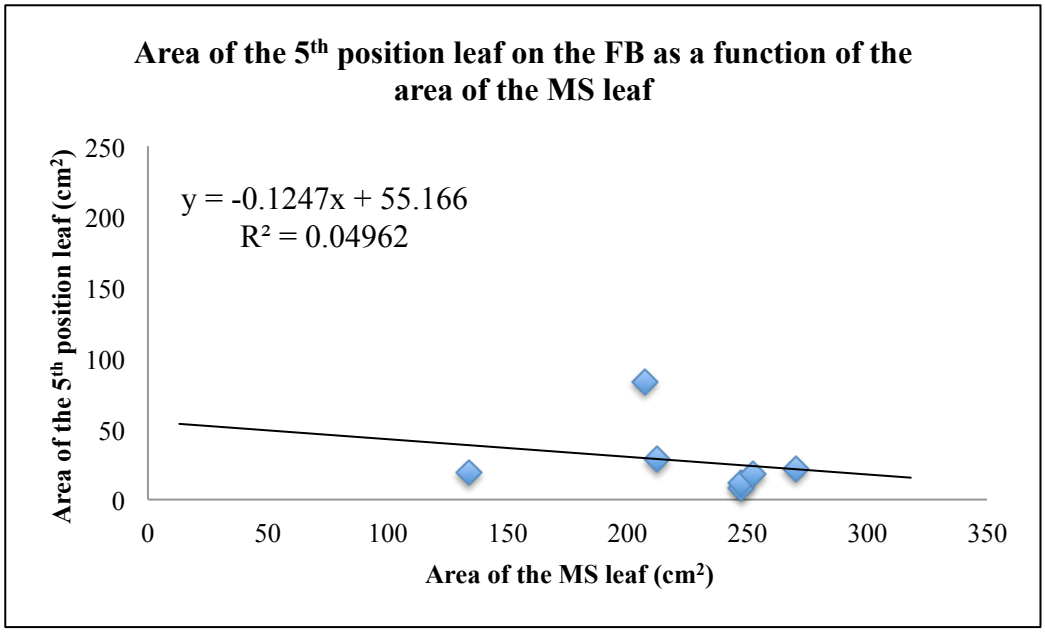

Figure A8. Regression equation: area of the main-stem leaf as a predictor of the area of the 5th position leaf on the fruiting branch; n = 7.

Therefore, based on this data, the model chosen for calculating the leaf area of the plants is made up of a two-step procedure. First, at a given node the length of the midrib of the MSL is measured and its area is calculated using the power function obtained from the regression equation. Then, the FB leaves relative to it are counted, and their areas are calculated by multiplying the MS area with the appropriate ratio, as shown in Table A4.

Table A4. Area calculation procedure for cotton leaves at the same node position.

Leaf type	Area formula (cm^2)
Main-stem leaf	$A = 0.7357*length^{2.1144}$
1st position leaf at the fruiting branch	$A= (0.7357*length^{2.1144})* 0.558461$
2nd position leaf at the fruiting branch	$A= (0.7357*length^{2.1144})* 0.466635$
3rd position leaf at the fruiting branch	$A= (0.7357*length^{2.1144})* 0.336048$
4th position leaf at the fruiting branch	$A= (0.7357*length^{2.1144})* 0.227003$
5th position leaf at the fruiting branch	$A= (0.7357*length^{2.1144})* 0.13024$

- In order to convert cm^2 to m^2 the values must be divided by a factor of 10000.

In order to calculate PLA this procedure must be applied at all the nodes. By summing the values it is possible to obtain PLA.

Two features of this model should be pointed out. The first is that the area value is just for one side of the leaf. The second is that the area values obtained for the leaves in the fruiting branches are necessarily smaller than their corresponding MS leaf. As the regression approach showed, this is not always the case, but for practical purposes it is a good approximation.

The linear regression equation relating PLA to PLM showed a very good fit to the data, as can be seen on Figure A9. The results also show that the equation obtained is significant at the 1% probability level (Table A5).

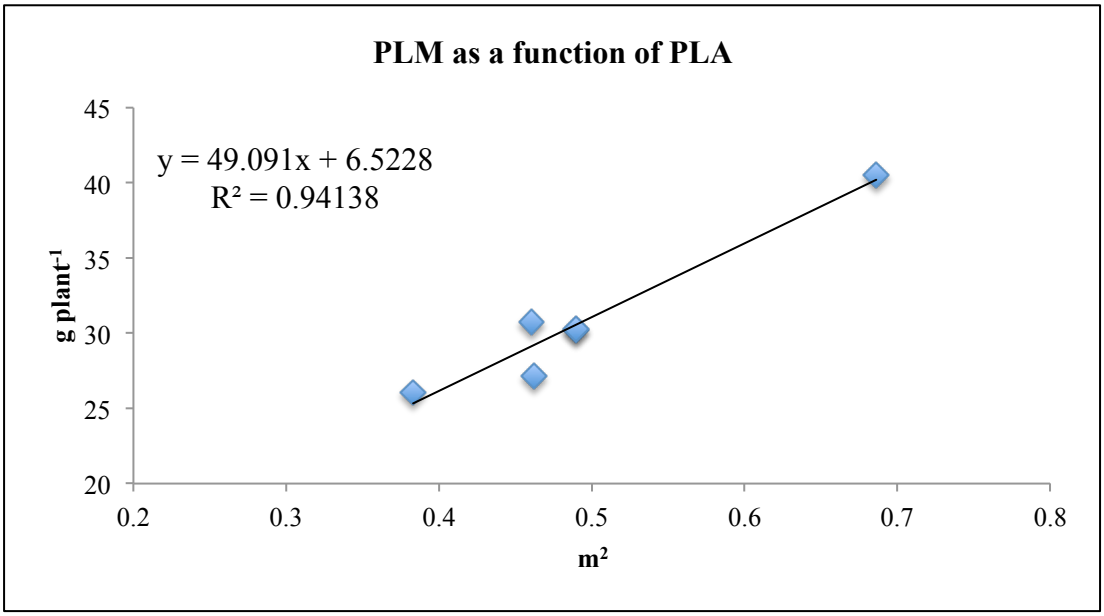

Figure A9. Regression equation: PLA as a predictor of PLW. PLA values were converted from cm^2 to m^2.

Table A5. ANOVA results for significance of the regression model.

Model	Regression Equation	Regression parameters	
		r^2	P-value for β
Linear	y = 49.091x + 6.5228	0.94	0.0013**

** Significant at the 1% probability level.

Knowing PLM is useful for increasing the precision of water use efficiency (WUE, g L^{-1}) calculations. This equation was used in the calculations of WUE for the studies presented at Chapters IV and V. Reversing the relationship to express the regression of PLA on PLM is useful for estimating PLA when PLM data is available.

Conclusions

Based on the data presented in this chapter it is possible to conclude that the power function depicts the relationship between length and area of cotton leaves with good precision; when estimating the area of FB leaves based on the area of MS leaves it is convenient to use the ratio approach rather than a regression approach, since the relationship between them may not be strong; and that the total dry weight of the leaves can be estimated based on the total leaf area of the plant with good precision as well.

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APPENDIX B

SIGNIFICANCE TESTS FOR REGRESSION SLOPES

All models (power, linear, polynomial) were using length as predictor of area for main-stem cotton leaves. The tables in this section show the significance of the analysis of variance (ANOVA) for the slopes of the models presented in Appendix A. For the linear and polynomial model (Tables B2 and B3) two degrees of freedom were used on the regression source of variation because the intercept was forced to go through the origin. For the power function that was not necessary (Table B1). Table B4 shows the ANOVA result for the slope of the model using PLA as a predictor of PLM.

Table B1. ANOVA for testing the slope of the power model that uses length as a predictor of area for main-stem leaves.

Source of variation	Degrees of freedom	Sum of Squares	Mean Squares	F	F-critical 5%	F-critical 1%
Regression	1	528031	528031	2101	3.9	6.9
Residual	88	22116	251			
Total	89	550147				

Table B2. ANOVA for testing the slope of the linear model that uses length as predictor of area for main-stem leaves.

Source of variation	Degrees of freedom	Sum of Squares	Mean Squares	F	F-critical 5%	F-critical 1%
Regression	2	187317	93658	22.4	3.1	4.8
Residual	87	362830	4170			
Total	89	550147				

Table B3. ANOVA for testing the slope of the polynomial model that uses length as a predictor of area for main-stem leaves.

Source of variation	Degrees of freedom	Sum of Squares	Mean Squares	F	F-critical 5%	F-critical 1%
Regression	2	497483	248741	410	3.1	4.8
Residual	87	52664	605			
Total	89	550147				

Table B4. ANOVA for testing the slope of the linear model that uses PLA as a predictor of PLM.

Source of variation	Degrees of freedom	Sum of Squares	Mean Squares	F	Prob. > F
Regression	1	123	123	64.2	0.0013
Residual	4	7	1.9		
Total	5	131			

APPENDIX C

STATISTICAL ANALYSES: ANOVA RESUTLS

The tables presented in this section show the results of the analyses of variance associated with each response variable (p-values and coefficients of variation are included) for the two studies discussed in Chapter III. Source of variation (SOV), degrees of freedom (df), Sum of Squares (SS), Mean Squares (MS), and coefficient of variation (CV) will be found abbreviated in these tables.

Effects of timing of water deficit on cotton water economy, growth, and yield

Table C1. CWPT (L) from match head to 1st bloom:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	16.95973212	5.65324404	2.25	0.1349
Error	12	30.15045520	2.51253793		
Total	15	47.11018732			

CV (%) = 10.76817

Table C2. CWPT (L) from 1st bloom to mid bloom:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	1221.429126	407.143042	88.08	<0.0001
Error	12	55.467982	4.622332		
Total	15	1276.897108			

CV (%) = 6.028485

Table C3. CWPT (L) from mid bloom to 1st cracked boll:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	1620.657157	540.219052	26.09	<0.0001
Error	12	248.471388	20.705949		
Total	15	1869.128545			

CV (%) = 14.28801

Table C4. CWPT (L) across the season:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	2980.796803	993.598934	12.29	0.0006
Error	12	969.964358	80.830363		
Total	15	3950.761161			

CV (%) = 8.780035

Table C5. PLA (m^2) when plants were at match head:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.00061358	0.00020453	2.09	0.1551
Error	12	0.00117470	0.00009789		
Total	15	0.00178828			

CV (%) = 9.850935

Table C6. PLA (m^2) when plants were at 1st bloom:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.04719371	0.01573124	4.85	0.0195
Error	12	0.03890248	0.00324187		
Total	15	0.08609619			

CV (%) = 11.74705

Table C7. PLA (m^2) when plants were at mid bloom:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.15846619	0.05282206	6.34	0.0080
Error	12	0.09996154	0.00833013		
Total	15	0.25842773			

CV (%) = 13.56437

Table C8. PLA (m^2) when plants were at 1st cracked boll:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.08702730	0.02900910	2.69	0.0932
Error	12	0.12931725	0.01077644		
Total	15	0.21634454			

CV (%) = 16.14554

Table C9. Daily transpiration per leaf area (L m^{-2}) when plants were at match head:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.08876171	0.02958724	0.72	0.5577
Error	12	0.49144287	0.04095357		
Total	15	0.58020457			

CV (%) = 26.97312

Table C10. Daily transpiration per leaf area (L m^{-2}) when plants were at 1st bloom:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	4.45553617	1.48517872	8.04	0.0033
Error	12	2.21791336	0.18482611		
Total	15	6.67344954			

CV (%) = 14.75075

Table C11. Daily transpiration per leaf area (L m^{-2}) when plants were at mid bloom:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	4.25758252	1.41919417	14.12	0.0003
Error	12	1.20605081	0.10050423		
Total	15	5.46363333			

CV (%) = 13.43835

Table C12. Daily transpiration per leaf area (L m^{-2}) when plants were at 1st cracked boll:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.85528072	0.28509357	10.64	0.0011
Error	12	0.32167609	0.02680634		
Total	15	1.17695681			

CV (%) = 11.80201

Table C13. Total dry biomass yield (g $plant^{-1}$):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	49560.11518	16520.03839	21.86	<0.0001
Error	12	9068.11398	755.67617		
Total	15	58628.22916			

CV (%) = 7.537605

Table C14. Vegetative dry biomass yield (g $plant^{-1}$):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	5215.2028	1738.4	4.366	0.0269
Error	12	4777.9969	398.17		
Total	15	9993.1997			

CV (%) = 7.973937

Table C15. Main-stem dry biomass (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	322.1525000	107.3841667	5.17	0.0160
Error	12	249.2450000	20.7704167		
Total	15	571.3975000			

CV (%) = 10.44989

Table C16. Branches dry biomass (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	1014.012500	338.004167	5.08	0.0169
Error	12	798.345000	66.528750		
Total	15	1812.357500			

CV (%) = 13.89822

Table C17. Burs dry biomass (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	4983.522500	1661.174167	20.69	<0.0001
Error	12	963.695000	80.307917		
Total	15	5947.217500			

CV (%) = 15.95632

Table C18. Roots dry biomass (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	861.526875	287.175625	5.07	0.0170
Error	12	679.802500	56.650208		
Total	15	1541.329375			

CV (%) = 14.01771

Table C19. Leaves dry biomass (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	209.7293734	69.9097911	2.69	0.0932
Error	12	311.6450286	25.9704190		
Total	15	521.3744020			

CV (%) = 13.38041

Table C20. Seedcotton yield (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	29644.87688	9881.62563	37.96	<0.0001
Error	12	3123.44250	260.28688		
Total	15	32768.31938			

CV (%) = 14.09570

Table C21. Lint yield (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	4608.304919	1536.101640	33.55	<0.0001
Error	12	549.415175	45.784598		
Total	15	5157.720094			

CV (%) = 14.51382

Table C22. Harvest index (%):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.08820994	0.02940331	27.65	<0.0001
Error	12	0.01275908	0.00106326		
Total	15	0.10096902			

CV (%) = 10.77187

Table C23. Boll retention per plant (%):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.50420575	0.16806858	30.67	<0.0001
Error	12	0.06575790	0.00547983		
Total	15	0.56996365			

CV (%) = 14.95287

Table C24. Total number of reproductive structures per plant:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	387.187500	129.062500	1.89	0.1854
Error	12	820.250000	68.354167		
Total	15	1207.437500			

CV (%) = 11.59356

Table C25. Number of bolls per plant:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	2213.687500	737.895833	24.01	<0.0001
Error	12	368.750000	30.729167		
Total	15	2582.437500			

CV (%) = 11.59356

Table C26. Number of aborted reproductive structures per plant:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	3318.500000	1106.166667	17.52	0.0001
Error	12	757.500000	63.125000		
Total	15	4076.000000			

CV (%) = 21.76746

Table C27. Total water use efficiency (WUE_{total}, g L^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.08439075	0.02813025	1.60	0.2409
Error	12	0.21086393	0.01757199		
Total	15	0.29525468			

CV (%) = 3.726244

Table C28. Economic water use efficiency ($WUE_{economic}$, g L^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	1.26364051	0.42121350	21.94	<0.0001
Error	12	0.23042196	0.01920183		
Total	15	1.49406247			

CV (%) = 12.79912

Table C29. Lint water use efficiency (WUE_{lint}, g L^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.19059433	0.06353144	18.11	<0.0001
Error	12	0.04209906	0.00350825		
Total	15	0.23269339			

CV (%) = 13.41387

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Table C30. CWPT (L) from match head to 1st bloom:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	25.24555378	8.41518459	9.05	0.0021
Error	12	11.15836265	0.92986355		
Total	15	36.40391643			

CV (%) = 8.505606

Table C31. CWPT (L) from 1st bloom to mid bloom:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	629.9637437	209.9879146	55.03	<0.0001
Error	12	45.7917664	3.8159805		
Total	15	675.7555101			

CV (%) = 8.902928

Table C32. CWPT (L) from mid bloom to 1st cracked boll:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	275.1921698	91.7307233	9.64	0.0016
Error	12	114.1336898	9.5111408		
Total	15	389.3258596			

CV (%) = 15.11292

Table C33. CWPT (L) across the season:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	972.996103	324.332034	9.55	0.0017
Error	12	407.373656	33.947805		
Total	15	1380.369759			

CV (%) = 9.157591

Table C34. PLA (m^2) when plants were at match head:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.00010331	0.00003444	0.32	0.8124
Error	12	0.00130072	0.00010839		
Total	15	0.00140403			

CV (%) = 11.32635

Table C35. PLA (m^2) when plants were at 1st bloom:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.06278301	0.02092767	10.75	0.0010
Error	12	0.02336108	0.00194676		
Total	15	0.08614409			

CV (%) = 12.57566

Table C36. PLA (m^2) when plants were at mid bloom:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.28100179	0.09366726	20.48	<0.0001
Error	12	0.05488506	0.00457375		
Total	15	0.33588685			

CV (%) = 14.51253

Table C37. PLA (m^2) when plants were at 1st cracked boll:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.04938329	0.01646110	2.76	0.0882
Error	12	0.07158456	0.00596538		
Total	15	0.12096785			

CV (%) = 16.54955

Table C38. Daily transpiration per leaf area (L m^{-2}) when plants were at match head:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.22717271	0.07572424	0.41	0.7481
Error	12	2.21088028	0.18424002		
Total	15	2.43805299			

CV (%) = 45.95457

Table C39. Daily transpiration per leaf area (L m^{-2}) when plants were at 1st bloom:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	7.42253401	2.47417800	11.46	0.0008
Error	12	2.59051291	0.21587608		
Total	15	10.01304693			

CV (%) = 21.19731

Table C40. Daily transpiration per leaf area (L m^{-2}) when plants were at mid bloom:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	3.04390403	1.01463468	8.94	0.0022
Error	12	1.36234008	0.11352834		
Total	15	4.40624411			

CV (%) = 16.81715

Table C41. Daily transpiration per leaf area (L m^{-2}) when plants were at 1st cracked boll:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.25919812	0.08639937	1.49	0.2666
Error	12	0.69492419	0.05791035		
Total	15	0.95412231			

CV (%) = 18.74646

Table C42. Total dry biomass yield (g $plant^{-1}$):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	14799.08352	4933.02784	8.42	0.0028
Error	12	7029.63151	585.80263		
Total	15	21828.71503			

CV (%) = 10.90442

Table C43. Vegetative dry biomass yield (g $plant^{-1}$):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	9823.093	3274.36	15.5134	0.0002
Error	12	2532.803	211.07		
Total	15	12355.896			

CV (%) = 9.27157

Table C44. Main-stem dry biomass (g $plant^{-1}$):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	585.2150000	195.0716667	16.01	0.0002
Error	12	146.2550000	12.1879167		
Total	15	731.4700000			

CV (%) = 11.96613

Table C45. Branches dry biomass (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	998.785000	332.928333	11.32	0.0008
Error	12	353.075000	29.422917		
Total	15	1351.860000			

CV (%) = 18.70445

Table C46. Burs dry biomass (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	330.2900000	110.0966667	2.34	0.1252
Error	12	565.2500000	47.1041667		
Total	15	895.5400000			

CV (%) = 20.67243

Table C47. Roots dry biomass (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	543.2568750	181.0856250	9.20	0.0020
Error	12	236.3225000	19.6935417		
Total	15	779.5793750			

CV (%) = 12.48529

Table C48. Leaves dry biomass (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	135.8757150	45.2919050	3.59	0.0465
Error	12	151.4767416	12.6230618		
Total	15	287.3524567			

CV (%) = 11.93132

Table C49. Seedcotton yield (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	795.527500	265.175833	0.93	0.4577
Error	12	3435.050000	286.254167		
Total	15	4230.577500			

CV (%) = 25.92461

Table C50. Lint yield (g plant^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	136.1815688	45.3938563	0.94	0.4505
Error	12	577.8233750	48.1519479		
Total	15	714.0049438			

CV (%) = 25.41177

Table C51. Harvest index (%):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.00726405	0.00242135	0.99	0.4287
Error	12	0.02923299	0.00243608		
Total	15	0.03649704			

CV (%) = 16.79400

Table C52. Boll retention per plant (%):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.00226507	0.00075502	0.07	0.9737
Error	12	0.12540927	0.01045077		
Total	15	0.12767434			

CV (%) = 22.01269

Table C53. Total number of reproductive structures per plant:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	918.500000	306.166667	6.25	0.0084
Error	12	587.500000	48.958333		
Total	15	1506.000000			

CV (%) = 15.21092

Table C54. Number of bolls per plant:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	161.1875000	53.7291667	2.83	0.0832
Error	12	227.7500000	18.9791667		
Total	15	388.9375000			

CV (%) = 20.68372

Table C55. Number of aborted reproductive structures per plant:

SOV	df	SS	MS	F	Prob. > F
Treatments	3	323.1875000	107.7291667	1.96	0.1740
Error	12	659.7500000	54.9791667		
Total	15	982.9375000			

CV (%) = 29.73351

Table C56. Total water use efficiency (WUE_{total}, g L^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.34204997	0.11401666	4.06	0.0332
Error	12	0.33704253	0.02808688		
Total	15	0.67909250			

CV (%) = 4.810767

Table C57. Economic water use efficiency ($WUE_{economic}$, g L^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.07942553	0.02647518	0.62	0.6158
Error	12	0.51309169	0.04275764		
Total	15	0.59251723			

CV (%) = 20.14292

Table C58. Lint water use efficiency (WUE_{lint}, g L^{-1}):

SOV	df	SS	MS	F	Prob. > F
Treatments	3	0.02122949	0.00707650	0.94	0.4539
Error	12	0.09081654	0.00756805		
Total	15	0.11204603			

CV (%) = 20.21166