DEFINING DROUGHT SENSITIVITY AND ASSESSING ALTERNATIVE IRRIGATION PRACTICES FOR *GOSSYPIUM HIRSUTUM* GROWN IN THE SOUTHEASTERN UNITED STATES

by

DARYL CHASTAIN

(Under the Direction of John L. Snider)

ABSTRACT

Drought represents a major threat to cotton production worldwide. In the current dissertation, three experiments were conducted under field conditions to assess 1) the underlying limitations to net photosynthesis under drought, 2) the potential for plant-based irrigation scheduling to improve water productivity in Georgia cotton production, and 3) the impact of leaf development on drought and heat tolerance. For experiment 1, predawn water potential (Ψ_{PD}) ranged from -0.31 to -0.95 MPa, and midday water potential (Ψ_{MD}) ranged from -1.02 to -2.67 MPa for the 2012 and 2013 growing seasons combined for irrigated and dryland cotton. Cotton responded to water deficit by decreasing stomatal conductance, increasing photorespiration, and increasing the ratio of dark respiration to gross photosynthesis, thereby limiting P_N and decreasing lint yield. Even extreme water deficit did not negatively affect primary photochemistry. For experiment 2, *G. hirsutum* plants were grown under fully-irrigated, dryland, and three predawn water potential (Ψ_{PD}) thresholds (-0.5, -0.7, -0.9 MPa). Ψ_{PD} was an effective means of determining the need for irrigation in cotton, and in the current study, yield and water productivity were maximized at a season-long average Ψ_{PD} threshold of -0.5 MPa. Canopy temperature-derived crop water stress index (CWSI) exhibited a non-linear relationship with Ψ_{PD} between -0.4 and -0.7 MPa ($r^2 = 0.81$) and with lint yield ($r^2 = 0.81$). For experiment 3, increased photosystem II thermotolerance was observed for young leaves early in the growing season. P_N in young leaves was not negatively impacted by leaf temperatures as high as 37°C or by extreme drought. For example, in young leaves, as T_{Leaf} increased from 31 to 37°C, no decline in P_N was observed whereas, P_N in more mature leaves declined by 66% over the same temperature range when high T_{leaf} was drought induced. The substantial differences in heat and drought tolerance between two different stages of leaf development may provide opportunities to improve drought and heat tolerance by regulating pre-existing genes within the same genotype.

INDEX WORDS: chlorophyll fluorescence, drought stress, *Gossypium hirsutum*, leaf water potential, photorespiration, photosynthesis, respiration, cotton, heat stress, leaf expansion, drought, water deficit, thermotolerance

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DEDICATION

This document is dedicated to my family, Leslie, Lily, and Hank Chastain. Many sacrifices were made to get to this point. In addition, this document is dedicated to my sister, Cherri Chastain with hopes and prayers for a full recovery from recent illness.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Need for efficient use of water resources under climatic uncertainty:

The future success of agriculture has been said to mainly be limited by water availability (Kramer and Boyer, 1995). Agricultural use accounts for approximately 70% of global fresh water use (Postel et al., 1996). In many areas, food and fiber production relies heavily on irrigation from ground and surface water resources; however, in locations such as the humid southeastern United States, rainfall can supply much of the water needed for profitable crop production. Despite high rainfall, the benefits of supplemental irrigation such as increased yield and avoidance of the negative effects of environmental unpredictability, as well as higher crop yields, lead many farmers to adopt an as-needed irrigation approach (Farahani and Munk, 2012). This has resulted in concerns over the sustainability of current irrigation practices.

Drought has been described as the most severe of natural disasters (Wilhite, 2000; Humphries and Baldwin, 2003), and has been linked to the total collapse of past agricultural societies (Kennett et al., 2012). Recently, limits on surface and groundwater withdrawals have been increasing in the United States in an effort to prevent negative environmental impacts of excessive surface water diversion, such as those seen in the Aral Sea (Micklin, 1988) as well as to prevent losses of biodiversity and ecosystem function (Tilman, 1999). Excessive irrigation has been linked ocean dead zones formed by large marine algal blooms due to excessive phosphorus leaching (Beman et al., 2005) and declines in endemic mussel populations (Golladay et al. , 2009). Additionally, recent increases in severity and scale of drought in the United States has prompted many states to enact (or discuss) legislation designed to anticipate and mitigate the negative impacts of decreased rainfall (Wilhite et al., 2000). In Georgia, new regulations have been enacted that set minimum flows for both surface and groundwater resources in the Flint River basin (GDNR-EPD, 2006). Specifically, Georgia's water resources in the future "shall promote the conservation and reuse of water within the state, guard against a shortage of water within the state, promote the efficient use of the water resource, and be consistent with the public welfare of the state." (O.C.G.A. 12-5-31(h)). To ensure future sustainability while still attracting business, Georgia State Representative Harry Geisinger is attempting to acquire water from bordering states. Specifically, legislation has been proposed to redraw Georgia state boundaries to include a portion of southern Tennessee for the purpose of obtaining water rights from lake Nickajack (Hatcher Hurd, 2013).

Furthermore, future climate models suggest increased global temperatures as well as decreased and unpredictable rainfall due to the effects of atmospheric CO_2 enrichment. Elevated CO_2 levels have been shown to increase carbon fixation, decrease stomatal density (Wagner et al., 2006), partially reduce stomatal aperture (Morison, 1998), thereby indirectly influencing leaf surface temperature through decreased transpirational cooling. The benefits of CO_2 enrichment (through increased carbon fixation) are not expected to outweigh the decrease in yield due to thermal stress (Reddy et al. , 2002). For example, cotton production modeling for Stoneville, Mississippi projects a decrease in yield of as much as 9%, despite projected yield increases due to CO_2 enrichment alone (Reddy et al., 2002). This effect was mainly due to increased boll abscission during heat stress. It is also important to note that elevated air temperatures can increase evapotranspiration by increasing atmospheric demand (Hargreaves and Allen, 2003). The combination of heat-induced declines in yield and increases in evaporative water loss under elevated temperature would likely limit water use efficiency.

When rainfall deficits necessitate irrigation, uncertainty about the effect that overuse of water resources has on human and non-human ecosystems necessitates a better understanding of the underlying mechanisms that allow for drought tolerance as well as investigations into techniques that allow for decreased water use and maintenance of profitable yields. Furthermore, dealing with the consequences of a changing climate will require efforts to increase crop tolerance to abiotic stress (Wassmann et al. , 2009). An understanding of how agronomic crops respond to this projected environment is essential for continued stability of feed and fiber supplies. Negative societal and environmental effects due to runoff or over-withdrawal of water resources can be mitigated by decreasing either the amount of water a crop needs, or improving methods for determining when and where water should be applied. Additionally, identification of variation in drought tolerance amongst cultivars can potentially be used to decrease yield and profit losses under dryland and deficit irrigation regimes (Pace et al., 1999), thereby decreasing grower risks associated with extreme year-to-year variation in precipitation.

A brief review of methods used to schedule irrigation in agricultural crops:

Irrigation scheduling is the process of determining when to irrigate, how much water to apply, and where to apply it. Triggers for scheduling irrigation can be grouped into two basic categories: environmental triggers and plant-based triggers (Jones, 2004). Environmental triggers have been the primary method used in agriculture for most of history. The use of rainfall, soil water content measurements, and evapotranspiration (ET, the sum of soil water evaporation and plant transpiration) rates for a "checkbook" approach to irrigation scheduling is well documented (Lundstrom and Stegman, 1983; Allen et al. 1998; Fisher and Udeigwe, 2012) and has been applied over a wide variety of crops and geographic regions. In this approach, ET is first measured for a reference surface, such as a grass. This data, along with climactic data such as wind speed, vapor pressure deficit, incident solar radiation, and air temperature, etc., are used in the Pennman-Monteith equation (see Monteith, 1965) to calculate a region specific reference ET (ET_o). Next, water loss from the crop (ET_c) in question is measured using lysimeters and related to ET_o by $ET_{o*}K_c = ET_c$, where K_c is referred to as the crop coefficient. ET_c values are calculated throughout the growing season to produce developmentally specific K_c values (such as pre/post-flowering, and fruiting). ET_o values (calculated from climatic data) can then be used to estimate ET_c by adjusting with the appropriate K_c. This has the advantage of allowing producers to schedule irrigation based on climatic data alone, when K_c values are known for a given region. Over time, when year to year variation in crop water use is low, budgets that are designed to replace water loss (either by rainfall or supplemental irrigation) from ET_c can be developed, such as that used by the

University of Georgia (Table 1.1). Using this method, irrigation is used to supplement rainfall for a given week such that rainfall + irrigation meet the weekly water requirement for cotton at a given stage of development. This approach only accounts for growth stage-specific cotton water requirements and does not account for adjustments due to prevailing environmental parameters (i.e. temperature, wind speed, relative humidity).

Table 1.1 Typical checkbook schedule for cotton irrigation in Georgia

Growth Stage	cm per week
Pre-Bloom	2.54
1st Bloom (week 1)	2.54
2nd week after 1st bloom	3.81
3rd week after 1st bloom	5.08
4th week after 1st bloom	5.08
5th week after 1st bloom	3.81
6th week after 1st bloom	3.81
7th week after 1st bloom	2.54

Such simplified scheduling approaches have traditionally been effective in producing maximal yields; however, other factors can also influence crop water demands at a given stage of development. These include variety, soil physical characteristics, and atmospheric demand (vapor pressure deficit). Because the aforementioned factors can vary substantially from one farm to another or from one area of a field to another, a substantial amount of research has been aimed at identifying irrigation scheduling approaches that would allow producers to apply water only where it is needed and in the amounts needed at a given time.

To this end, other, environmental irrigation triggering methods such as remote sensing of soil moisture status offer 1) the promise of timely irrigation scheduling based on crop need and 2) the possibility of complete automation. However, these methods are also problematic due to factors such as heterogeneity in soil composition, accuracy of the method used for their calculation, and the need for site-specific calibration (Leib et al., 2003; Jones, 2007). These methods, while useful and easily applied, are limited due to their lack of information on the stress level of the crop in question.

Ideally, an irrigation trigger should provide information on soil water status, as well as a stress level to the plant itself. Leaf gas exchange (net photosynthesis and stomatal conductance of water vapor, $A_{N,}$ g_s, respectively) has been used to provide evidence of water stress (Ennahli and Earl, 2005). Response time for g_s can be very rapid to the onset of water stress; however, the need for excessive manpower limits its use (Jones, 2004). A_N is less sensitive to water stress (Hsiao, 1973; Jones, 2004). Additionally, by the time reductions in A_N are observed, negative impacts on yield may have already occurred. Often, techniques combining gas exchange and chlorophyll fluorometry are needed to detect differences between stress levels. For example, a study done by Ennahli and Earl (2005) on cotton failed to detect differences between four levels of relative soil water content (RSWC, 5, 15, 25, 75%). When only gas exchange comparisons were made, stress levels were grouped into two categories (5 and 15% versus 25 and 75% RSWC), despite dramatic differences in leaf water potential. When chlorophyll fluorometry was used concurrently with infrared gas analysis, differences were found among all treatment levels, suggesting the potential for use in irrigation scheduling. However, the need for expensive measurement devices and the expertise to operate the instruments and interpret the data is also likely to prevent methods such as gas exchange and chlorophyll fluorescence from being adopted as routine methods for scheduling irrigation.

The water status of a plant is the integration of all factors that determine if the plant needs irrigation, i.e.; soil characteristics, rainfall, environmental demands. Water status measures fall into two general categories: 1) water content and 2) water potential (Jones, 2007). Although measures of relative water content accurately characterize plant water status, they do not provide information on the effect of that water on plant function (Jones 2007). Leaf water potential (Ψ_l) has been correlated to productivity in many crops. However, the time chosen to measure plant water status complicates this approach, as plant water status is temporally variable. Fluctuations due to environmental factors, such as intermittent cloud cover (Jones, 1990; So, 1979) can make measurements of water status during the day unreliable. Water potentials (as measured with a pressure bomb) generally are at a maximum immediately before sunrise, after which they fall to a minimum around mid-day before steadily rising through the afternoon and leveling off to values similar to pre-sunrise values overnight (Jones, 1990). A study in cotton showed that the degree of water stress, as measured using midday (1200 to 1400 h) Ψ_l, affected fiber characteristics in a non-linear fashion. There was no effect of leaf water potential on fiber weight or length for values as low as -2.7 MPa. Ψ_l below this level produced dramatic decreases in fiber length and weight (Grimes and Yamada, 1982), and stem elongation was drastically reduced at Ψ_l at ~-2.4 MPa. Additionally, mid-day leaf water potential was shown to vary with soil type, strengthening the argument that plant-based measurements can be used to provide information on water deficit conditions. For example, soils with diminished water retention capabilities such as sandy loams produced plants with a greater rate of decrease in leaf water potentials over time after irrigation was ceased, when compared to soils with high clay contents (Grimes and Yamada, 1982).

Predawn water potential (Ψ_{PD}) has been considered the best available measurement of crop water status (Ameglio et al., 1999). Assuming no transpiration at night, water potential gradients between the soil and the plant disappear, and Ψ_{PD} can be considered a measure of the water potential of the soil in contact with the roots (Ameglio et al., 1999). However, recent research indicates that nighttime transpiration can occur, creating disequilibrium between the soil and plant (Caird, et al., 2007; Donovan, et al., 2001; Snyder, et al., 2003). Ψ_{PD} measurements have the advantage of being less sensitive to environmental fluctuations (i.e. cloud cover). Furthermore, Ψ_{PD} has been shown to correlate with relative evapotranspiration, and has been used to schedule irrigation in tree species (Ameglio et al., 1999). However, there is some evidence that under certain circumstances, Ψ_{PD} values may not fully equilibrate with soil water potential overnight, where Ψ_{PD} has been reported to be significantly lower than the wettest portion of the root zone (Jordan and Ritchie et al., 1971; Klepper et al., 1973). This may be due to Ψ_{PD} providing information on the water potential of the soil in direct contact with the entire root system, rather than in specific portions of the soil profile.

In the late 1970's, it was found that leaf temperature could provide information on plant water status (Ehrler et al. , 1978). This information was used to develop the stress degree day (SDD) as an indication of whether or not irrigation should be triggered under a given vapor pressure deficit (VPD) (Idso et al. , 1981). Briefly, if canopy temperature minus air temperature (T_C-T_A) was negative then the plant was well watered. This works well in arid environments, where VPDs are conducive to effective transpirational cooling; however, there is evidence that well watered crops in humid regions can frequently exhibit leaf temperatures well above ambient air temperature (Hall et al., 2014). The SDD method was empirically modified to account for variation due to regional effects to include a well-watered baseline (Idso, 1982). Advances in (recently reviewed by Maes and Steppe, 2012) estimating canopy temperature using infrared canopy sensors are promising in that calculation of total evapotranspiration, crop canopy temperature, and stomatal conductance of water vapor can be achieved despite leaf to leaf variability in temperature. Additionally, the relative affordability of new sensors increases the potential for implementation in production rather than pure research (Maes and Steppe, 2012). In cotton, the use of the biologically identified optimal temperature interactive console (BIOTIC; Upchurch et al., 1996) has been shown to be a powerful indication of the need for irrigation. Specifically, lint yield was shown to be negatively associated with total time above a predetermined critical canopy temperature (28°C) (Wanjura et al., 2006). In the same study, other estimates of water stress such as crop water stress index (CWSI, Jackson et al. 1981, Idso et al., 1981), and T_c-T_a, were shown to be less sensitive.

One caveat of using a plant-based water stress indication is that a stress level does not give information as to how much water needs to be applied to relieve the stress (Jones, 2004). This would suggest that any study attempting to use leaf water status as a trigger for irrigation scheduling would need references from multiple stress levels, as well as a well-watered baseline for determination of irrigation need (Idso, 1982). Additionally, due to narrow limits on the severity of water deficits that can be imposed on crops before losses in productivity are observed, manual monitoring methods, such as the determination of leaf water status via pressure bomb, while inexpensive in terms of equipment and easy to do, may be impractical due to the cost associated with hiring more manpower to continually monitor plant water status (Jones, 2004). Due to temporal limits such as using standardized measurement times or allocating manpower, resolution on crop stress can be lost (Jones, 2004). This implies that by the time stress is identified, negative effects on yield, and thus profit could already be incurred. For this reason, automated monitoring of factors such as leaf temperature (Jones, 1999; Saha et al. , 1986), stem diameter (Fereres and Goldhamer, 2003) or leaf turgor pressure (Zimmermann et al. , 2008) could be used as a proxy for plant water status, eliminating the need for excessive human resources. Irrespective of the method used to schedule irrigation; a reliable and rapid method of determining when (and where) water should be applied is vital to the success of irrigation management programs (Jones, 2004). Despite concerns listed above, plant-based irrigation triggers could be useful, especially when correlation of on-the-ground data with data from automated sensors is possible.

Growth and yield of cotton under water stress:

The factor affected most by water stress in agricultural crops is growth. Water deficit stress decreases cell turgor and reduces the strength of the mechanical force necessary for cell expansion (Hsiao, 1973). Plant water status in cotton has been shown to have a linear relationship between mid-day leaf water potential and main-stem elongation rate, with little to no elongation for leaf water potentials below -2.4 MPa (Grimes and Yamada, 1982). Additionally, drought has been shown to decrease stem height, stem dry weight, leaf area, leaf dry weight, and total number of main stem nodes in cotton (Pace et al. , 1999).

Root growth is also affected by water stress. In cotton, total taproot length was shown to increase by ~23% in response to imposition of water stress, suggesting an avoidance mechanism that would allow a stressed plant to reach deeper water resources (Grimes and Yamada, 1982). Additionally, drought has been shown to dramatically reduce root:shoot ratios in cotton (Pace et al. , 1999). Root:shoot ratios (when measured at first bloom) have been shown to be positively associated with boll abscission and negatively associated with lint yield in dryland cotton (Cook and El-Zik, 1992).

Decreases in lint yield under water deficit are primarily due to decreases in source strength due to limited leaf area and poor photosynthetic efficiency of individual leaves, diminished plant growth, decreased numbers of vegetative and sympodial nodes, and decreases in the total number of fruiting sites (Kreig and Sung, 1986; Turner et al. 1986). The effect water stress has on flowering was recently reviewed by Loka and Oosterhuis (2012). They noted that water deficit prior to flowering can result in abscission of very young squares (cotton floral buds); however, flowers are buffered from water stress and will not abscise. During the first two weeks after flowering, young cotton bolls are extremely sensitive to drought stress due to limited source strength, as mentioned above and changes in hormone balance and will typically abort if water deficit is experienced during this time (Reviewed in Loka et al., 2011, Loka and Oosterhuis, 2012).

Fiber length and thickness appear to respond to drought stress. Under optimal growing conditions, fiber development occurs in three stages: fiber initiation, fiber elongation, and fiber thickening. Fiber cell initiation occurs on the day of anthesis (Oosterhuis, 1991; Lee et al., 2007), the elongation phase occurs immediately following initiation and continues for 20-30 days past anthesis (Lee et al. 2007). Fiber thickening continues from 16 to 42 days past anthesis (Oosterhuis, 1991; Gokani and Thaker 2002). Fiber length and weight have been shown to be negatively affected by mid-day leaf water potentials below -2.8 MPa, with no affect above this value when compared to fully hydrated plants (Grimes and Yamada, 1982).

Stomatal conductance under water deficit:

Stomatal conductance to water vapor (g_s) is one of the first indicators of water stress in plants (Hsiao, 1973). For example, moderately drought stressed cotton leaves showed a more rapid decrease in g_s throughout the day, when compared to well watered leaves (Massacci et al. , 2008). Additionally, in *Rosa rubiginosa*, decreases in g_s were cited as the primary factor accounting for decreases in photosynthesis (Meyer and Genty, 1999). High g_s under heavily irrigated conditions has been linked to increased lint yield through selection for more thermotolerant cotton cultivars (Lu et al., 1994). This may be due to creation of favorable microclimates (leaf temperatures near 30°C) produced by excessive evaporative cooling (Jarman, 1959; Lu et al., 1994). Factors influencing g_s are diverse; however, air moisture content, air temperature, plant water status, and leaf age seem to play a major role. Vapor pressure deficit (VPD), or the difference between the amount of water vapor present in the air and the amount the air is capable of holding at a given temperature, has been shown to be negatively correlated with g_s; however, this seems to only be true for VPDs >2 kPa (Day, 2000). There is evidence stoma respond more to the rate of transpiration, rather than VPD itself; however, this relationship is not fully understood (Monteith, 1995). High air temperature tends to increase g_s. For example, cotton plants (in controlled environments) stressed to 38°C daytime temperatures exhibited g_s values significantly higher than those grown at 30°C (Snider et al. , 2009). Leaf water potential, as well as a balance of phytohormones (abscisic acid and possibly cytokinins) are known to regulate stomatal guard cell turgor and stomatal aperture. Interestingly, cotton stomates appear to stay open during substantial leaf water deficits (as low as -3.0 MPa) (Ackerson and Krieg, 1977) yet do not appear to lose significant amounts of water because of the presence of an internal cuticle that limits direct water loss through guard cell membranes (Wullschleger and Oosterhuis, 1989).

Davies et al. (1991) present an argument that the primary factor governing stomatal conductance is ABA that is synthesized in the roots in response to soil drying and is transported to the leaf via the transpiration stream (transported in the xylem). A general mechanism of ABA-induced stomatal closure is presented in Taiz and Zeiger (2010), where 1) ABA binds to receptors in guard cell plasma membranes, 2) ROS production is stimulated, causing an influx of Ca^{2+} across the plasma membrane, 3) the influx of Ca^{2+} stimulates the release of calcium from the vacuoles, 4) the rise in intracellular calcium blocks K^+_{in} channels and opens Cl^-_{out} channels, 5) Cl^- efflux, coupled with Ca-induced inhibition of proton pumps, depolarizes the plasma membrane, 6) K^+_{out} channels are activated and K+ is released across the membrane, 7) water moves out of the cell, Ψ_P decreases, and the stoma closes. Confirming the relationship between soil drying and stomatal closure, White and Raine (2009) reported that cotton grown in a split pot experiment, where one side of the root system was allowed to dry while the other side remained well-watered, showed a four-fold increase in xylem sap ABA concentration during partial root zone drying down to -2.36 MPa. However, stomatal conductance was not significantly different for plants receiving the soil drying treatment relative to plants for which the entire root system was well watered.

Maintenance of plant water status:

Water stress, in plants, occurs due to soil moisture deficits or when excessive amounts of water are lost due to evaporation. Avoidance of the negative effects associated with tissue dehydration is accomplished through either a reduction in water loss or an increase in water uptake (Chaves et al. , 2003). Reducing water loss is accomplished though decreased transpiration by reducing the number of stoma, or decreasing stomatal size/aperture. For example; two olive cultivars grown under drought conditions tended to respond by increasing stomatal, mesophyll, and dermal cell density (the result of decrease in cell size) as well as increasing cuticle thickness. Additionally, stoma were smaller and had decreased aperture size (Bosabalidis and Kofidis, 2002). Decreased cell size is due to reduced mechanical force needed for cell expansion from loss of turgor pressure (Hsiao, 1973).

Increased water uptake is accomplished by adjusting cell solute concentrations (primarily though accumulation of photosynthates) (Handa et al., 1983). This has the effect of lowering root cell water potential relative to that of the soil and allowing for water movement into the root, provided that soil-root contact is maintained. There is some evidence for root shrinkage due to desiccation resulting in low soil-root contact and a decreased potential for water uptake (Carminati et al., 2009). Solute accumulation coupled with decreased stomatal conductance can ameliorate negative effects of low soil water content, provided pressure potentials are not as low as to facilitate xylem cavitation (Tyree and Sperry, 1989).

Effect of water stress on photosynthesis:

Photosynthesis has been shown to be negatively affected by plant water status. There is some evidence that, at least in cotton, photosynthesis is not a sensitive measurement for determining moderate water stress because a number of other processes are negatively impacted prior to photosynthesis (cell expansion and stomatal conductance; Hsiao, 1973). On a whole plant basis, net assimilation is limited by water stress through decreases in light interception due to decreased total leaf area (Wells and Stewart, 2010); however, changes on an individual leaf basis are more complicated. Generally, as water stress increases, stomatal conductance and photosynthesis decrease with decreasing plant water status (Turner et al, 1986). Stomatal limitations (discussed below) appear to be the primary source of photosynthetic inhibition under moderate drought stress; however, metabolic impairment as a result of water stress has been observed.

Stomatal limitations—The effect water stress has on CO_2 availability is debated; however, most can agree that as water stress increases, g_s decreases, resulting in a decreased internal leaf CO_2 concentration (C_i) (gaseous CO_2 within the leaf) and consequently a decrease in CO_2 concentration at the carboxylation site (Cc) (CO_2 in the dissolved in the stroma) of RuBP carboxylase/oxygenase (Rubisco), thereby decreasing net photosynthesis (A_N) (Flexas et al. , 2004; Quick et al. , 2006). Traditionally, water stress was demonstrated by decreased slopes of A_N/C_i curves relative to well-watered plants, suggesting metabolic (non-stomatal) impairment of photosynthesis (discussed in Flexas et al., 2002; Ennahli and Earl, 2005). A major limitation to this approach is that, under drought stress, C_i is often overestimated for a number of reasons (Long and Bernacchi, 2003). For example, until recently, it was assumed that CO_2 diffusion into the leaf was limited and subject to the same resistances as the diffusion of water vapor (i.e. all diffusion was assumed to be through the stomata). Under drought stress, a substantial amount of water vapor diffuses through the cuticle, where resistance to CO_2 is substantially greater than for H_2O (Ennahli and Earl, 2005). Consequently, Ci would likely be overestimated, and could incorrectly implicate non-stomatal limitations to photosynthesis (Ennahli and Earl, 2005). Additionally, some authors have argued that patchy stomatal closure under water stress can result in overestimation of Ci (Ennahli and Earl, 2005); however, in cotton there is evidence (based on chlorophyll fluorescence imaging) that leaves respond fairly uniformly to the onset of water stress (Massacci et al., 2008). It is also important to note that even when Ci can be accurately determined, it may not be an accurate indicator of the CO_2 concentration in the chloroplasts (C_C). New evidence suggests a complex pathway for CO_2 diffusion to the carboxylation site, where increased mesophyll resistance to CO_2 diffusion under drought stress can greatly limit C_C (Flexas et al., 2002, 2012).

Due to the aforementioned issues, using gas exchange alone to indicate water stress can limit the ability to distinguish between stress levels. For example, a study by Ennhali and Earl (2005) on cotton showed that when relative soil water content (RSWC) was decreased (with treatment levels of 75, 25, 15 and 5% RSWC), differences between treatments were difficult to detect for stomatal conductance to water vapor (g_s) and leaf net CO_2 assimilation rate (A_N) despite significant differences in leaf water potential (Ψ_L). Using g_s and A_N alone (as measured by infrared gas analysis, IRGA), treatments were grouped into two categories; with mild stress (75% and 25% RSWC) being similar and extreme stress (15% and 5% RSWC) being similar; however, for mild stress, CO_2 concentration at the carboxylation site (C_C, measured by chlorophyll fluorometry and gas exchange combined) showed significant decreases even under mild stress (25% RSWC) relative to the control (75% RSWC). Carboxylation efficiency (the slope of A_N/C_C), however, was unchanged under mild stress. Differences between extreme stress treatments (15 and 5% RSWC) were not detected by gas exchange (g_s and A_N) measurements, but were detected as decreases in electron flux through photosystem II (J_e) (as measured by chlorophyll fluorometry) and as decreases in carboxylation efficiency (Slope of A_N/C_C), as RSWC decreased. Carboxylation efficiency and J_e were significantly lower in the severe stress treatments than in the control. The above indicates that photosynthesis is governed by C_C under mild drought stress, indicating diffusional (g_s and g_m) control over photosynthesis at this stress level. Conversely, decreased carboxylation efficiency in the severe stress treatments indicates metabolic (non-stomatal) impairment of photosynthesis under these conditions. Additionally, after rewatering, mild stress treatments returned to pre-stress A_N levels, whereas severe stress treatments were inhibited despite reaching pre-stress C_C levels, suggesting lasting metabolic impairment (non-stomatal inhibition of A_N).

Non-stomatal limitations—In addition to stomatal limitations mentioned above, there is evidence for non-diffusional limitations to photosynthesis under drought stress. Some researchers have provided evidence that g_m may not be a purely diffusional process. For example, a study of tobacco using gas analysis and chlorophyll fluorometry found that as temperatures increased in the leaf, g_m decreased, suggesting that g_m may be enzymatically regulated (Bernacchi et al. , 2002). Additionally, CO2? availability is limited by the activity of carbonic anhydrase, which is known to respond negatively to drought stress (Jones, 1973).

Substrate limitations are not exclusively related to CO_2 availability. For example, a study done on sunflower showed that RuBP concentration was positively correlated with Ψ_l while rubisco concentrations remained constant, suggesting negative effects on regeneration of RuBP in the carbon reactions (Tezara et al. , 1999).

Non-stomatal limitations are not limited to substrate availability. For example, a study done on tobacco found that rubisco activity was positively correlated to leaf relative water content (RWC). They found evidence that levels of tightly bound, reversible daytime rubisco inhibitors increased as leaf RWC decreased (Parry et al., 2002). A study on *Rosa rubiginosa* found that, while stomatal limitations were the major contributor to photosynthetic decline, a decrease in maximal electron-transport rates resulted in a significant reduction of RuBP carboxylase/oxygenase activity after leaves were allowed to recover from water stress, suggesting a lingering effect of drought exposure (Meyer and Genty, 1999). In a study done on sunflower large decreases in net photosynthesis, adenosine triphosphate (ATP) concentrations, and ATP synthase were observed as Ψ_L decreased (Tezara et al. , 1999). The authors suggested that decreased ATP production via photophosphorylation was the primary cause of reduced photosynthesis under water stress rather than a decrease in the carbon reactions (ATP would increase if the rate of the carbon reactions decreased) (Tezara et al. , 1999). Additionally, there is some evidence for increased ATP synthesis via photoreduction of O_2 (Mehler reaction) under times of high O_2 exposure (Makino et al. , 2002) (which can be expected following stomatal closure). This suggests a possible mechanism for maintenance of ATP levels for carbon fixation under water-stressed conditions.

The effect of drought stress on electron transport rate (ETR) seems to vary with species. For example, grape vines responded to severe drought with a reduction in ETR (Flexas et al. , 2002). In cotton, Massacci et al. (2008) found that moderate water stress led to a slight increase in ETR, relative to well watered leaves as C_i was artificially increased; however, this effect was not detected for low C_i treatments (below 100 µmol mol^{-1}) . In the same study, ETR was found to be slightly higher in drought stressed leaves, relative to well watered leaves at moderate PAR values (500-1500 PAR); however, ETR values were similar under high irradiance (2000 PAR). Additional reports with field-grown cotton have shown, similarly, that ETR was either increased or unaffected by drought stress conditions (Kitao and Lei, 2007; Zhang et al., 2011), suggesting insensitivity of this process to drought stress in cotton. However, Pettigrew (2004) reported a significant decline in Φ_{PSII} and ETR for dryland versus irrigated cotton under only moderate stress conditions, suggesting that the response of ETR to water deficit in cotton needs to be examined further.

Effect of water stress on Photorespiration:

Photorespiration (R_l), or the oxygenation reaction of Rubisco, results in decreased carbon assimilation rates under times of limited CO_2, such as after stomatal closure, times of high light exposure, or thermal stress. The complex, multi-organelle pathway needed for conversion of glycolate to 3-phosphoglycerate results in increased metabolic cost, relative to direct carboxylation or ribulose bis-phosphate. Flexas et al. (1999) reported a significant decrease in photorespiration for grapevines exposed to severe water deficit conditions along with a concurrent decline in steady-state fluorescence yield and photosynthetic electron transport (ETR). Similar declines in photorespiration and primary photochemistry have also been reported for soybean

(Zhang et al., 2011). In contrast, photosynthetic parameters in *Quercus* sp. appear to be completely insensitive to drought stress conditions (i.e. no decline in A_N or change in R_l even under Ψ_l = -3.0; Valentini et al., 1995). Some conflicting results on the effects of drought stress on R_l exist for cotton. For example, early work by Perry et al. (1983) showed that R_l, expressed per unit leaf area, decreased under dryland conditions, but increased as a proportion of net photosynthesis. Zhang et al. (2011) also reported significant decreases in R_l under field conditions although ETR increased significantly under drought stress. In contrast, a study done on cotton showed elevated R_l for moderately drought stressed, relative to fully hydrated leaves. They showed that, for both water stressed and well watered leaves, R_l increased as C_i decreased to a point (around 50 μmol $m^{-2}s^{-1}$), after which it drastically decreased (Massacci et al. , 2008). In the same study, high light intensity was also shown to increase R_l in drought stressed leaves. The authors suggest that, at least in cotton, elevation of ETR and R_l are used as a protective measure during drought stress to prevent oxygen radical formation and reduce oxidative damage.

Effect of water stress on dark respiration:

The effect water stress has on dark respiration (R_D) was recently reviewed and found to either increase or decrease depending on the severity of water stress, plant organ, and the species being investigated (Loka et al., 2011); however, there is a general trend for a decrease in R_D for moderate water stress followed by an increase under severe water stress (Atkin and Macherel, 2009; Flexas et al., 2005). Information regarding the effect water deficit has on cotton is limited. In greenhouse grown cotton leaves, R_D tended to initially decrease after imposition of drought stress before increasing 5-10 days after irrigation was ceased (Pallas et al. , 1967). Other studies on field-grown cotton have shown either no significant effect of moderate drought stress on R_D (Massacci et al. , 2008) or a significant decrease in R_D (Zhang et al., 2011). This effect varies with plant organ in cotton. For example, cotton bolls showed no change in R_D due to moderate water stress; however, severe water stress caused a decrease in R_D (Wullschleger and Oosterhuis, 1990).

Reactive Oxygen Species:

A common, negative response of plants to water-deficit and high temperature stress, which often occur concurrently, is enhanced production of reactive oxygen species (ROS; Suzuki and Mittler, 2006; Miller et al., 2010). ROS include singlet excited oxygen ($^1O_2$), the superoxide ion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (HO_*), all of which are capable of damaging every organic component of a living cell (nucleic acids, proteins, cell membranes, etc. Iba, 2002; Agarwal et al., 2005). Under drought stress, limited CO_2 availability due to stomatal closure coupled with a continual input of photosynthetically active radiation results in increased electron flow to O_2, stimulating ROS production by PSII and PSI (Mehler Reaction) in the chloroplasts (Asada, 2006; Miller et al., 2010). Increased photorespiration rates under drought stress result in enhanced glycolate oxidation in the peroxisomes, producing elevated H_2O_2 levels (Miller et al., 2010). Additionally, mitochondrial ROS production has been shown to increase under drought stress conditions, contributing to increased cellular ROS production (Miller et al., 2010).

Optimal growth temperature for cotton:

Upland cotton (*Gossypium hirsutum*) is a perennial shrub, native to arid, hot regions of Mesoamerica (Wendel et al. , 2010). Contrary to its evolutionary history; hot, arid environments are not optimal for cotton growth and fiber production. For example, high growth temperatures during flowering have been show to negatively affect lint yield by limiting key reproductive processes, including square retention, gametophyte development (pollen and embryo sac), pollen germination, pollen tube growth, fertilization, seed production, boll size, and boll number (Hodges et al., 1992; Oosterhuis, 1999; Snider et al. , 2009; Snider et al., 2011; Snider and Oosterhuis, 2011). The optimal growth temperature regime for cotton is a 30/20°C day/night temperature regime (Reddy et al. , 1991) which is cooler than temperatures observed during the growing season for many cotton producing regions in the United States. Recent attempts at determining optimal temperatures for use as biologically relevant irrigation triggers have indicated thermal optima of 28 ± 2°C for field grown cotton (Conaty et al., 2012). As noted above, high air temperatures during flowering decrease yields for field grown cotton (Lu et al. , 1997; Oosterhuis, 1991). Under drought stress, reduced stomatal conductance coupled with a continual input of solar radiation can also be expected to cause high leaf temperatures. Additionally, high relative humidity is well-known to limit transpirational cooling (Gates, 1968). Consequently, dryland *G. hirsutum* cultivars grown in humid regions of the southeast would likely experience both drought and heat stress at various times during the growing season. Because drought stress and high temperature stress often occur concurrently, it is essential to characterize the thermal environment in studies dealing with crop water status.

Chlorophyll Fluorescence:

Chlorophyll fluorescence (CF) has promise in characterizing crop responses to drought and possibly functioning as a drought tolerance screening tool. CF methodologies are rapid, and provide a measure of the efficiency of primary events in photosynthesis. Consequently, CF methods have been widely used to characterize abiotic stress responses in crop plants. For example, the ratio of variable fluorescence to minimum fluorescence in dark adapted leaves (Fv/Fo) has been use as a measure of photosynthetic temperature tolerance in a number of species (Burke, 1990). Similarly, maximum quantum efficiency of PSII (Fv/Fm), ETR, Φ_{PSII}, steady state fluorescence (Fs), non-photochemical quenching (NPQ), etc. have all been used to quantify plant responses to abiotic stress (Valentinini et al., 1995; Flexas et al., 1999; Woo et al., 2008; Snider et al., 2009, 2010; Zhang et al., 2011). For example, Massacci et al. (2008) showed increases in actual quantum yield (Φ_{PSII}) in cotton at low light intensities (<1000PAR) for moderately drought stressed leaves, relative to well watered leaves (the author uses to explain increases in photorespiration, relative to net photosynthesis). Similar results were found for the efficiency of open PSII reaction centers (Fv'/F_m'). At moderate to high light intensities (>1000PAR), significant increases in non-photochemical quenching (NPQ, an indicator of heat dissipation) and the quantum efficiency of dissipation by down-regulation (Φ_{NPQ}) were seen in drought stressed leaves, relative to well watered leaves. Increases in NPQ have been linked to pH changes across thylakoid (Makino et al. , 2002). The quantum efficiency of other non-photochemical losses were (Φ_{NO}) found to be higher in well watered leaves under all light intensities (0-2000PAR). As discussed previously, ΦPSII and ETR may not be sensitive to water deficit stress under field conditions (Kitao and Lei, 2007; Massacci et al. 2008; Zhang et al., 2011), so the success of using chlorophyll fluorescence-based parameters to screen for drought tolerance in cotton will likely hinge on the ability to identify methods with the ability to accurately detect the onset of drought stress. Recent methods that provide a kinetic description of the fluorescence trace from Fo to Fm, allow for calculation of traditional fluorescence parameters (Fv/Fm, Φ_{PSII}). Moreover, the fluorescence trace is broken down into the O step (F_o), the J step (occurring at 2 ms), the I step (occurring at 30 ms), and the P step (Fm, regardless of time; Strasser et al., 2000). Using all the information obtained in the fluorescence trace, a number of parameters can be calculated, including the performance index, which is arguably more sensitive than traditional fluorescence parameters to abiotic stress (Strasser et al., 2000). A comparison of these methods along with simultaneous measurement of plant water status, A_N, and lint yield should improve fluorescence-based drought tolerance screening protocols in cotton.

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CHAPTER 2

WATER DEFICIT IN FIELD-GROWN *GOSSYPIUM HIRSUTUM* PRIMARILY LIMITS NET PHOTOSYNTHESIS BY DECREASING STOMATAL CONDUCTANCE, INCREASING PHOTORESPIRATION, AND INCREASING THE RATIO OF DARK RESPIRATION TO GROSS PHOTOSYNTHESIS[1].

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Abstract

Much effort has been expended to improve irrigation efficiency and drought tolerance of agronomic crops; however, a clear understanding of the physiological mechanisms that interact to decrease source strength and drive yield loss has not been attained. To elucidate the underlying mechanisms contributing to inhibition of net carbon assimilation under drought stress, three cultivars of cotton (*Gossypium hirsutum*) were grown in the field under contrasting irrigation regimes during the 2012 and 2013 growing season near Camilla, Georgia, USA. Physiological measurements were conducted on three sample dates during each growing season (providing a broad range of plant water status) and included, predawn and midday leaf water potential (Ψ_{PD} and Ψ_{MD}), gross and net photosynthesis, dark respiration, photorespiration, and chlorophyll *a* fluorescence. End-of-season lint yield was also determined. Ψ_{PD} ranged from -0.31 to -0.95 MPa, and Ψ_{MD} ranged from -1.02 to -2.67 MPa, depending upon irrigation regime and sample date. *G. hirsutum* responded to water deficit by decreasing stomatal conductance, increasing photorespiration, and increasing the ratio of dark respiration to gross photosynthesis, thereby limiting P_N and decreasing lint yield (lint yield declines observed during the 2012 growing season only). Conversely, even extreme water deficit, causing a 54% decline in P_N, did not negatively affect actual quantum yield, maximum quantum yield, or photosynthetic electron transport. It is concluded that P_N is primarily limited in drought-stressed *G. hirsutum* by decreased stomatal conductance, along with increases in respiratory and photorespiratory carbon losses, not inhibition or down-regulation of electron transport through photosystem II. It is further concluded that Ψ_{PD} is a reliable indicator of drought stress and the need for irrigation in field-grown cotton.

Keywords: chlorophyll fluorescence, drought stress, *Gossypium hirsutum*, leaf water potential, photorespiration, photosynthesis, respiration

Abbreviations: C_c, CO_2 concentration at the carboxylation site; C_i, internal leaf CO_2 concentration; E, leaf transpiration rate; ETR, electron transport rate through photosystem II; F’, steady state fluorescence of illuminated leaves; F_o, minimum fluorescence of dark adapted leaves; F_m, maximum fluorescence of dark-adapted leaves; Fm’, maximum fluorescence of illuminated leaves; F_v/F_m, maximum quantum yield of photosystem II; g_s, stomatal conductance to water vapor; PAR, photosynthetically active radiation; P_G, gross photosynthesis; P_N, net photosynthesis; PSII, photosystem II; R_D, dark respiration; R_l, photorespiration; R_n, single-leaf nocturnal respiration rate; T_L, leaf temperature; T_A, air temperature; Ψ_l, leaf water potential; Ψ_{MD}, midday leaf water potential; Ψ_{PD}, predawn leaf water potential; and Φ_{PSII}, actual quantum yield of electron transport through PSII.

Introduction

Climactic uncertainty, concerns about negative environmental impacts of excessive irrigation (Micklin, 1988; Beman et al., 2005), competition between agriculture and municipalities for water resources (Wilhite et al., 2000), and the economics associated with bringing irrigation water from source to field has given impetus to research on improving irrigation efficiency. Despite extensive research on crop response to water deficit, a clear understanding of how underlying physiological mechanisms interact to decrease source strength and drive yield loss has not been attained. Elucidation of these factors is necessary to provide a selection source for breeders to improve drought adaptation strategies (Loka et al., 2011), and to improve water use efficiency under water-limited conditions.

For cotton, decreases in lint yield under water deficit are due to factors such as decreases in total source strength due to diminished leaf area for light interception and decreased average photosynthetic efficiency (Kreig and Sung 1986; Pettigrew, 2004; Wells and Stewart, 2010), diminished plant growth, decreased numbers of vegetative and sympodial nodes, as well as sink limitations such as decreases in the total number of fruiting sites (Turner et al. 1986). At the individual leaf level, the mechanisms by which water deficit inhibits photosynthesis are heavily debated (reviewed in Loka et al., 2011); however, there is strong evidence for a stomatal limitation under mild to moderate drought stress for most species (reviewed by Cornic and Fresneau, 2002). Generally, as drought stress increases, stomatal conductance to water vapor (g_s) and net photosynthesis (P_N) decline (Hsiao, 1973; Turner et al, 1986; Ennahli and Earl, 2005; Zhang et al., 2011). That is to say that as water deficit increases, g_s decreases, resulting in a low internal leaf CO_2 concentration (C_i) and consequently, a decrease in CO_2 concentration at the carboxylation site (C_C) of ribulose-1, 5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco), thereby decreasing net photosynthesis (P_N) (Flexas et al., 2004; Ennahli and Earl, 2005; Quick et al., 2006). Other, non-stomatal limitations such as regeneration of RuBP (Tezara et al., 1999), Rubisco inhibition (Meyer and Genty 1999; Parry et al., 2002;

Carmo-Silva et al., 2012), and decreases in adenosine triphosphate (ATP) and ATP synthase concentration (Tezara et al., 1999) have also been reported. The effect drought stress has on photosystem II (PSII) is also debated. Interestingly, the rate of electron transport through PSII (ETR) and actual quantum yield of electron transport through PSII (Φ_{PSII}) are not negatively impacted by water deficit stress in many studies with field-grown cotton (Kitao and Lei, 2007; Massacci et al., 2008; Zhang et al., 2011; Snider et al. 2013, 2014;) although decreased ETR (Pettigrew, 2004; Ennahli and Earl, 2005; Carmo-Silva et al., 2012) under moderate water deficit has been observed for greenhouse and field-grown cotton.

Mitochondrial respiration (R_D) has been found to either increase or decrease under water deficit depending on the severity of stress, plant organ, and the species being investigated (Loka et al., 2011). Flexas et al. (2005) proposed a biphasic response to drought stress, where growth and maintenance respiration components respond differently as the stress severity increases. Specifically, the growth R_D component decreases as mild drought stress develops, due to decreases in growth and the concomitant downregulation of metabolism. Under more severe stress, R_D has been shown to increase. The authors attribute this effect to enhanced senescence, solute accumulation, and energy dissipation by the mitochondria to prevent oxidative damage by excess reductants from the light reactions. Similarly, Pallas et al. (1967) observed a biphasic response of R_D to drought progression in cotton, whereas other investigators demonstrated a general decrease in R_D under water deficit (Massacci et al., 2008; Zhang et al., 2011). In an extensive review on drought stress effects on respiration, Atkin and Macherel (2009) suggested that the ratio of R_D to gross photosynthesis (P_G) increases under drought stress in nearly all instances due to the differences in magnitude between the two processes. Information on the response of R_D/P_G to drought stress is lacking for field-grown cotton. Additionally, the oxygenation reaction of Rubisco and associated photorespiration (R_l) have been shown to increase (Cornic and Fresneau, 2002; Ennahli and Earl, 2005; Massacci et al., 2008) under water deficit although some studies have indicated that R_l can decrease (Perry et al., 1983; Zhang et al., 2011) or remain stable in response to drought, and that the effect varies with cultivar (Perry et al., 1983).

The aforementioned studies on physiological responses of cotton to drought stress have varied widely in soil type and growth environment (field versus greenhouse) in addition to timing, duration, and severity of stress. Consequently, a standard, accurate measurement of plant water status is needed to characterize physiological responses to water deficit (Jones, 2007). Although the most appropriate measure of plant water status is debated, leaf water potential (Ψ_l) has been one of the most heavily utilized plant water status parameters in drought stress research (Jones, 2007). Additionally, Ψ_l relationships with? evapotranspiration (Ameglio et al., 1999), growth (Grimes and Yamada, 1982; Pace et al. , 1999), ETR (Flexas et al., 1999), net photosynthesis (Pettigrew, 2004; Snider et al., 2014), and cotton fiber length and thickening (Grimes and Yamada, 1982) have been reported. Predawn leaf water potential (Ψ_{PD}) has been considered the best available measurement of crop water status (Ameglio et al., 1999). Assuming no transpiration at night, water potential gradients between the soil and plant disappear, and Ψ_{PD} can be considered a measure of the water potential of the soil in contact with the roots (Ritchie and Hinckley, 1975; Ameglio et al., 1999). However, there is some evidence that plants transpire at night (Snyder et al., 2003; Caird et al., 2007) and do not fully equilibrate with soil water potential by sunrise (Donovan et al, 2001). Despite this, Ψ_{PD} represents maximum leaf water potential, and low Ψ_{PD} levels are known to inhibit P_N and g_s (Snider et al., 2014), boll and leaf retention (McMichael et al., 1973), and leaf and stem expansion (Jordan et al., 1970). Despite the plethora of information available regarding drought stress effects on net photosynthesis, many well respected studies concerning drought stress have been conducted in controlled environments atypical of field conditions (Ennahli and Earl, 2005; Meyer and Genty 1999; Parry et al., 2002; Tezara et al., 1999), have not accounted for seasonal or year to year variation in photosynthetic response to drought (Carmo-Silva et al., 2012; Kitao and Lei, 2007; Massacci et al., 2008; Zhang et al., 2011), have not accounted for carbon loss mechanisms under drought stress, such as R_D and R_l (Carmo-Silva et al., 2012; Kitao and Lei, 2007; Snider et al., 2013, 2014; Turner et al., 1986), or were conducted on species other than *G. hirsutum* (Cornic and Fresneau, 2002; Medrano et al., 2002; Meyer and Genty, 1999; Parry et al., 2002; Tezara et al., 1999). In our current study, we sought to bridge this knowledge gap by comprehensively investigating the effect diminished plant water status has on leaf gas exchange and metabolism over two growing seasons under typical cotton production conditions. Consequently, the objectives of our current study are to 1) quantify changes in Ψ_l (predawn and midday) for field-grown cotton under contrasting irrigation regimes and 2) to determine the effect of plant water status on underlying processes affecting P_N. We hypothesize that decreased Ψ_l will primarily limit P_N, by decreasing g_s, increasing R_l, and increasing the ratio of R_D to P_G without negatively impacting primary photochemistry.

Materials and Methods

Plant Material and Study Site

To address cotton's physiological response to drought stress, experiments were conducted at C.M. Stripling Irrigation Research Park near Camilla, Georgia (31°16'55.5"N, 84°17'39.9"W) in 2012 and 2013. Seeds of three commercially-available *Gossypium hirsutum* cultivars [PHY499 WRF (PhytoGen, Dow AgroSciences, Indianapolis IN), DP 0912 B2RF, and DP 1050 B2RF (Delta and Pine Land, Monsanto Company, St Louis MO)] were sown on May 5, 2012 and May 6, 2013 (Camilla, GA) at a 0.91m inter-row spacing and at a rate of 11 seeds m^{-1} row. Plots for each cultivar (n = 4 for 2012 and n = 3 for 2013) were two rows wide, 12.2 m long, and had 3 m bare-soil alleys. Soil type was classified as Lucy loamy sand (loamy, kaolinitic, thermic Arenic Kandiudults). Fertilization and pest management practices were conducted according to University of Georgia Cooperative Extension Service recommendations (Collins et al., 2012). Climatic data were provided by the Georgia Automated Environmental Monitoring Network (www.georgiaweather.net) weather station located at C.M. Stripling Irrigation Research Park, near Camilla, GA.

Irrigation Treatments

All cultivars were grown under both dryland and well-watered conditions to generate variation in leaf water supply at different times during the growing season.

Dryland plots are defined as those plots only receiving water via rainfall during the growing season, and well-watered plots received supplemental irrigation to meet weekly water requirements for cotton as defined using University of Georgia Cooperative Extension Service ‘Checkbook’ recommendations (Collins et al., 2013, Fig. 2.1). Using this approach, a growth stage-specific, weekly crop water requirement is met by subtracting rainfall from the weekly amount and using irrigation to supply the balance. Irrigations were applied three times per week. A variable-rate, linear-move, overhead, sprinkler irrigation system supplied each plot with the specified amount of water at each application time (Valley Irrigation; Valley, NE). In this study, we employed a split-plot, randomized complete block design with 4 and 3 replicate blocks for 2012 and 2013, respectively. Irrigation treatments (the main-plot) were randomized within each block. Individual cultivars (the split-plot) were randomized within each irrigation main-plot. In each plot, physiological measurements were conducted on fully expanded main-stem leaves at the fourth main-stem node below the shoot terminal. Measurements were taken on three sample dates during each growing season: July 9, July 19, July 23, for 2012 and June 26, July 9, and July 18 for 2013. These sample dates provided a broad range of crop water status.

Leaf Water Potential

Leaf water potentials (Ψ_l) were measured pre-dawn (Ψ_{PD}) and midday (Ψ_{MD}). All pre-dawn measurements were conducted between 0400 and 0600 h, whereas all midday measurements were conducted between 1200-1400 h. For all Ψ_l

measurements, leaves were excised from the plant and the leaf petiole was immediately sealed in a compression gasket with the cut surface of the petiole exposed. The leaf blade was then sealed in a pressure chamber (Model 615; PMS Instruments, Albany, OR) and the chamber was pressurized using compressed nitrogen at a rate of 0.1 MPa s^{-1} until water first appeared at the cut surface of the petiole. The total elapsed time from when the leaf was cut from the plant to the initial pressurization of the chamber was 5-10 s.

Chlorophyll fluorescence, leaf temperature, and gas exchange

Maximum quantum yield of photosystem II (F_v/F_m) was determined by obtaining fluorescence transients from the uppermost fully expanded leaves with a portable, pulse amplitude modulated fluorometer (Model OS5p, Opti-Sciences, Hudson, NH). Ground state fluorescence intensity (F_o) was measured *in situ* during predawn hours (0500 to 0600 h) using a modulation light intensity of approximately 1 μmol m^{-2} s^{-1} followed by maximal fluorescence intensity (F_m) determinations using an excitation light intensity of approximately 15 000 μmol m^{-2} s^{-1} for 0.8 s. F_v/F_m was calculated according to Maxwell and Johnson (2000) [$F_v/F_m = (F_m-F_0)/F_m$]. Actual quantum yield of electron transport through photosystem II (Φ_{PSII}) was measured *in-situ* using the OS5p Modulated Fluorometer (Opti-Science, Tyngsboro, MA) using the fourth main-stem leaf below the apical meristem at midday (1200 to 1400 h). During each reading leaves were positioned so that angle of incidence of solar radiation was perpendicular to the adaxial leaf surface. *F'* was measured under field irradiance and a modulated red light intensity of ~1μmol m^{-2} s^{-1}. Subsequently, the adaxial leaf surface was illuminated with a stepwise exposure to three light intensities (2850, 5700, and 8550 µmol m^{-2} s^{-1}) for a combined duration of 0.95 s, to allow for estimation of Fm' when all reaction centers are closed (at saturating light intensity) (Earl and Ennahli, 2004). Φ_{PSII} was calculated according to the equations given in Maxwell and Johnson (2000). Electron transport rate through photosystem II (ETR) was calculated for each leaf by multiplying ΦPSII × PAR (at the leaf surface) × 0.5 (excitation energy is divided between two photosystems) × 0.84 (a common leaf absorbance coefficient for C_3 plants) (Flexas et al., 1999). PAR values at the adaxial leaf surface were obtained using a gallium arsenide, cosine corrected PAR sensor, factory calibrated (by Opti-Science, Tyngsboro, MA) to a Licor-L-190 quantum sensor (Li-Cor, Lincoln, NE) positioned on the fluorometer leaf clip, immediately adjacent to the portion of the leaf surface being measured.

Pre-dawn, single-leaf respiration (R_n) was determined using an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE), where all leaves were measured before sunrise (PAR = 0 µmol m^{-2} s^{-1}) at a reference CO_2 concentration of 380 p.p.m. and a flow rate of 500 mmol m^{-2} s^{-1}. Steady-state respiration rates were obtained approximately 120 s after the leaf was enclosed in the leaf chamber. Because solar radiation-induced increases in leaf temperature would not be expected during predawn hours, block temperature was not controlled, and leaf temperature was measured using a type K thermocouple in contact with the abaxial surface of each leaf prior to conducting respiration measurements. Midday dark respiration rates (R_D) were estimated using the equations of Valentini et al. (1995). Midday leaf temperatures were obtained using a type K thermocouple in contact with the abaxial leaf surface immediately prior to gas exchange measurements. Midday air temperatures were obtained using a type K thermocouple placed in a shaded portion of the canopy.

Midday (1200 to 1400 h), single-leaf gas exchange measurements were performed immediately following midday chlorophyll fluorescence measurements within the same plot and on leaves at the same developmental stage as those sampled for fluorescence analysis using an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE). Midday gas exchange and chlorophyll fluorescence measurements (described above), were conducted under natural irradiance when PAR ≥ 1200 μmol m^{-2} s^{-1} which is above the light saturation point for net photosynthesis in cotton (Constable and Rawson, 1980; Krizek, 1986; Ehleringer and Hammond, 1987; Constable and Oosterhuis, 2010; Wells, 2010). Furthermore, no significant differences in PAR levels were observed between irrigation treatments on any sample date (data not shown). All leaves were measured under chamber CO_2 concentration of 380 p.p.m. and a flow rate of 500 mmol m^{-2} s^{-1}. Block temperature was maintained at ambient air temperature (as measured by type K thermocouple in a shaded location immediately adjacent to the leaves being measured). Gas exchange parameters measured were transpiration (E), net photosynthesis (P_N), stomatal conductance to water vapor (g_s) and leaf internal CO_2 concentration (C_i, in 2013 only). Gross photosynthesis (P_G) was estimated as the sum of P_N and R_D. The proportion of P_G lost to R_D was calculated as R_D/P_G. Photorespiration (R_l) was estimated according the method described in Valentini et al. (1995) using the relationship $R_l=1/12[ETR-4(P_N + R_D)]$.

Lint yield estimate

To estimate lint yield, seedcotton was harvested with a two-row spindle cotton picker (September 24, 2012; October 10, 2013) from two, 14 m (28 m total) rows of each plot after defoliation. Seedcotton was then ginned at the University of Georgia Micro Gin (Tifton, GA). Lint yields were the extrapolated to kg ha^{-1}, based on a 0.91 m inter-row spacing.

Statistical analysis

Data were analyzed by JMP Pro 10.0 (SAS, Cary, NC). All parameters were analyzed by three-way mixed effects ANOVA using standard least squares, restricted maximum likelihood, according to a split-plot, randomized complete block design. Blocks were treated as a random effect. Fixed effects were: 1) cultivar 2) irrigation treatment, and 3) sample date. Post-hoc differences were determined using Fisher's Least Significant Difference (alpha = 0.05). For all parameters, no significant cultivar effect or cultivar interactions with other main effects were observed ($P>0.05$). Consequently, data from all cultivars were pooled within a given irrigation treatment and sample date for graphical representation.

Results

Climactic conditions and irrigation

Daily maximum and minimum air temperatures as well as precipitation events for the 2012 and 2013 growing seasons are presented in Figure 2.1 Season total rainfall amounts for 2012 and 2013 were 43.17 and 72.19 cm, respectively (equivalent to dryland total water). For irrigated treatments, total seasonal water was 61.72 and 95.07 cm for 2012 and 2013, respectively (Fig. 2.2). Checkbook target, rainfall, irrigation, and total water for irrigation treatments for each growth week are illustrated in Figure 2.2. For 2012 sample dates, days since rainfall were 3, 2, and 1(1.12, 0.23, 1.52 cm, respectively), for July 9, 19, and 23, respectively. For 2013 sample dates, days since rainfall were 1, 2, and 1 (0.03, 3.2, 0.03 cm, respectively), for June 26, July 9, 18, respectively. Average daytime maximum temperature during the pre-sample rainless periods noted previously were 34.6, 33.6, 34.2 °C for July 9, 19, and 23, 2012, respectively and 33.6, 32.6, 32.6 °C, for June 26, July 9, 18, 2013, respectively. Irrigation was terminated 11 and 12 weeks after planting for 2012 and 2013, respectively.

Leaf water potential

Both measures of leaf water status (Ψ_{PD} or Ψ_{MD}) were found to have a significant sample date by irrigation treatment interaction ($P < 0.001$ for Ψ_{PD} and Ψ_{MD}). Specifically, Ψ_{PD} was lower for dryland plants, relative to plants grown under the 100% checkbook regime on July 19 (40% lower) and 23 (23% lower), 2012 and June 26 (23% lower), 2013 (Fig. 2.3 A, B). The lowest Ψ_{PD} observed was -0.95 MPa on June19, 2012 in dryland compared to -0.58 MPa in irrigated treatments. Values ranged from -0.95 to -0.31 MPa in dryland and between -0.76 and -0.35 MPa for irrigated treatments. Measurements of Ψ_{MD} were found to be lower for dryland treatments, relative to irrigated treatments on July 9, 19 and 23, 2012 (39, 49, and 44% lower, respectively) and June 26, 2013 (42%; Fig. 2.3 C, D). The lowest Ψ_{MD}

observed was -2.67 MPa on July 19, 2012 in dryland compared to -1.37 MPa in irrigated treatments. Values ranged from -2.67 to -1.01 MPa in dryland and between -1.37 and -1.02 MPa for irrigated treatments.

Single leaf gas exchange

For P_N, P_G, R_D, E, gs, WUE, and R_l, a significant treatment by date interaction was observed (P<0.001 in all cases) Dryland P_N was found to be significantly lower than irrigated on July 19 and 23, 2012 (54 and 14% lower, respectively) and June 26, 2013 (29 % lower; Fig. 2.4 A, B). Large differences in P_N were observed between irrigation treatments on July 19, 2012 (15.3 to 33.2 µmol m^{-2} s^{-1} for dryland and irrigated, respectively). Dryland P_G was found to be significantly lower than irrigated on July 19 and 23, for 2012 (46 and 16%, respectively) and June 26, 2013 (18 %; Fig. 2.4 C, D). A significant date by treatment interaction was observed for C_i (P=0.003). Internal leaf CO_2 was found to be lower in dryland, relative to irrigated cotton on June 26 and July 18, 2013 (Table 1.2; this parameter was not measured in 2012). Measurements of R_D were lower in dryland, relative to irrigated leaves for July 19 and 23, 2012; however, no treatment effect was observed for 2013 (Fig. 2.4 E, F). When respiration was expressed as a ratio of R_D/P_G, a significant treatment by date interaction was observed (P=0.006). Specifically, R_D/P_G was significantly higher for dryland, relative to irrigated cotton on July 19, 2012, and June 26, 2013 (38, 40% higher, respectively; Fig 2.5 A, B). Dryland leaves also exhibited lower E and g_s, relative to irrigated leaves on July 19, 2012, and June 26, 2013 (42 and 39% lower E, and 72 and 58% lower g_s, respectively; Fig. 2.6 A, B, C, D). Water use efficiency was significantly lower for dryland leaves on July 19, 2012 and higher for dryland on June 26, 2012; however, in both 2012 and 2013, WUE declined as the season progressed (Fig. 2.6 E, F). Photorespiration was shown to increase in dryland leaves, relative to irrigated leaves on July 19 and 23, 2012 (34 and 19% higher; Fig.2.7).

Chlorophyll fluorescence

There were significant responses to both irrigation treatment (P=0.002) and date (P<0.001) for F_v/F_m, Φ_{PSII}, and ETR. As the season progressed, significant declines were observed in Φ_{PSII} and ETR for both irrigated and dryland treatments, whereas F_v/F_m slightly increased (2012, both irrigated and dryland) or remained stable (2012, both irrigated and dryland, Table 1.1). Equipment failure prevented midday fluorescence measurement in 2013. For 2012, significant differences due to irrigation treatment were observed for F_v/F_m on July 19 and 23; however, these differences were marginal (1% increase in dryland, relative to irrigated), and neither Φ_{PSII} nor ETR were significantly affected by irrigation treatment in 2012. No differences due to irrigation treatment were observed for F_v/F_m, in 2013.

Leaf Microclimate and Temperature

Ambient air temperatures (Fig. 2.8 A, B) were similar across each date and irrigation treatment, for both the 2012 and 2013 growing season. Leaf temperatures (Fig 2.8 C, D) were somewhat higher for dryland, relative to irrigated treatments on July 19, 2012 (34.2 and 33.2 °C, respectively) and on June 26, 2013 (32.6 and 29 °C, respectively). Leaf – air temperature differences (Fig. 2.8 E, F) were all positive and similar across treatments and dates for 2012; however, in 2013 dryland cotton T_L-T_A values were significantly higher than irrigated treatments on June 26, 2013 (Fig. 2.8 F). All other T_L-T_A values were negative and no differences due to irrigation treatment were observed.

Lint yield

A significant treatment by year interaction was observed ($P=0.025$). Dryland treatments produced lower lint yields, relative to irrigated cotton in 2012 (976 to 1490 kg ha^{-1}, respectively; a 35% decline; Fig. 2.9); however, no treatment effect was observed in 2013.

Discussion

The current study supports the hypotheses that water deficit will primarily limit P_N, by decreasing g_s, increasing R_l, and increasing the ratio of R_D to P_G, as opposed to photosynthetic downregulation or inhibition upstream of the carbon reactions. We reinforce the observations of Tardieu and Simonneau (1998) that Ψ_L in cotton exhibits an anisohydric response to water availability. Specifically, Ψ_{PD} and Ψ_{MD} were found to decrease in dryland, relative to irrigated treatments (Fig. 2.3) when rainfall was insufficient to reach checkbook target amounts (Fig. 2.2). Furthermore, the Ψ_{MD} values causing significant declines in P_N for dryland relative to irrigated plants in the current study during the 2012 growing season (-1.95 to -2.67 MPa) were comparable to those reported to limit net carbon assimilation in previous reports with field and greenhouse-grown cotton (-2.0 to -3.1MPa, Ennahli and Earl, 2005; -2.0 to -3.6 MPa, McMichael and Hesketh, 1982; -2.63MPa, Pettigrew, 2004). Additionally, during the 2012 growing season, the Ψ_{MD} values observed on July 19 (-1.95 MPa) and 23 (-2.67 MPa) were lower than those reported by Grimes and Yamada (1982) to limit lint yield (>1.9 MPa). Similarly, lint yields in the present study were negatively impacted in dryland treatments in 2012; whereas, no effect due to irrigation treatment was observed in 2013, due to high rainfall for the majority of the growing season (Fig. 2.1 and 2.2). Interestingly, P_N and P_G (Fig. 2.4 A, B, C, D) were not shown to respond to low Ψ_{MD} for all sample dates (Fig 2.3 C, D). For example, on July 09, 2012, dryland Ψ_{MD} was 39% lower than irrigated cotton (-2.10, -1.28 MPa, respectively Fig. 2.3 C); however, no decline in P_G or P_N was observed (Fig. 2.4 A, C). Osmotic adjustment could potentially explain the irrigation treatment effect on midday water potential (Cutler et al., 1980) for plants that have acclimated to drying soil, although water potential components were not measured in this study. Conversely, low Ψ_{PD} in dryland treatments (Fig. 2.3 A, B) was found to be a strong indicator of midday photosynthetic declines (Fig. 2.4 A, B, C, D), similar to results reported by Snider et al. (2014); in other words, when Ψ_{PD} was significantly lower for dryland relative to irrigated plants, P_N was also lower. This effect supports and strengthens the argument that Ψ_{PD} can be a powerful indicator of drought stress (Ameglio et al., 1999; Jones, 2007) and physiological limitations to end of season lint yield.

Our results indicate that photosynthetic processes upstream of the carbon reactions are apparently insensitive to drought stress in field-grown cotton, within the range of water stress observed in this study (similar to the results of: Cornic and

Fresneau, 2002; Kitao and Lei, 2007; Zhang et al., 2011; Snider et al. 2013, 2014). Under extreme differences in Ψ_{PD} (as much as 40%, -0.575, -0.954 MPa, for irrigated and dryland, respectively, for July 19, 2012; Fig. 2.3 A), no effect of irrigation treatment was observed for either Φ_{PSII} or ETR (Table 1.1). This effect was consistent for all sample dates (Fig 2.3). During this sample period, dryland plants were visibly wilted and P_N was 54% lower for dryland than irrigated plants (15.3 to 33.2 μmol m^{-2} s^{-1} for dryland and irrigated, respectively; Fig. 2.3A). While significant increases in F_v/F_m (0.86, 0.85, for dryland and irrigated cotton, respectively; Table 1.1) due to irrigation treatment were observed, these differences were marginal (1%), and all values were near or above the non-stressed optimum of most species ($F_v/F_m = 0.83$, Maxwell and Johnson, 2000). Similarly, Kitao and Lei (2007) found that drought adapted cotton cultivars maintain electron flow through PSII as an adaptive mechanism to prevent over excitation of PSII reaction centers and production of reactive oxygen species (ROS, Asada, 2006; Miller et al., 2010). The lack of a response of photochemical efficiency or photosynthetic electron transport to extreme drought stress suggests that drought-induced decreases in P_N are due to factors downstream of the light reactions.

As drought stress progresses and leaf water status declines, g_s tends to decline (Hsiao, 1973; Kitao and Lei, 2007, Snider et al., 2014). In the current study, Ψ_{PD} (our measure of plant water status; Fig. 2.3 A, B) was a strong indicator of midday trends for g_s, and E (Fig. 2.6, C D, A B, respectively). Additionally, when g_s was negatively impacted, R_l was shown to increase, similar to the results of other researchers (Cornic and Fresneau, 2002; Ennahli and Earl, 2005; Massacci et al., 2008). This increase in the Rubisco oxygenation reaction, along with the lack of a drought-induced inhibition of photosynthetic electron transport, suggests that decreases in P_G are primarily due to a reduction in the Rubisco carboxylation reaction in response to a lack of substrate (Cornic and Fresneau, 2002; Ennahli and Earl, 2005; Flexas et al., 2004; Quick et al., 2006), rather than a downregulation of Rubisco (as reported by Tezara et al., 2002), as both processes would be affected similarly if this were the case. In addition to an overall decrease in carbon assimilation, we show for the first time that the authors are aware of, in field-grown cotton, when g_s was negatively impacted by drought, carbon lost by R_D as a proportion of P_G tends to increase (similar to results derived in Atkin and Macherel, 2009; Fig. 2.5), further exacerbating the negative effects of water deficit on P_N. Interestingly, after water deficit was alleviated by rainfall, as evidenced by loss of significant Ψ_{PD} or g_s (Fig. 2.2A, B; 3A, B, respectively), dryland leaves performed similarly (in terms of P_N, P_G, P_N/P_G, and R_l) to pre-stress levels, suggesting that no lasting metabolic impairment occurred as a result of previous drought stress.

Increases in leaf temperature have been observed under drought stress (Hsiao 1973) due to limitations to transpirational cooling of leaf surfaces; therefore, the effects of high temperature stress can often confound drought stress effects under field conditions. Factors affecting net carbon gain associated with heat stress include Rubisco activase activity (Haldimann and Feller 2005; Salvucci and Crafts-Brandner 2004; Sharkey 2005), thylakoid membrane thermostability (Haldimann and Feller 2005; Kim and Portis, 2005; Sharkey, 2005; Hall et al., 2014), and changes in ETR (Haldimann and Feller 2005; Wise et al., 2004). In the current study, confounding temperature effects on photosynthesis are unlikely, as all leaf temperatures (Fig. 2.8

C, D) were: within ~3°C of each other at the time of measurement, near optimal temperatures for P_N and lint yield in cotton (Salvucci and Crafts-Brandner 2004; Conaty et al., 2012), and well below temperatures known to negatively affect PSII in cotton (Salvucci and Crafts-Brandner 2004; Snider et al., 2013).

Although physiological measurements were not conducted during the pre-flowering period in 2012 due to logistical constraints, during the 2012 growing season, in two of the three weeks prior to flowering, rainfall was less than amounts prescribed by the checkbook irrigation approach (Fig. 2.2 A), whereas in 2013, water received by dryland treatments met or exceeded checkbook recommendations (Fig. 2.2 B). Previous research suggests that water deficit prior to flowering affects square retention negatively (Loka et al., 2011; Loka and Oosterhuis, 2012), potentially accounting for a portion of the yield loss observed in the 2012 growing season (Fig. 2.9). Also of note; it appears that plants sampled early in the growing season may be more sensitive to declines in leaf water status than those sampled later in the season, similar to the reported results by Ackerson and Krieg (1977) for cotton, corn and sorghum. In their study, cotton leaf resistance, when measured during vegetative growth, increased as minimum leaf water potentials (equivalent to Ψ_{PD}) decreased from -1.2 to -2.2 MPa: however, resistance was not unaffected by the same water potential range during reproductive growth (Ackerson and Krieg, 1977). Our findings further support the hypothesis that leaves sampled prior to flowering are more sensitive to water deficit than leaves sampled after flowering. Specifically, leaves sampled prior to flowering (June 26) in the dryland treatment during the 2013 growing season were under severe water deficit stress, as evidenced by visible wilting along with significant declines in photosynthesis (Fig. 2.4) and g_s (Fig. 2.6) relative to irrigated plants. However, the Ψ_{PD} values of dryland leaves on this sample date were higher than well-watered leaves sampled after flowering in 2012 (Fig. 2.3). On this pre-flowering sample date, our values for Ψ_{MD} (-1.0, -1.8 MPa, for irrigated and dryland, respectively) were similar to those reported by Ackerson and Krieg (1977) to negatively affect stomatal resistance in younger plants. Furthermore, decreased g_s in dryland cotton resulted in a lower C_i (Table 1.2), relative to irrigated cotton, strengthening the argument for a greater sensitivity early in the growing season. An irrigation effect on C_i was observed later in the growing season; however, C_i values were similar to those of unstressed plants from previous sample dates (Table2).

In this article, we have presented what is arguably the most comprehensive assessment of drought's effect on the underlying physiological mechanisms that ultimately limit net photosynthesis in field-grown cotton. Specifically, we present the results of a two-year study, with multiple metrics of plant water status that accounts for photorespiratory and respiratory carbon loss in addition to the response of primary photochemistry to drought stress. We conclude the following about drought stress effects on field-grown cotton: 1) under water deficit, net photosynthesis (P_N) is limited by decreased gross photosynthesis, resulting from declines in g_s and an increase in photorespiration (R_l) similar to the results of previous investigators (Cornic and Fresneau, 2002; Medrano et al., 2002; Baker et al., 2007); 2) For the first time in field grown cotton, we showed that P_N is further limited under water deficit because dark respiration increases as a ratio of R_D/P_G; 3) we found no evidence for photosynthetic limitations due to downregulation of PSII or photosynthetic electron transport rate within the level of water stress observed; 4) Ψ_{PD} is highly responsive to water availability in cotton and, therefore, exhibits potential for usage as a reliable indicator of drought stress, and thus the need for irrigation in field grown cotton; 5) care should be taken early in the growing season to prevent water stress due to the potentially increased sensitivity to slight declines in water potential for plants prior to flowering.

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Figure Captions

Fig. 2.1 Daily maximum (Max) and minimum (Min) (A,B) air temperatures and precipitation (B,C) during the 2012 (left panel) and 2013 (right panel) growing season at the Stripling Irrigation Research Park in Camilla, Georgia. Downward facing arrows indicate actual sample dates.

Fig. 2.2 Weekly water amounts for checkbook irrigation targets, rainfall (equivalent to dryland), irrigation, and checkbook total water applied to cotton field grown cotton during 2012 (Panel A), 2013 (Panel B) at the Stripling Irrigation Research Park. Asterisks indicate bloom initiation for each growing season.

Fig. 2.3 Pre-dawn (A, C) and midday (B, D) leaf water potential for irrigated (closed symbols) and dryland (open symbols) cotton during the 2012 (left panel) and 2013 (right panel) growing seasons in Camilla, Georgia. Data are means ± standard errors (n = 12 for 2012 and 9 for 2013).

Fig. 2.4 Net photosynthesis (A, B), gross photosynthesis (C, D), midday dark respiration rates (E, F), for irrigated (closed symbols) and dryland (open symbols) cotton during the 2012 (left panel) and 2013 (right panel) growing seasons in Camilla, GA. Data are means ± standard errors (n = 12 for 2012 and 9 for 2013).

Fig. 2.5 Ratio of dark respiration to gross photosynthesis for irrigated (closed symbols) and dryland (open symbols) cotton during the 2012 (left panel) and 2013 (right panel) growing seasons in Camilla, GA. Data are means ± standard errors (n = 12 for 2012 and 9 for 2013).

Fig. 2.6 Transpiration rate (A, B), stomatal conductance to water vapor (C, D), and water use efficiency (E, F) for irrigated (closed symbols) and dryland (open symbols) cotton during the 2012 (left panel) and 2013 (right panel) growing seasons in Camilla, Georgia. Data are means ± standard errors (n = 12 for 2012 and 9 for 2013).

Fig. 2.7 Photorespiration rates for dryland and irrigated cotton during the 2012 growing season in Camilla, Georgia. Data are means ± standard errors (n = 12 for 2012 and 9 for 2013).

Fig. 2.8 Air temperature at the plant canopy (A, B), abaxial leaf temperature (C, D), and leaf – air temperature differences (E, F) for irrigated (closed symbols) and dryland (open symbols) cotton during the 2012 (left panel) and 2013 (right panel) growing season in Camilla, Georgia. Data are means ± standard errors (n = 12 for 2012 and 9 for 2013).

Fig. 2.9 Lint yield for irrigated (black symbols) and dryland (grey symbols) cotton during the 2012 (left panel) and 2013 (right panel) growing season in Camilla, Georgia. Bars sharing a letter are not significantly different. Data are means ± standard errors (n = 12 for 2012 and 9 for 2013).

Table 2.1 Maximum quantum yield (Fv/Fm), actual quantum yield (Φ_{PSII}), and electron transport rate (ETR) for irrigated and dryland cotton for 2012 and 2013. Data are means ± SE (n = 12 and 9 in 2012 and 2013, respectively). For a given parameter, values sharing the same number of asterisks vertically or the same letter horizontally are not significantly different (Fisher's LSD, $P < 0.05$).

			2012	
Parameter	Treatment	July 9	July 19	July 23
Fv/Fm	Irrigated	0.83 ± 0.001 a	0.85 ± 0.001 a	0.85 ± 0.002 b
	Dryland	0.83 ± 0.000 a	0.86 ± 0.002 *b	0.86 ± 0.002 *b
Φ_{PSII}	Irrigated	0.51 ± 0.001 a	0.46 ± 0.011 b	0.45 ± 0.001 b
	Dryland	0.48 ± 0.015 a	0.44 ± 0.019 b	0.45 ± 0.016 ab
ETR	Irrigated	306 ± 6.8 a	302.10 ± 8.0 a	268.23 ± 5.3 b
	Dryland	304 ± 6.1 a	288.32 ± 12.2 ab	269.60 ± 10.8 ab
			2013	
		June 26	July 9	July 18
Fv/Fm	Irrigated	0.83 ± 0.003 a	0.83 ± 0.002 a	0.82 ± 0.002 b
	Dryland	0.82 ± 0.003 a	0.83 ± 0.001 b	0.82 ± 0.003 a

Table 2.2 Midday internal leaf CO_2 concentration (C_i) for irrigated and dryland cotton in 2013. Data are means ± SE (n=9). Values sharing the same number of asterisks vertically or the same letter horizontally are not significantly different (Fisher's LSD, $P < 0.05$).

Parameter	Treatment	June 26	July 9	July 18
C_i	Irrigated	241.56 ± 2.42 a	279.67 ± 1.92 b	274.78 ± 2.92 ab
	Dryland	155.27 ± 25.56 *a	279.44 ±2.51 b	241.5 ± 14.28 *c

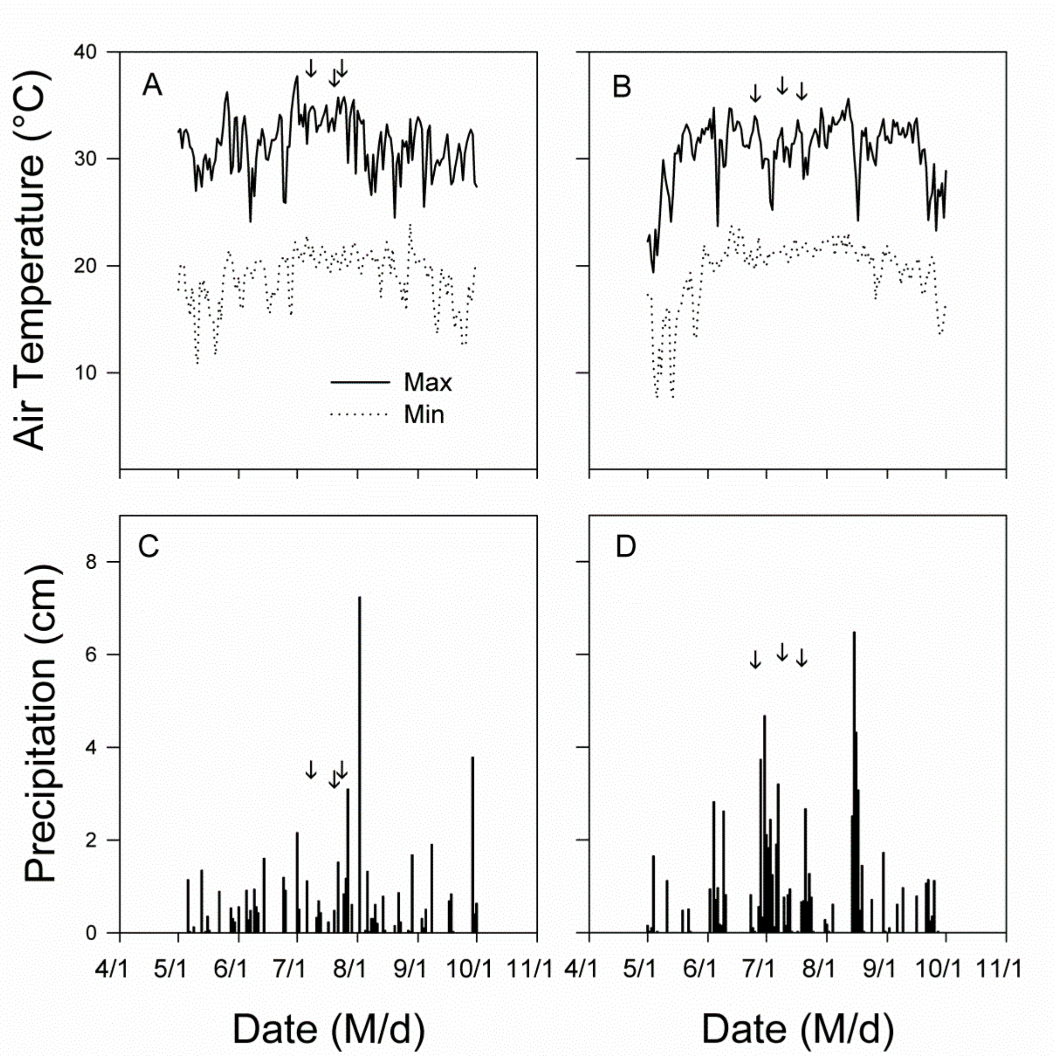

Air Temperature (°C)
40
30
20
10
A
B
Max
Min
Precipitation (cm)
8
6
4
2
0
C
D
4/1
5/1
6/1
7/1
8/1
9/1
10/1
11/1
Date (M/d)
Date (M/d)

Figure 2.1

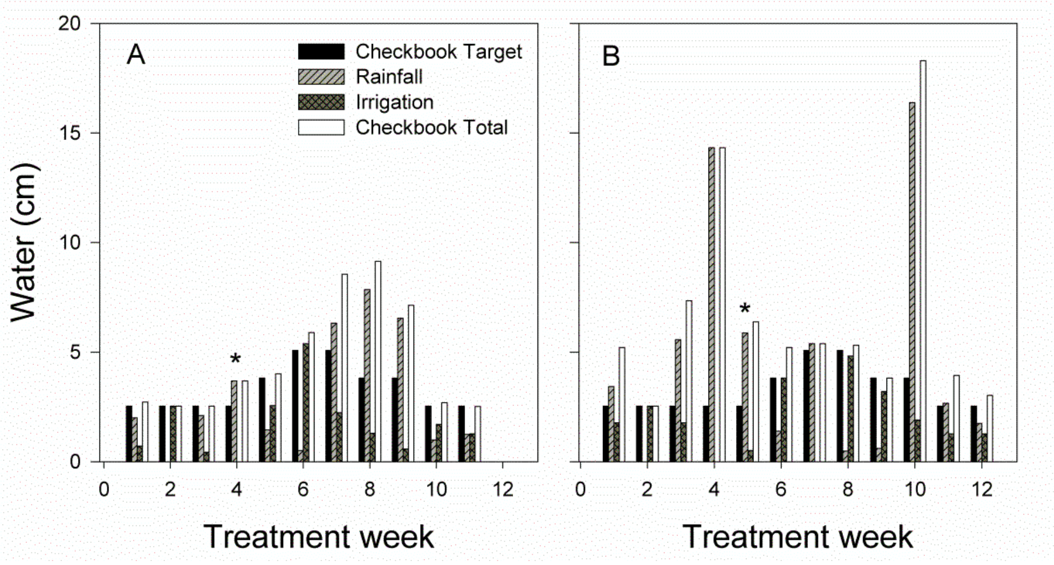

A
B
Checkbook Target
Rainfall
Irrigation
Checkbook Total
Water (cm)
0
5
10
15
20
*
0
2
4
6
8
10
12
Treatment week

Figure 2.2

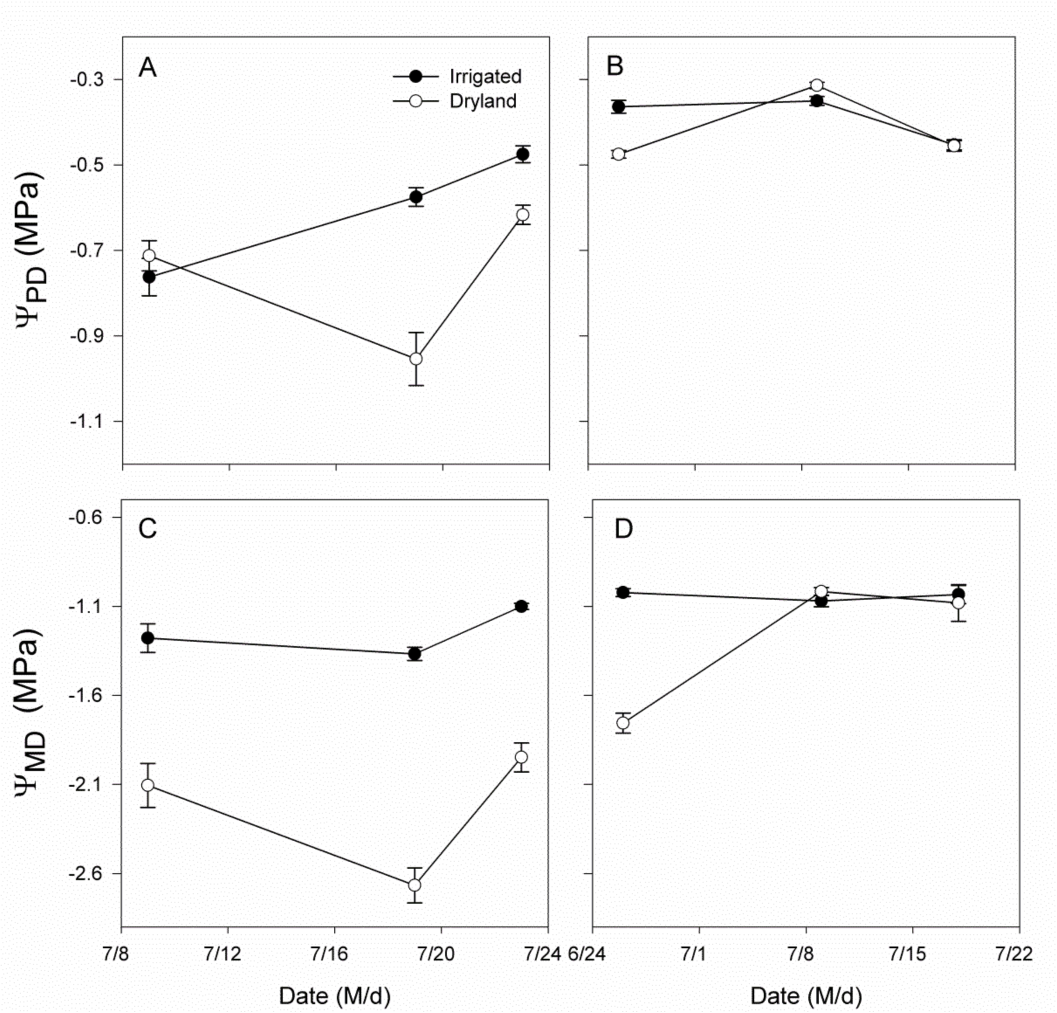
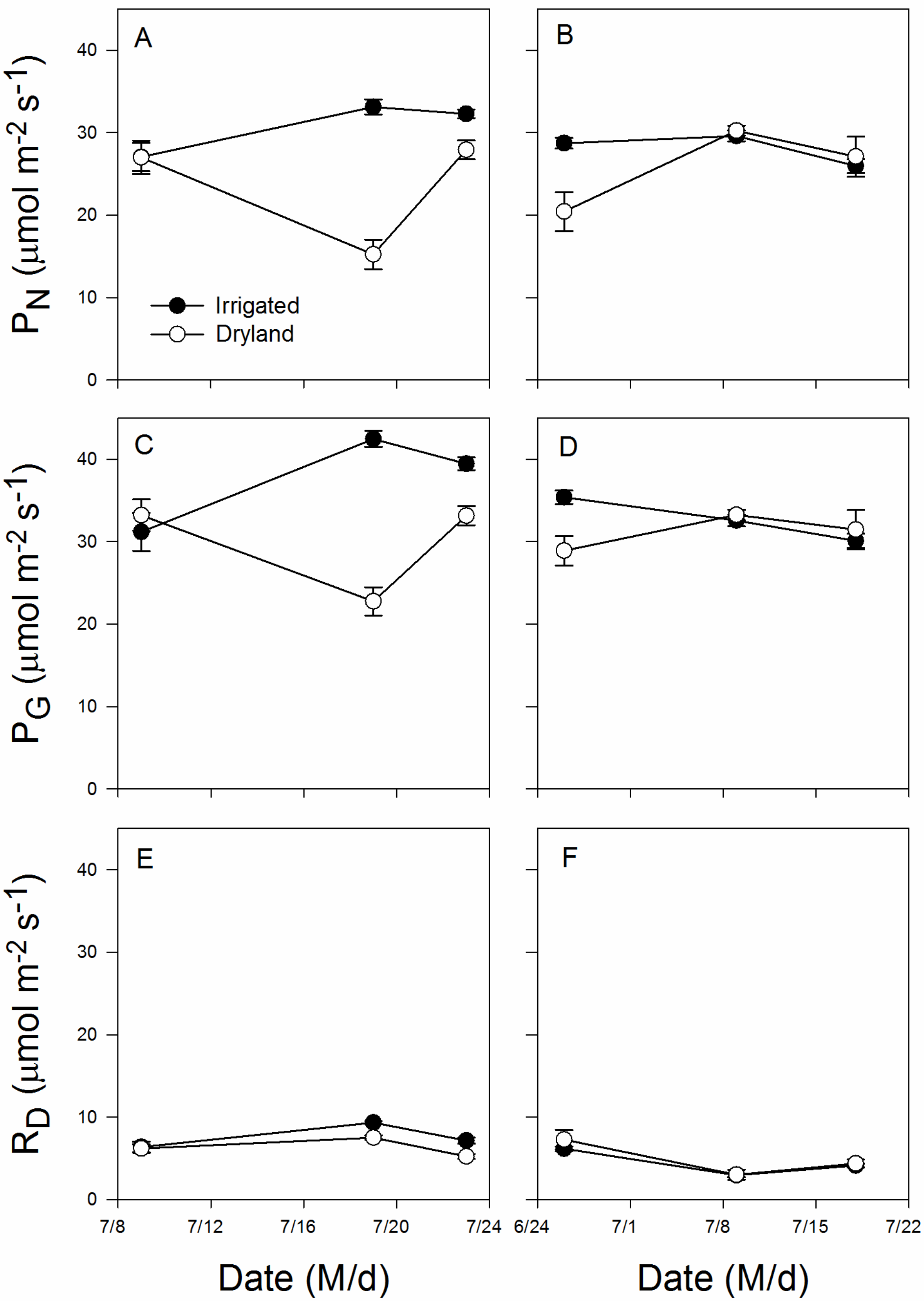

A
B
C
D
Irrigated
Dryland
Ψ_PD (MPa)
Ψ_MD (MPa)
-0.3
-0.5
-0.7
-0.9
-1.1
-0.6
-1.1
-1.6
-2.1
-2.6
7/8
7/12
7/16
7/20
7/24
6/24
7/1
7/8
7/15
7/22
Date (M/d)
Date (M/d)

Figure 2.3



A
B
C
D
E
F
Irrigated
Dryland
P_N (µmol m^-2 s^-1)
P_G (µmol m^-2 s^-1)
R_D (µmol m^-2 s^-1)
0
10
20
30
40
7/8
7/12
7/16
7/20
7/24
6/24
7/1
7/8
7/15
7/22
Date (M/d)
Date (M/d)

Figure 2.4

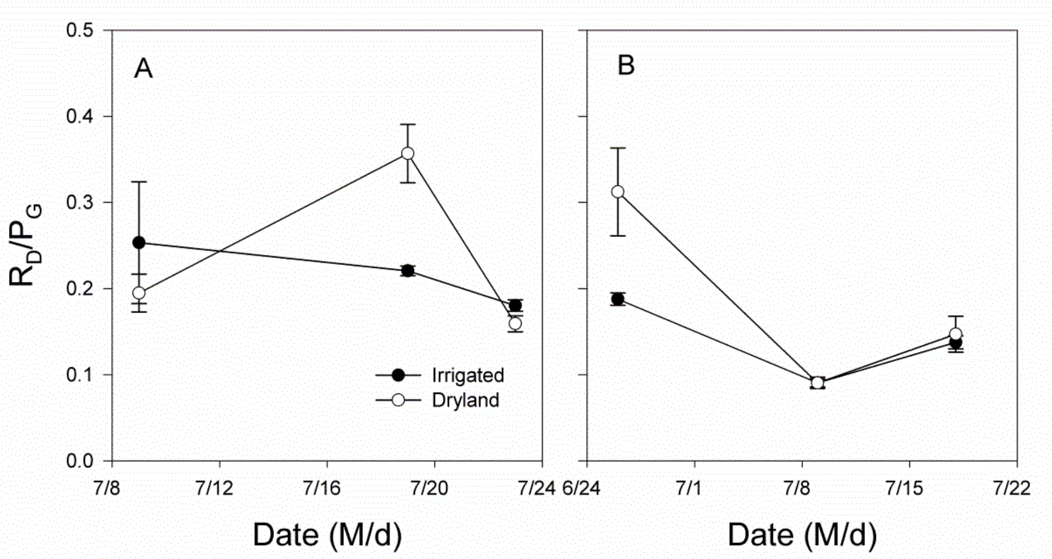
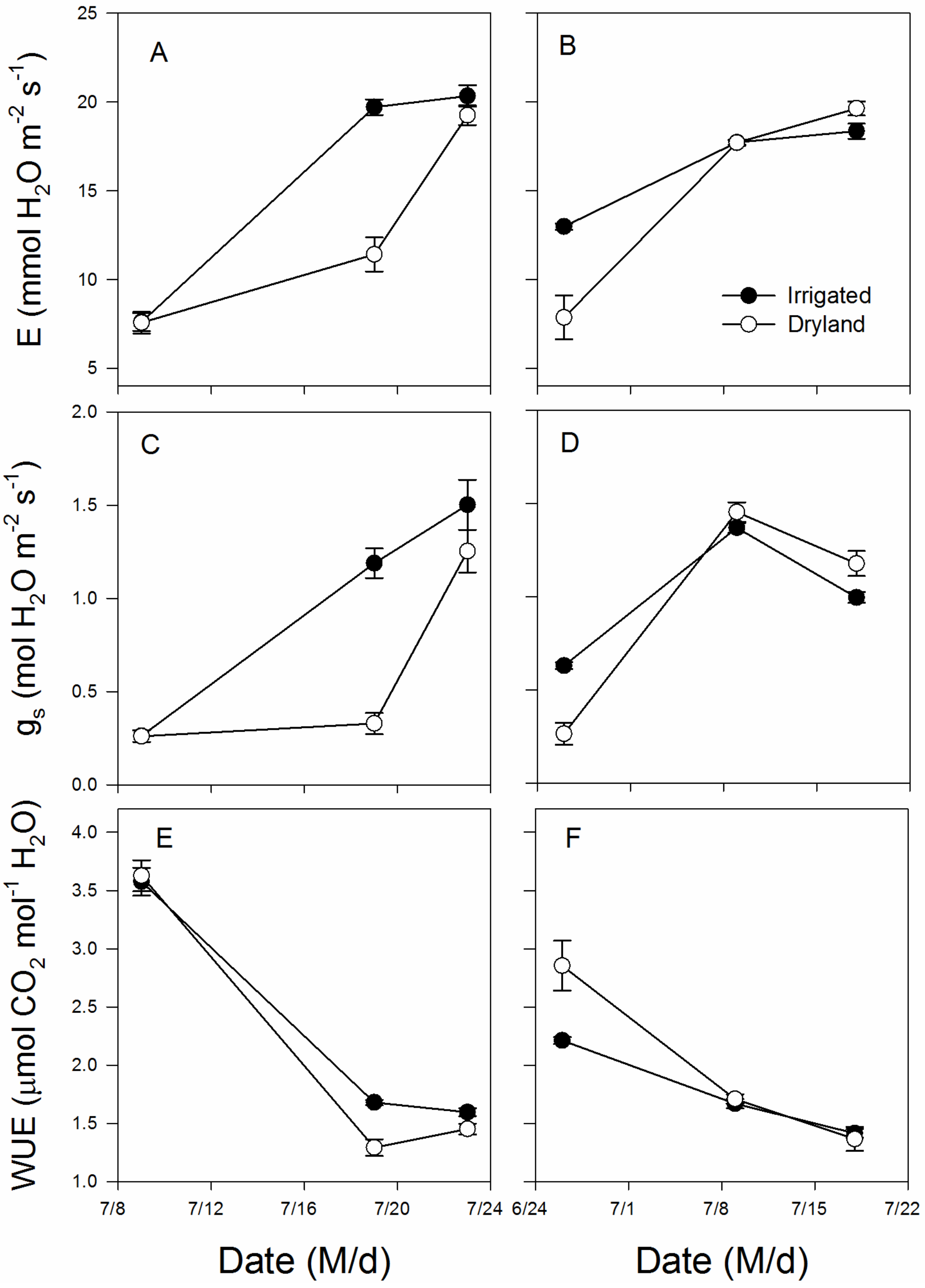

A
B
R$_D$/P$_G$
0.0
0.1
0.2
0.3
0.4
0.5
Irrigated
Dryland
7/8
7/12
7/16
7/20
7/24
6/24
7/1
7/8
7/15
7/22
Date (M/d)
Date (M/d)

Figure 2.5


A
B
C
D
E
F
E (mmol H_2O m^{-2} s^{-1})
g_s (mol H_2O m^{-2} s^{-1})
WUE (µmol CO_2 mol^{-1} H_2O)
25
20
15
10
5
2.0
1.5
1.0
0.5
0.0
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Irrigated
Dryland
7/8
7/12
7/16
7/20
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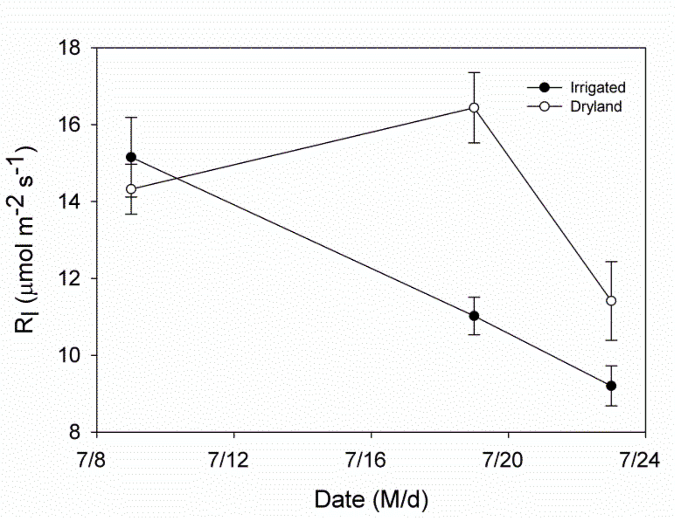
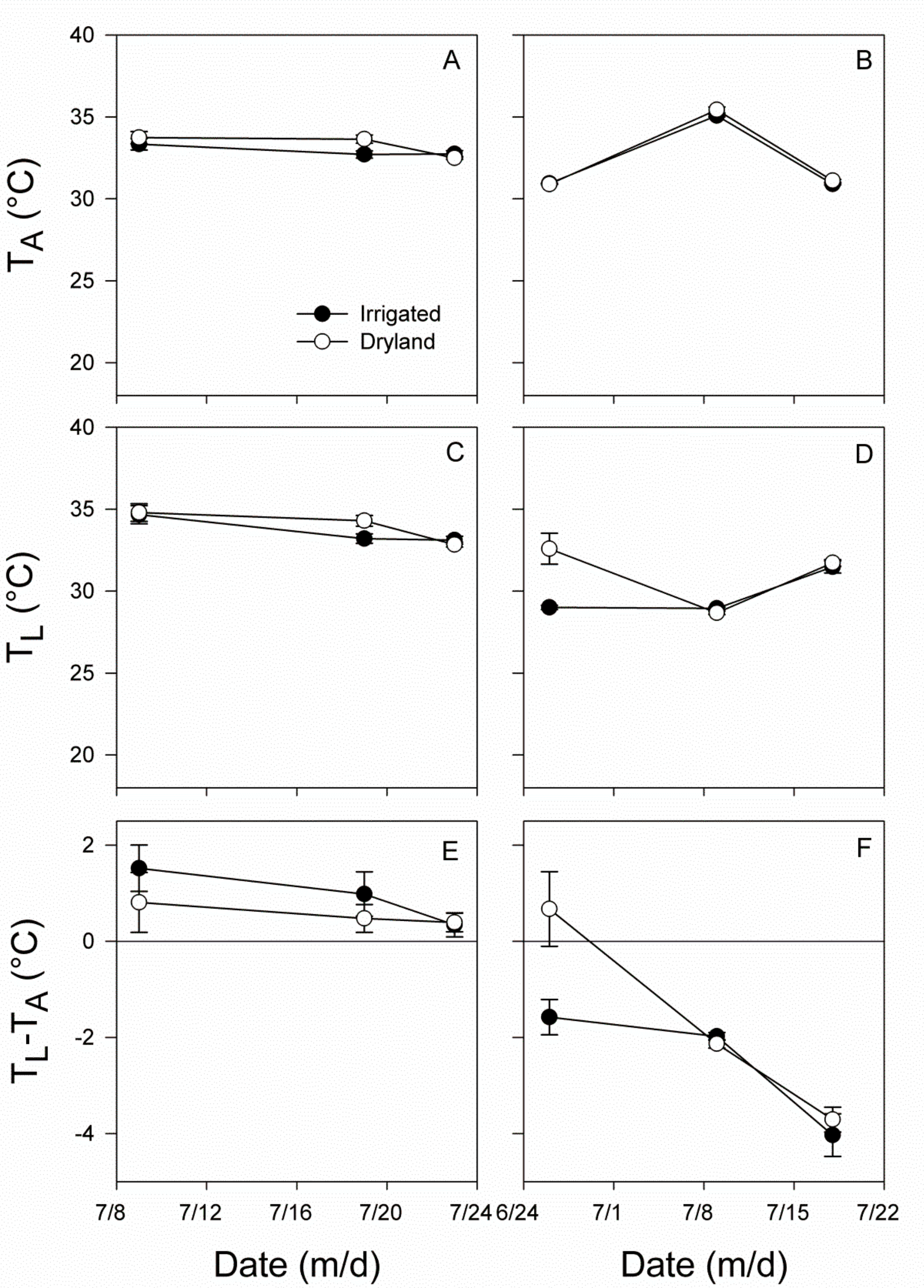
6/24
7/1
7/8
7/15
7/22
Date (M/d)
Date (M/d)

Figure 2.6



18
16
14
12
10
8
R$_l$ (μmol m^{-2} s^{-1})
7/8
7/12
7/16
7/20
7/24
Date (M/d)
● Irrigated
○ Dryland

Figure 2.7


A
B
C
D
E
F
Irrigated
Dryland
T_A (°C)
T_L (°C)
T_L-T_A (°C)
40
35
30
25
20
2
0
-2
-4
7/8
7/12
7/16
7/20
7/24
6/24
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7/22
Date (m/d)
Date (m/d)

Figure 2.8

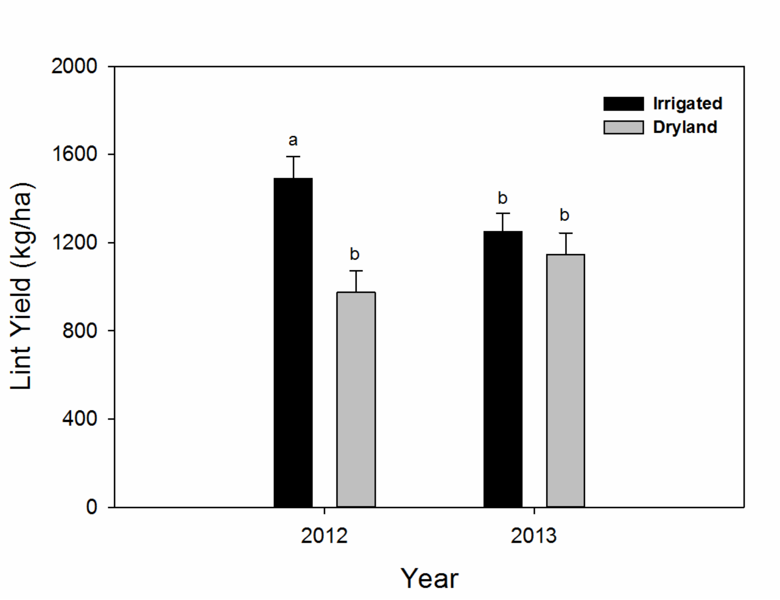
2000
1600
1200
800
400
0
Lint Yield (kg/ha)
Irrigated
Dryland
a
b
b
b
2012
2013
Year

Figure 2.9

CHAPTER 3

IRRIGATION SCHEDULING USING PREDAWN LEAF WATER POTENTIAL IMPROVES WATER PRODUCTIVITY IN DRIP IRRIGATED COTTON[2].

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[2] To be submitted to Crop Science

Abstract

To address the effectiveness of predawn leaf water potential in plant-based irrigation scheduling, cotton (*G. hirsutum*) plants were grown under fully irrigated and dryland conditions and under three predawn water potential (Ψ_{PD}) thresholds (-0.5, -0.7, and -0.9 MPa). Measurements included Ψ_{PD}, growth characteristics, lint yield, yield components, HVI fiber quality, and continuous crop canopy temperature. We found that Ψ_{PD} produced similar yields to current practices, while decreasing overall water use from 7-31 %, depending upon rainfall levels and treatment utilized. When the cotton crop was exposed to drought (2014), using a -0.5 MPa Ψ_{PD} produced the highest water productivity (40.7 kg ha^{-1} cm^{-1}), produced the highest yields (1995 kg ha^{-1}), and did not negatively impact fiber quality relative to traditional practices. In addition, decreased fiber yield in drought-stressed treatments was primarily due to decreased bolls m^{-2} (as much as a 43 % decline in severely stressed treatments). Using a well-watered baseline developed in 2013, we calculated a crop water stress index (CWSI) that exhibited a very strong, non-linear relationship with Ψ_{PD} values between ~-0.4 and -0.7 MPa (r^2 = 0.81. A strong, non-linear relationship was seen between CWSI and lint yield (r^2 = 0.81). Ψ_{PD} appears to be an effective means of determining the need for irrigation in cotton, and in the current study, yield and water productivity were maximized at a season-long average Ψ_{PD} threshold of -0.5 MPa.

Introduction

Arguably the most limiting factor in agricultural crop production is drought. While drought induced metabolic limitations (Chastain, et al., 2014; Cornic and

Fresneau, 2002; Ennahli and Earl, 2005; Hsiao, 1973; Pettigrew, 2004; Tezara, et al., 1999; Turner, et al., 1986; Zhang, et al., 2011) contribute to yield loss in cotton, decreases in individual leaf area and total number of leaves (Krieg and Sung, 1986) are the primary causes of decreased assimilate available for fiber growth and development. In addition, sink limitations such as deceased fruiting sites (Loka and Oosterhuis, 2012; Turner, et al., 1986) have been shown to be a major source of yield loss under drought. Predawn leaf water potential (Ψ_{PD}) values lower than -0.4 MPa have been shown to increase boll abscission rates in cotton (McMichael, et al., 1973). Decreases in overall plant growth are among the first negative responses associated with drought (Hsiao, 1973). Drought can decrease stem height, stem dry weight, leaf area, leaf dry weight, and total number of main stem nodes in cotton (Pace, et al., 1999); extreme drought can result in decreased boll size (Gerik, et al., 1996; Lokhande and Reddy, 2014). Midday leaf water potential (Ψ_{MD}) has been shown to have a positive linear relationship with cotton mainstem elongation rate. Specifically, little to no elongation was observed for Ψ_{MD} values below -2.4 MPa (Grimes and Yamada, 1982). Ψ_{PD} values below -0.8 MPa have been shown to negatively impact cotton mainstem growth (Jordan, 1970). Recently, a study conducted in controlled environments found mainstem elongation rate and node addition rate to decrease as water stress increased when water stress was imposed at flowering (Lokhande and Reddy, 2014). In the same study, plant biomass, number of bolls per plant, seedcotton per plant, seed mass per plant, seedcotton per boll, and seed mass per boll were shown to decrease as water stress increased, relative to non-deficit conditions. In addition, cotton fiber length, strength, and uniformity have been shown to decrease, whereas micronaire increases as Ψ_{MD} decreases from ~ -1.6 to -2.4 MPa (Lokhande and Reddy, 2014).

Supplemental irrigation can mitigate the negative effects associated with low rainfall; however, deciding when and where water should be applied has proved problematic. Producers have adopted a wide scope of irrigation scheduling methods. These range from calendar type methods, to rainfall budget approaches, and the use of environmental sensors. Irrigation triggers based on environmental parameters such as remote sensing of soil moisture status offer 1) the promise of timely irrigation scheduling based on crop need and 2) the possibility of complete automation (Snider et al., 2015). However, these methods are also problematic due to factors such as heterogeneity in soil composition, accuracy of the method used for their calculation, and the need for site-specific calibration (Jones, 2007; Leib, et al., 2003). These methods while useful and easily applied, are limited due to their lack of information on the stress level of the crop in question. For cotton grown in Georgia, many producers use a "checkbook" approach, where each phenological growth stage is allotted a weekly amount of water (see Collins et al., 2014); rainfall amounts are supplemented with irrigation to reach the weekly target. While the previously mentioned approaches consistently result in high yields, recent interest in adopting more sustainable irrigation practices for large-scale agriculture spawned research into maximizing water productivity (defined as agricultural product per unit total water; WP; reviewed in Ali and Talukder, 2008).

Beginning in the latter quarter of the last century, many crop researchers sought to enhance irrigation efficiency by using indirect measures of plant water status such as crop canopy temperature as an indicator of water deficit (Conaty, et al., 2012; Ehrler, et al., 1978; Idso, et al., 1981; Mahan, et al., 2010). Briefly, as water becomes limiting, transpiration decreases as a result of active and passive stomatal closure (Hsiao, 1973), ultimately resulting in elevated canopy temperatures. One of the more successfully demonstrated canopy temperature-based methods for detecting water deficit, the Crop Water Stress Index (Idso, et al., 1981), compares the canopy temperature response to vapor pressure deficit (VPD) of a given crop to that of a well-watered crop to indicate the severity of drought stress. While this approach is very effective in arid regions (high VPD), application in more humid regions, like the southeastern United States, has been limited (Jones, 2004).

The environmental factors limiting the use of indirect measures of plant water status can potentially be accounted for by region-specific calibration using a direct measure of plant water status (Jones, 2004). Predawn water leaf potential (Ψ_{PD}), a widely used measure of crop water status (Ameglio et al., 1999), may be the most useful for calibration purposes for several reasons. Water potentials (as measured with a pressure bomb) generally are at a maximum immediately before sunrise (Jordan, 1970; N Turner, et al., 1986), after which they fall to a minimum around mid-day before steadily rising through the afternoon and leveling off to values similar to pre-sunrise values overnight (Jones, 1990). Ψ_{PD}, although not an estimate of soil moisture (Caird, et al., 2007; Donovan, et al., 2001; Snyder, et al., 2003), may be the most feasible direct measure of plant water status available for usage in irrigation scheduling. Ψ_{PD} measurements have the advantage of being less sensitive to environmental fluctuations (i.e. cloud cover) as well as leaf position on the plant

(Jordan, 1970). Furthermore, Ψ_{PD} has been shown to correlate with relative evapotranspiration, and has been used to schedule irrigation in tree species (Ameglio et al., 1999). In addition, Ψ_{PD} has been shown to be highly predictive of midday metabolic trends (Chastain, et al., 2014). One caveat of using a plant-based water stress indication is that a stress level does not indicate how much water needs to be applied to relieve the stress (Jones, 2004).

In the current study, we sought to couple the use of Ψ_{PD} as an irrigation threshold with modified use of the Georgia Checkbook irrigation recommendations. By using this combined approach, our plant-based thresholds gave information on when to apply water, whereas the Georgia Checkbook provided information on irrigation quantity. We hypothesized that the use of Ψ_{PD} as an irrigation trigger can lower water use, and increase crop water productivity, without reducing lint yield or penalizing fiber quality. In addition, through continuous monitoring of crop canopy temperature and season long measurement of Ψ_{PD} during our study, we hypothesized that a CWSI derived from a well-watered baseline, would be closely associated with Ψ_{PD} and predictive of water-induce variability in lint yield.

Materials and Methods

Plant Material

To evaluate the usage of predawn leaf water potential as an irrigation trigger, research plots were established at C.M. Stripling Irrigation Research Park near Camilla, Georgia (31°16’55.5”N, 84°17’39.9”W) in 2013 and 2014. A rye cover crop was established in the off season and subjected to a glyphosate burn down and mowing prior to planting. Fields were then strip tilled to a 0.45 m depth. On 06 May, 2013 and 02 June 2014, seeds of two commercially-available *Gossypium hirsutum* cultivars [PHY 499 WRF (Dow AgroSciences), FM 1944 GLB2 (Bayer CropScience)] were sown at an inter-row spacing of 0.91 m and a rate of 11.5 seeds m^{-1}. Plots were 12.2 m long with 2.4 m alleys. To promote uniform stand establishment, supplemental irrigation (14.9 cm in 2013 and 18.5 cm in 2014) was applied via overhead sprinklers until squaring at which time irrigation treatments were initiated. Upon initiation of irrigation treatments, plots were irrigated via subsurface drip tape placed at 0.3 m deep, in alternating rows. Fertilization and pest management practices were conducted according to University of Georgia Cooperative Extension Service recommendations (Collins, et al., 2014). Rainfall and environmental data were provided by the Georgia Automated Environmental Monitoring Network (www.georgiaweather.net) weather station located on site.

Irrigation Treatments

In this study, irrigation treatments consisted of the Georgia Cooperative Extension Checkbook recommendations (T1; Collins, et al., 2014), dryland (T5; only receiving rainfall) and three modifications of the checkbook using a direct measure of plant water status as an irrigation trigger. Plant based irrigation triggers were based on Ψ_{PD} (-0.5, -0.7, and -0.90 MPa; T2-4, respectively) thresholds previously shown to relate to midday photosynthetic trends (Snider et al., 2014). Ψ_{PD} was measured between 0400 and 0600 h in each plot three times per week (M, W, and F), beginning at squaring (squares present in each plot). When a given irrigation treatment average reached the above thresholds, 1/3 of the weekly requirement was applied.

Regardless of irrigation treatment, Ψ_{PD} was measured in every plot using a Scholander pressure chamber (Model 615; PMS Instruments, Albany, OR). Measurements were conducted on the uppermost, fully expanded leaf. Cut leaves were sealed in the chamber within 5 s of excision and pressure was applied at 0.1 MPa s^{-1} until xylem sap reached the cut surface of the petiole.

Canopy Temperature and Crop Water Stress Index

Beginning at initiation of irrigation treatments, 15 minute average canopy temperatures (T_C) were monitored using an infrared thermometer in each plot (SmartField, Lubbock, TX) until irrigation was terminated. Sensors were maintained 20 cm above the canopy at 60° relative to the horizontal plane. The field of view (~ 20 cm diameter) was oriented towards the east, down a given row. Prior to analysis, data were filtered to include only T_C averages from 12-1400 h, when solar radiation was above 600 W/m^2. Dates when sensors were down due to fertilizer, growth regulator or pesticide application were also excluded.

In order to calculate a Crop Water Stress Index (Idso, et al., 1981) or CWSI, canopy to air temperature differentials ($T_{Canopy} - T_{Air}$) were plotted against ambient vapor pressure deficit (VPD) for 100% checkbook plots (in 2013 only). Data were subjected to linear regression to estimate a non-water stressed ($T_{NWS} – T_A$) baseline. $T_{Canopy} - T_{Air}$ for a non-transpiring crop ($T_{dry}- T_A$) were estimated by regression according to that described in Idso et al., (1981). CWSI for each plot was then estimated using the equation: $CWSI = [(T_{Canopy} - T_{Air}) - (T_{NWS} - T_{Air})] / [(T_{dry} - T_{Aair}) - (T_{NWS} - T_{Air})]$, where CWSI = 0 would represent a crop without any water limitations and CWSI = 1 would be a completely non-transpiring, drought stressed crop.

Growth and Yield Characteristics

In addition to Ψ_{PD} and T_C, crop growth characteristics were monitored throughout each growing season and included mainstem length from the soil surface to the apical meristem and total number of mainstem nodes per plant (measured on five plants per plot). After defoliation, two 1 m sections from each of the two center rows of a plot were harvested by hand. From each of these samples, bolls were counted, samples were ginned, seed and lint weight were determined, and the average weight of 500 seed was recorded. From these data, yield characteristics such as bolls m^{-2}, bolls per plant, individual seed weight, number of seeds per boll, lint weight per boll, ling weight per individual seed, and average boll size were calculated. Plots were then harvested mechanically using a two-row spindle picker. Samples were processed at the University of Georgia's Micro Gin in Tifton, GA. A 0.45 kg fiber sample was sent to the USDA Classing Office in Macon, GA for HVI analysis (fiber length, strength, micronaire, and uniformity index).

Statistical Analysis

Plots were organized according to a split-plot, randomized complete block design, where irrigation treatment represented the whole plot factor with cultivar being the sub plot factor. Main effects (irrigation treatment and cultivar) were analyzed by two-way mixed effects ANOVA using standard least squares and restricted maximum likelihood. Blocks were treated as a random effect. Post-hoc differences were determined using Fisher's Least Significant Difference (alpha = 0.05). Second-order, polynomial regression was used to determine the relationships between season average Ψ_{PD}, CWSI, and lint yield. In all cases, the quadratic component of the polynomial regression was found to be significant ($P < 0.05$). All analyses were conducted using JMP Pro 11.0 (SAS, Cary, NC). For both years in this study, no cultivar effect or cultivar by irrigation treatment interaction was observed for lint yield. As a result, the remainder of this paper will only evaluate effects due to irrigation treatment. In addition, figures presented represent data pooled from both cultivars in this study.

Results

Climatic Conditions

Daily minimum and maximum air temperatures are illustrated in Figure 3.1. In 2013, total rainfall (Table 2.1) from planting to defoliation was 66.9 cm, or ~ 21 cm greater than what is recommended by the Checkbook approach (Collins, et al., 2014). In contrast, the 2014 growing season total rainfall was less than Checkbook total recommendations (10.82 cm less; Table 2.1). In 2014, only 3.3 cm (9.5 % of the season total rainfall) occurred between June 24 and August 8, a time encompassing pre-bloom to peak bloom.

Predawn Water Potential and Growth

In 2013, Ψ_{PD} trends were similar amongst all treatments (Fig. 3.2A), and not all Ψ_{PD} irrigation thresholds were reached. In contrast, all Ψ_{PD} irrigation thresholds were reached in 2014 (Fig. 3.2B). In 2013, Ψ_{PD} ranged from -0.8 to -0.1 MPa, across all treatments and sample dates. In 2014, the maximum observed Ψ_{PD} during the growing season for T1-5 were -0.15, -0.25, -0.2, -0.1, and -0.2 MPa, respectively. T1-

5 minimum observed Ψ_{PD} during the entire growing season were -0.8, -0.9, -1.1, -1.7, and -2.1 MPa, respectively. When expressed as season-long treatment averages (Fig. 3.3), all treatments were similar in 2013; however, in 2014, only treatments 1 and 2 were similar and had the highest water potentials of all treatments. Treatments 3-5 had Ψ_{PD} values 17, 22, and 30 % lower than average Ψ_{PD} for T1-2 (Fig. 3.3B).

Similar to Ψ_{PD} trends, total mainstem height and total mainstem node number did not respond to our irrigation treatments throughout the 2013 growing season (Fig. 3.4A, C). In contrast, growth parameters were significantly affected by irrigation treatment at multiple times during the 2014 growing season (Fig. 3.4B, D). This is particularly evident on the August 08 sample date ($P < 0.001$ for height and mainstem node number). On this date, mainstem heights were similar for treatments 1-2 (120.69 cm) and treatments 3-5 (94.04 cm). Furthermore, the number of mainstem nodes per plant was numerically highest in T2 on this same sample date (18.48; no significant difference between T1 and T2) and lowest in T5 (14.2; Fig. 3.4D).

Yield and Yield Components

For both years included in this study, lint yield did not vary due to cultivar. As a result, cultivar effects will not be assessed for any parameter discussed below. In 2013, irrigation treatments did not affect total lint yield (Fig. 3.4A), fiber length, micronaire, or strength. Fiber length uniformity did respond to irrigation treatment; however, all treatments were within 1 % of each other and greater than 83 % (considered high uniformity: USDA Agricultural Marketing Service, 2001). Similarly, no effect of irrigation treatment was observed for number of bolls per plant, individual seed weight, seeds per boll, lint weight per boll, seed weight per boll, boll size, or lint weight per individual seed. Irrigation did have a significant effect on bolls m^{-2} ($P = 0.04$), with T3, having the greatest boll density (Table 2.2, 2.3).

In 2014, irrigation treatments had a strong effect on lint yield ($P < 0.001$). Specifically, our highest yielding treatments (T1, 2) had 23, 43, and 58 % higher lint yields than T3-5, respectively (Fig. 3.4B). Fiber quality parameters varied by treatment in 2014. Fiber length ($P < 0.001$) was greatest in T1-3 (3.04 cm), with T4-5 being 4.6 and 7.2 % shorter (Table 2.2). Micronaire ($P < 0.001$) was highest in T4-5 (5.12) with T4 and T1-2 being 8.8 and 18.8 % lower, respectively (Table 2.2). Fiber strength also responded to irrigation treatment ($P = 0.02$; 1.6 g tex^{-1} higher in T3, relative to T1, with T2, T4, and T5 being intermediate; Table 2.2). No effect of irrigation was observed for uniformity in 2014, and no difference in number of bolls per plant were found in T1-4 during the 2014 growing season; however, T5 was found to have, on average, 2.4 fewer bolls per plant than the mean of T1-4 ($P = 0.003$; Table 2.2). Individual seed weight ($P < 0.001$) was highest in T2-3, with T1 and 4 having ~ 5% less mass than the aforementioned treatments. T5 seeds were ~9.6 % lighter than T2-3 (Table 2.2). Seed weight per boll also responded to irrigation treatment ($P = 0.005$). T1 had the highest number of seeds per boll (27.4), T5, the lowest (24.71), with T2-4 having intermediate seed numbers (Table 2.3). No effect of irrigation treatment was observed for lint weight per boll (Table. 2.3) in 2014. Seed weight per boll ($P < 0.001$) was highest in T1-3 (2.82 g), with T5 being ~ 15 % lower. Boll size (seedcotton weight per individual boll) ($P = 0.002$) was similar amongst T1-

4 (4.79 g), with T5 being 9.8 % smaller than the aforementioned treatments (Table 2.3). Bolls per square meter also responded to irrigation treatment in 2014 ($P < 0.001$). Numerically, T2 had the highest number of bolls per unit area (83.3; no difference was seen between T1 and T2). T4 and T5 had 21.5 and 35.9 fewer bolls per square meter than T2, respectively.

Water Productivity

The use of Ψ_{PD} irrigation triggers decreased water usage 7 to 9% (T2; -0.5 MPa threshold) and 21 to 31% (T5: -0.9 MPa threshold) relative to the Checkbook for the 2013 and 2014 growing seasons, respectively. In 2013, water productivity (WP) was lowest in T1 (Georgia Checkbook). T2 WP increased by 2 kg ha^{-1} cm^{-1}, relative to T1. T3-4, the treatments with numerically the highest WP, had 20 % higher WP, relative to T1. T5, while not significantly different from T2, had 19.4 % higher WP, relative to T1 ($P < 0.001$; Fig 2.6A). In 2014 (Fig. 3.6B), WP was numerically highest for T2 ($P < 0.001$; 35.83 kg ha^{-1} cm^{-1}), with no difference detected between T2 and T3. T1 had 12 % lower WP, relative to T2. T4 WP was similar to T1, with T5 having, numerically, the lowest WP (30.23 kg ha^{-1} cm^{-1}; Fig. 3.6B).

Crop Water Stress Index

In order to develop a well-watered baseline for calculation of CWSI, $T_{Canopy} - T_{Air}$ data were regressed with VPD (Fig. 3.7). A strong, negative relationship was found between VPD and $T_{Canopy} - T_{Air}$ ($r^2 = 0.79$), representing our well-watered baseline (WWB = -0.2196 + -1.53 X). Season average CWSI was then calculated for all irrigation treatments for both 2013 and 2014 (Fig. 3.8). In 2013, no differences were found across T1-5 (Fig. 3.8A). In 2014, T1 and T2 were similar, and near zero. T3-5 CWSI values were 0.35, 0.66, and 0.84, respectively (Fig. 3.8B).

Relationships amongst Ψ_{PD}, Lint Yield, add CWSI

To assess the relationship between Ψ_{PD} and lint yield, season average Ψ_{PD} was plotted against end-of-season lint yield and subjected to second order polynomial regression. A strong, positive relationship was observed between the two variables ($P = 0.001$; $r^2 = 0.81$; Fig. 3.9). Season average Ψ_{PD} was then plotted against season average CWSI for each irrigation treatment before conducting second-order polynomial regression. A strong, negative relationship was found between the two variables ($P < 0.001$; $r^2 = 0.93$; Fig. 3.10). Finally, average CWSI was plotted against average lint yield and subjected to second-order polynomial regression and a strong, negative relationship was observed ($P = 0.003$; $r^2 = 0.81$; Fig. 3.11)

Discussion

Our current study supports the hypothesis that Ψ_{PD} can be used as an effective irrigation trigger to maximize water productivity in drip-irrigated cotton. The use of Ψ_{PD} resulted in an overall decreased water usage 7 to 9% (T2; -0.5 MPa threshold) to 21 to 31% (T5: -0.9 MPa threshold) relative to the Checkbook for the 2013 and 2014 growing season, respectively. Additionally, WP was increased, relative to Georgia Checkbook recommendations during both an extremely high rainfall (2013; Fig. 3.6A) and low rainfall year (2014; Fig. 3.6B). WP increased by as much as 20% in 2013, with no detectable difference in fiber yield (Fig. 3.5A). During this year, season average Ψ_{PD} (Fig. 3.3A) remained above our lowest irrigation threshold (-0.5 MPa). In addition, for the majority of the growing season, all irrigation treatments were near or above this threshold (Fig. 3.2A). This confirms the observations of McMichael et al. (1973), where fruit abscission (a major driver of yield loss; Loka and Oosterhuis, 2012) should be limited at Ψ_{PD} values above -0.5 to -0.4 MPa. In addition to lint yield, our growth data agrees with the observations of Jordan (1970), where Ψ_{PD} values greater than -0.8 MPa can inhibit mainstem growth. Specifically, growth was not affected by irrigation treatment in 2013 (a low stress year; Fig. 3.4A, C); however, in 2014, water limited treatments' (T3-5) average Ψ_{PD} were below -0.8 MPa on several sample dates (Fig. 3.2B), and mainstem height and total mainstem node number were significantly lower than non-stressed treatments (Fig. 3.4B, D).

The use of Ψ_{PD} as an accurate indicator of drought stress is a heavily debated topic. Some researchers argue against Ψ_{PD} due to the fact that it fails to accurately reflect soil water status, and is often lower than wetter soil portions in the rooting zone (Jordan and Ritchie, 1971; Klepper, et al., 1973). As stated previously, if one assumes no transpiration occurs overnight, Ψ_{PD} would be in equilibrium with soil in contact with the entire root system (Ameglio, et al., 1999; Jones, 2004). However, recent research indicates that nighttime transpiration can occur, creating disequilibrium between the soil and plant (Caird, et al., 2007; Donovan, et al., 2001; Snyder, et al., 2003). Irrespective of this disequilibrium, Ψ_{PD} represents the maximum water potential within a 24 hour period (Jordan, 1970; NC Turner, et al., 1986). In addition, Ψ_{PD} has been linked to changes in growth and boll retention in cotton (Jordan, 1970; McMichael, et al., 1973) as well as midday carbon metabolism

(Chastain, et al., 2014; JL Snider, et al., 2014). Furthermore, in our current study, the use of Ψ_{PD} as an irrigation trigger resulted in decreased water usage in both a wet and a dry year (Table 2.1), without negatively impacting fiber yield (Fig. 3.5). In addition, without rewatering, Ψ_{PD} represents the sum of all water loss mechanisms by a crop from the time the stress was initiated to the time of measurement (Jordan, 1970), thereby giving an accurate indication of cumulative water stress. Furthermore, Ψ_{PD} showed a strong, non-linear relationship with cotton fiber yield for both 2013 and 2014 (Fig. 3.9; $r^2 = 0.81$), strengthening the argument for the utility of Ψ_{PD} as an indicator of water stress and the need for irrigation.

In 2014, pronounced drought stress (Fig 2.2B: Fig. 3.3B) led to significant yield loss (Fig. 3.5B), especially in T5 (dryland). T5 fiber yield loss appears to be primarily due to decreased number of bolls per plant (Table 2.2) and bolls per unit land area (Table 2.3). This reduction in bolls per unit area agrees with the previous findings of many researchers (Guinn and Mauney, 1984; Krieg and Sung, 1986; Lokhande and Reddy, 2014; Pettigrew, 2004; NC Turner, et al., 1986). This is likely due to shedding of young bolls during drought sensitive periods (bolls < 14 days old: McMichael et al., 1973). In addition, we did see a small (9.8 %) reduction in boll mass in our most stressed treatment in 2014 (T5; Table 2.3), which is similar in magnitude to that reported in the literature (Gerik, et al., 1996; Lokhande and Reddy, 2014). In addition, seed number per boll was shown to decrease in T3-5, relative to T1, with no significant difference in lint weight per boll (Similar to Lokhande and Reddy, 2014; Table 3). In a four year study conducted by Pettigrew (2004), this effect was not observed; however, in this study, yield differences due to irrigation treatment

(~283 kg ha^{-1}; 25 %) were much smaller than what was observed in the current study (1171 kg ha^{-1}; 59 %), possibly indicating a lower degree of drought stress. In addition, we observed slight negative relationship between drought and seed weight per boll (Table 2.3), similar to that reported by Lokhande and Reddy (2004), but in conflict with Pettigrew (2004). Individual seed weight trends were somewhat inconsistent for 2014. Specifically, seed weight was lowest for T5, however no difference was observed between T4 and T1. Pettigrew (2004) showed seed weight declines in dryland, relative to irrigated cotton for 1 of 4 years, suggesting this effect is real, but may only appear under severe stress. In addition, treatments with lower seed weights (Table 2.1) typically had lower lint weight per seed, possibly sue to decreased seed surface available for fiber initiation (Pettigrew, 2004).

During the 2014 growing season, as treatment average Ψ_{PD} decreased, fiber length decreased, whereas fiber strength and micronaire increased. Pettigrew (2004) noted similar trends; however, year to year variability in rainfall made predictions difficult. Lokhande and Reddy (2014), using controlled environments established strong positive relationships between Ψ_{MD}, fiber length, strength, and uniformity, whereas micronaire showed a negative relationship in agreement with our results. In our study micronaire in 2014 was within the premium range for T1-2, whereas T4-5 were in the penalty range (Bradow and Davidonis, 2000). From a physiological perspective, shorter, high micronaire fibers support the hypothesis for a limitation to fiber elongation under water stress, due to loss of cell turgor (Dhindsa, et al., 1975). In addition, thicker fibers under intermediate drought conditions indicate a reallocation of assimilate into secondary cell wall formation (Gordon and Hsieh, 2006).

Based on canopy temperature data our calculated CWSI values for 2013 and 2014 were highly reflective of season-long crop water status. Specifically, Ψ_{PD} and CWSI exhibited a very strong, non-linear relationship for Ψ_{PD} values between ~-0.4 and -0.7 MPa ($r^2 = 0.81$; Fig. 3.10). In addition, elevated CWSI in T3-5 during the 2014 growing season (Fig. 3.8B) agreed closely with yield losses observed in those same treatments relative to T1 and T2 (Fig. 3.5B). Furthermore, a strong, non-linear relationship was seen between CWSI and lint yield ($r^2 = 0.81$; Fig. 3.11), suggesting the possibility of using T_{Canopy}-derived CWSI during the flowering period in cotton as a potentially viable irrigation scheduling parameter for humid regions. Furthermore, the use of T_{Canopy} as an indication of water stress can allow for simultaneous, automated monitoring of multiple locations (reducing producer costs); however, short-term weather fluctuations such as cloud cover may limit its utility as a scheduling method (Jones, 2004).

In conclusion, Ψ_{PD} appears to be an effective means of determining the need for irrigation in cotton and in the current study, yield and water productivity were maximized at a season long average Ψ_{PD} threshold of -0.5 MPa. In addition, drought stress negatively impacts fiber yield primarily through total bolls per unit land area and decreased boll size (under severe drought). Drought negatively impacts fiber quality through decreases in fiber length and increases in micronaire. In addition, CWSI, when calibrated by means of a direct indicator of plant water status offers the potential for usage as an irrigation scheduling method for low VPD environments, assuming afternoon solar radiation is accounted for.

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Figure Captions

Fig 3.1 Maximum and minimum daily air temperature (A, B) and daily precipitation totals (C, D) during the cotton growing season at the field site near Camilla, GA in 2013 (left panel) and 2014 (right panel).

Fig. 3.2 Seasonal trends in predawn leaf water potential (Ψ_{PD}) for cotton grown in Camilla, GA during the 2013 (A) and 2014 (B) growing seasons for irrigation treatments 1-5. Dashed horizontal lines represent leaf water potential thresholds for treatments 2-4 (-0.5, -0.7, 0.9 MPa, respectively). Data represent means ± SE (n = 8).

Fig. 3.3 Season-long average predawn leaf water potential (Ψ_{PD}) for cotton grown near Camilla, GA during the 2013 (A) and 2014 (B) growing seasons in irrigation treatments 1-5. Within a given year, bars sharing the same letter were not significantly different ($P > 0.05$). Dashed horizontal lines represent Ψ_{PD} thresholds for treatments 2-4 (-0.5, -0.7, 0.9 MPa, respectively). Data are means ± SE (n = 8).

Fig. 3.4 Average mainstem height (A, B) and number of mainstem nodes per plant (C, D) for cotton grown near Camilla, GA during the 2013 (left panel) and 2014 (right panel) growing seasons in irrigation treatments 1-5. Data are means ± SE (n = 8), and five plants were measured per replicate plot to provide a representative value for each parameter within a given plot.

Fig. 3.5 Lint yield for cotton grown near Camilla, GA during the 2013 (A) and 2014 (B) growing seasons in irrigation treatments 1-5. Within a given year, bars sharing the same letter were not significantly different (*P* > 0.05). Data are means ± SE (n = 8).

Fig. 6 Water productivity (lint yield per hectare per cm of water applied) for cotton grown near Camilla, GA during the 2013 (A) and 2014 (B) growing seasons irrigation treatments 1-5. Within a given year, bars sharing the same letter were not significantly different (P > 0.05). Data are means ± SE (n = 8).

Fig. 3.7 Canopy to air temperature differential (T_{canopy} - T_{air}) versus air vapor pressure deficit (VPD) for well-watered cotton (Treatment 1) grown near Camilla, GA during the 2013 growing season in irrigation treatments 1-5. Data points represent mean midday (12-1400 h) values (n = 8) on days when solar radiation was greater than 600 W m^{-2} during the aforementioned time frame. Horizontal dashed lines represent hypothetical canopy to air temperature differences for a non-transpiring canopy at air temperatures (T_{air}) of 50 and 15 °C, respectively.

Fig. 3.8 Canopy temperature-derived crop water stress index (CWSI) for cotton grown near Camilla, GA during the 2013 (A) and 2014 (B) growing seasons irrigation in treatments 1-5. Within a given year, bars sharing the same letter were not significantly different (*P* > 0.05). Data are means ± SE (n = 8).

Fig. 3.9 Relationship between season-long average predawn leaf water potential (Ψ_{PD}) and lint yield for each irrigation treatment-year for cotton grown near Camilla, GA during the 2013 and 2014 growing seasons. The trend line represents a best fit, second order polynomial ($P < 0.05$). Data are means ± SE (n = 8). What do the error bars represent?

Fig. 3.10 Relationship between season-long average predawn leaf water potential (Ψ_{PD}) and crop water stress index for each irrigation treatment-year for cotton grown near Camilla, GA during the 2013 and 2014 growing seasons. Trend line represents a best fit, second order polynomial ($P<0.05$). Data are means ± SE (n = 8).

Fig. 3.11 Relationship between crop water stress index (CWSI) and lint yield for each irrigation treatment-year for cotton grown near Camilla, GA during the 2013 and 2014 growing seasons. The trend line represents a best fit, second order polynomial ($P < 0.05$). Data are means ± SE (n = 8).

Tables

Table 3.1. Irrigation total, rainfall total, and overall total water applied to cotton grown near Camilla, GA during the 2013 and 2014 growing seasons. Within a given year and parameter, values sharing the same letter were not significantly different (*P* > 0.05). Data are means ± SE (n=8).

Year	**Treatment**	**Irrigation (cm)**	**Rainfall (cm)**	**Total Water (cm)**
2013	T1	17.4	66.9	84.3
	T2	11.5	66.9	78.4
	T3	2.5	66.9	69.4
	T4	0	66.9	66.9
	T5	0	66.9	66.9
2014	T1	29.9	34.9	64.8
	T2	23.7	34.9	58.6
	T3	14.2	34.9	49.1
	T4	10.0	34.9	44.9
	T5	0	34.9	34.9

Table 3.2. Fiber length, micronaire, strength, uniformity, number of bolls per plant, and individual seed weight for cotton grown near Camilla, GA during the 2013 and 2014 growing seasons. Within a given year and parameter, values sharing the same letter were not significantly different (*P* > 0.05). Data are means ± SE (n=8).

Year	**Treatment**	**Fiber Length (cm)**		**Micronaire**		**Strength (g/tex)**		**Uniformity (%)**		**Bolls Plant-1**		**Seed Weight (g)**	
2013	T1	2.98	a	5.09	a	32.09	a	83.66	ab	9.32	a	0.095	a
	T2	2.98	a	4.96	a	31.33	a	83.04	c	7.27	a	0.100	a
	T3	2.98	a	5.10	a	31.14	a	83.74	ab	9.43	a	0.100	a
	T4	3.01	a	5.16	a	31.53	a	83.20	bc	8.82	a	0.099	a
	T5	2.99	a	5.06	a	30.85	a	83.91	ab	9.60	a	0.100	a
2014	T1	3.03	a	4.18	c	33.66	c	83.88	a	6.39	a	0.104	b
	T2	3.07	a	4.14	c	34.11	bc	84.00	a	7.04	a	0.109	a
	T3	3.02	a	4.68	b	35.25	a	83.76	a	7.15	a	0.110	a
	T4	2.90	b	5.10	a	34.93	ab	83.74	a	6.11	a	0.104	b
	T5	2.82	c	5.14	a	34.23	bc	83.66	a	4.29	bc	0.099	c

Table 3.3. Average seed number per boll, lint per boll, seed weight per boll, boll size, bolls per square meter, and lint weight per individual seed for cotton grown near Camilla, GA during the 2013 and 2014 growing seasons. Within a given year and parameter, values sharing the same letter were not significantly different (*P* > 0.05). Data are means ± SE (n=8).

Year	**Treatment**	**Seeds Boll^{-1}**		**Lint weight Boll^{-1} (g)**		**Seed Weight Boll^{-1} (g)**		**Boll Size (g)**		**Boll m^{-2}**		**Lint weight Seed^{-1} (g)**	
2013	T1	25.71	a	1.95	a	2.44	a	4.39	a	49.80	abc	0.076	a
	T2	25.62	a	2.06	a	2.56	a	4.62	a	38.16	c	0.081	a
	T3	26.33	a	2.06	a	2.63	a	4.69	a	55.21	a	0.078	a
	T4	27.01	a	2.11	a	2.66	a	4.77	a	42.59	bc	0.078	a
	T5	26.60	a	2.12	a	2.67	a	4.79	a	53.85	ab	0.080	a
2014	T1	27.24	a	2.01		2.83	a	4.84	a	78.57	ab	0.074	c
	T2	26.27	ab	1.99		2.87	a	4.86	a	83.30	a	0.076	bc
	T3	25.39	bc	2.03		2.78	ab	4.81	a	68.88	bc	0.080	a
	T4	25.40	bc	2.00		2.64	bc	4.64	a	61.82	c	0.079	ab
	T5	24.71	c	1.88		2.44	C	4.32	b	47.39	d	0.076	bc

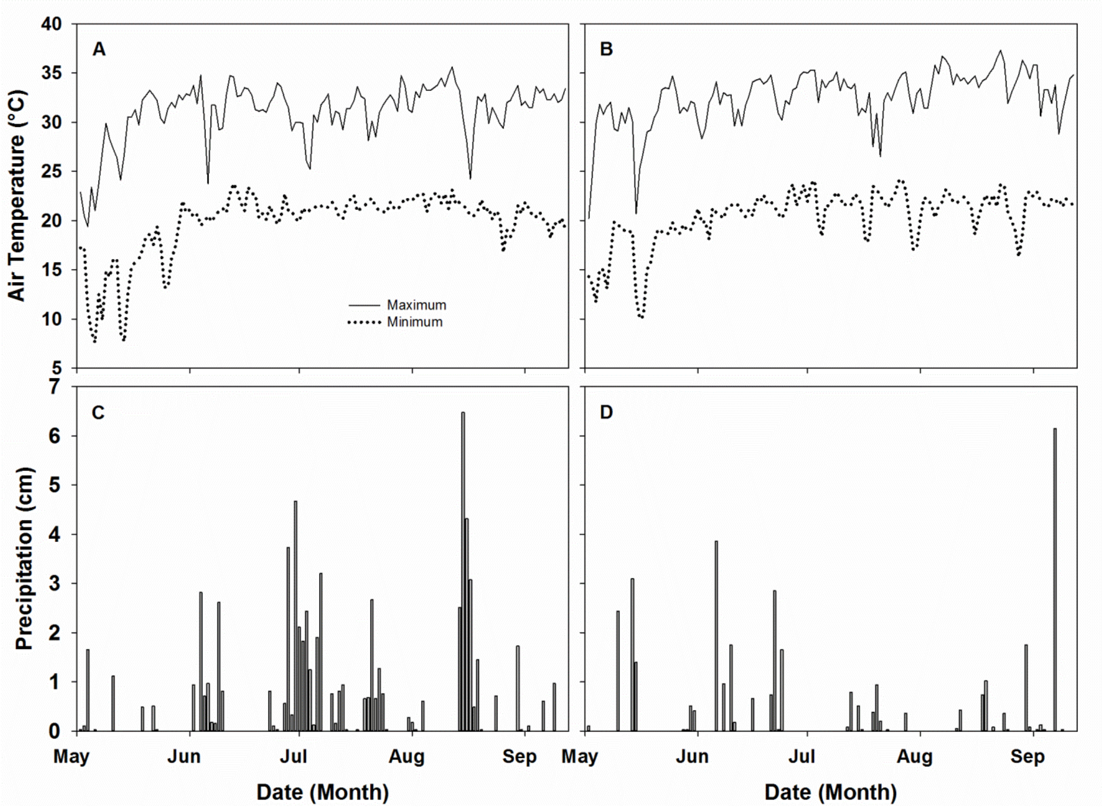
A
B
C
D
Air Temperature (°C)
Precipitation (cm)
40
35
30
25
20
15
10
5
7
6
5
4
3
2
1
0
Maximum
Minimum
May
Jun
Jul
Aug
Sep
Date (Month)

Figure 3.1

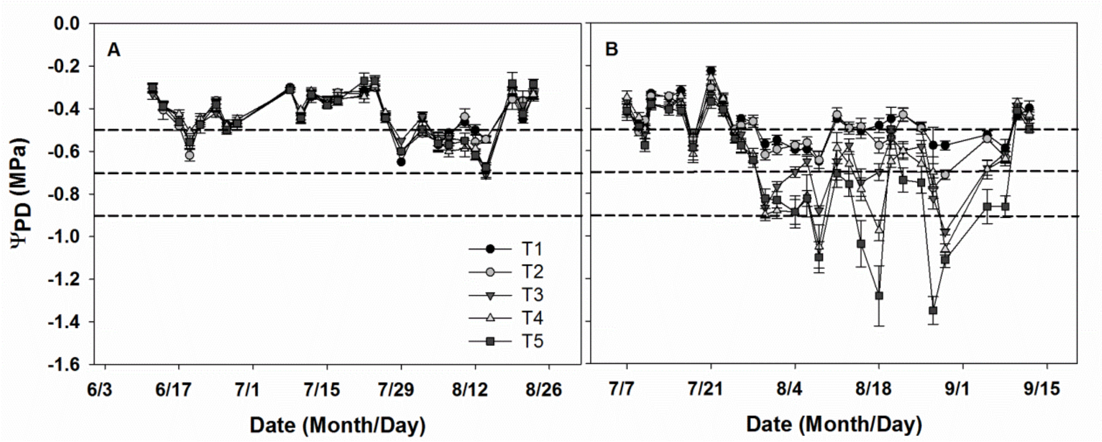

A
B
ΨPD (MPa)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
6/3
6/17
7/1
7/15
7/29
8/12
8/26
7/7
7/21
8/4
8/18
9/1
9/15
Date (Month/Day)
Date (Month/Day)
T1
T2
T3
T4
T5

Figure 3.2

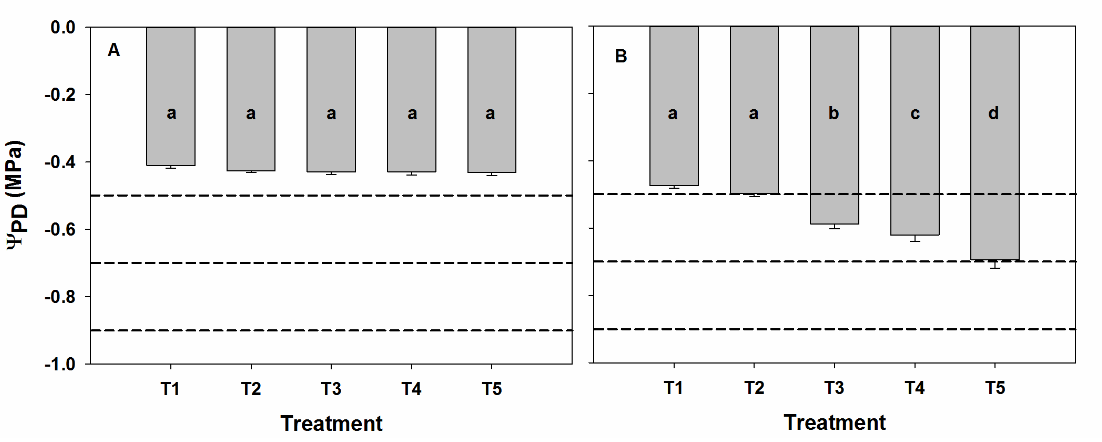
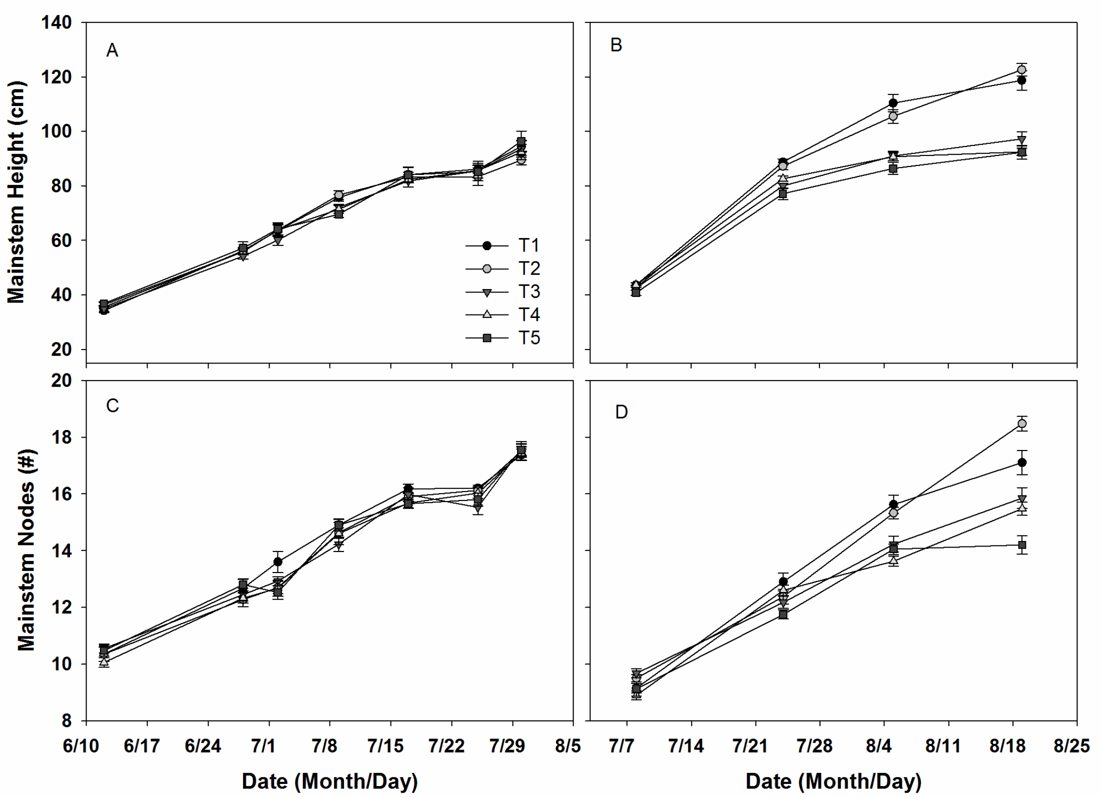

A
B
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Ψ_{PD} (MPa)
a
a
a
a
a
a
a
b
c
d
T1
T2
T3
T4
T5
Treatment
T1
T2
T3
T4
T5
Treatment

Figure 3.3



A
B
C
D
Mainstem Height (cm)
140
120
100
80
60
40
20
Mainstem Nodes (#)
20
18
16
14
12
10
8
T1
T2
T3
T4
T5
6/10
6/17
6/24
7/1
7/8
7/15
7/22
7/29
8/5
7/7
7/14
7/21
7/28
8/4
8/11
8/18
8/25
Date (Month/Day)
Date (Month/Day)

Figure 3.4

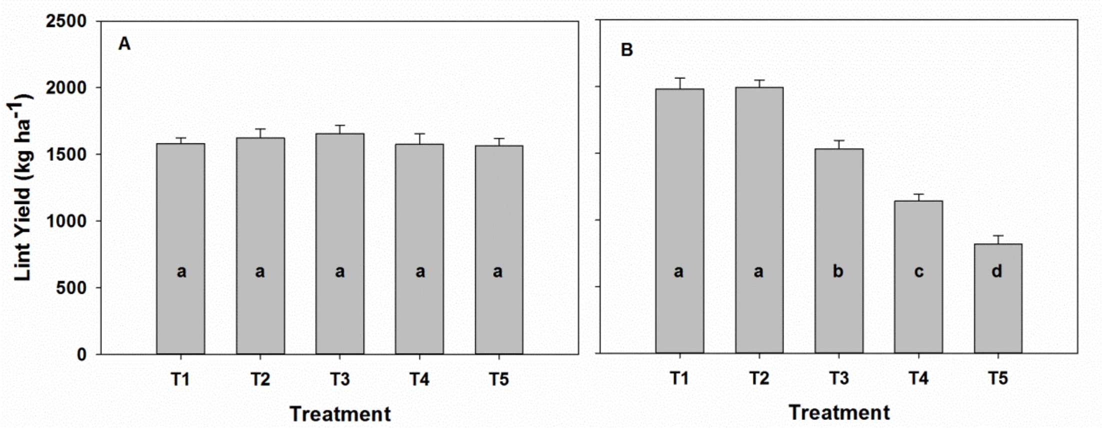

A
B
Lint Yield (kg ha-1)
2500
2000
1500
1000
500
0
a
a
a
a
a
a
a
b
c
d
T1
T2
T3
T4
T5
Treatment
T1
T2
T3
T4
T5
Treatment

Figure 3.5

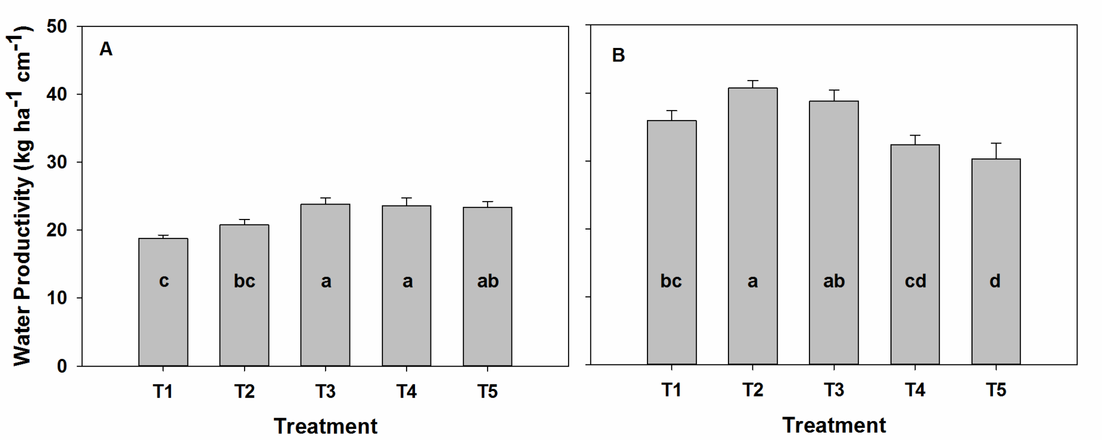

Water Productivity (kg ha^{-1} cm^{-1})
0
10
20
30
40
50
A
c
bc
a
a
ab
T1
T2
T3
T4
T5
Treatment
B
bc
a
ab
cd
d
T1
T2
T3
T4
T5
Treatment

Figure 3.6

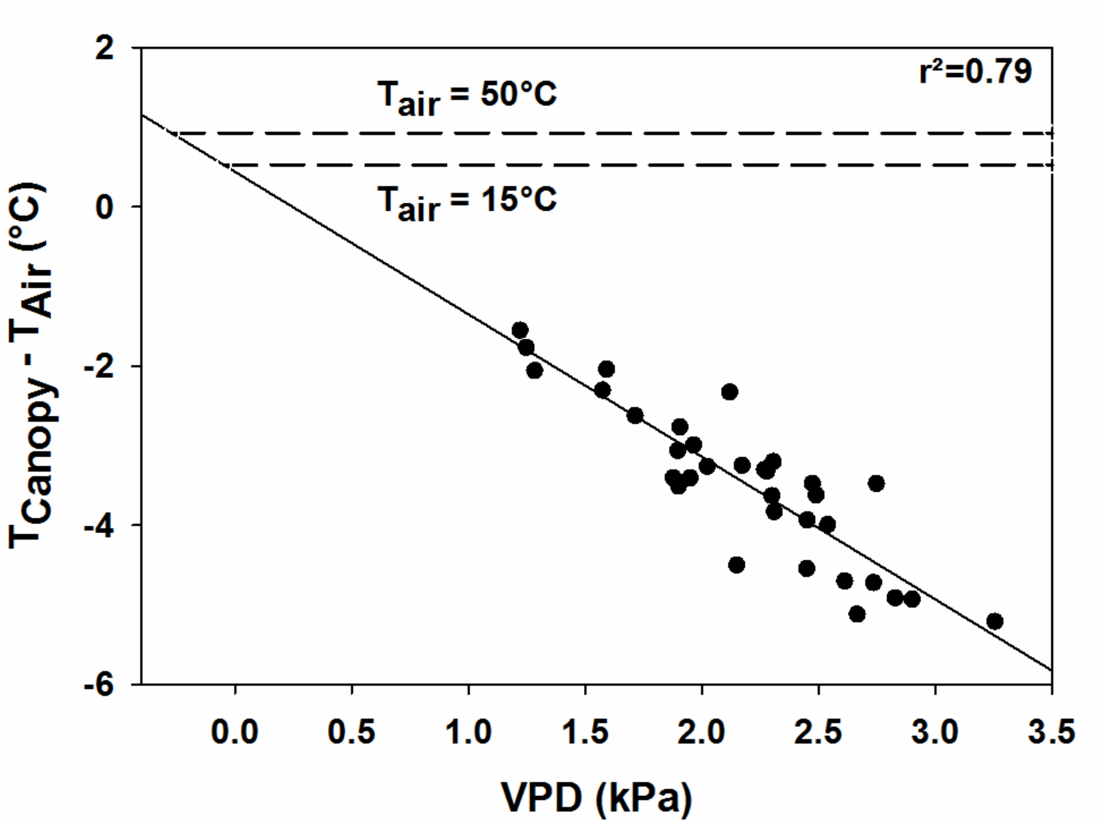

2
0
-2
-4
-6
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
$T_{Canopy} - T_{Air}$ (°C)
VPD (kPa)
T_{air} = 50°C
T_{air} = 15°C
r^2=0.79

Figure 3.7

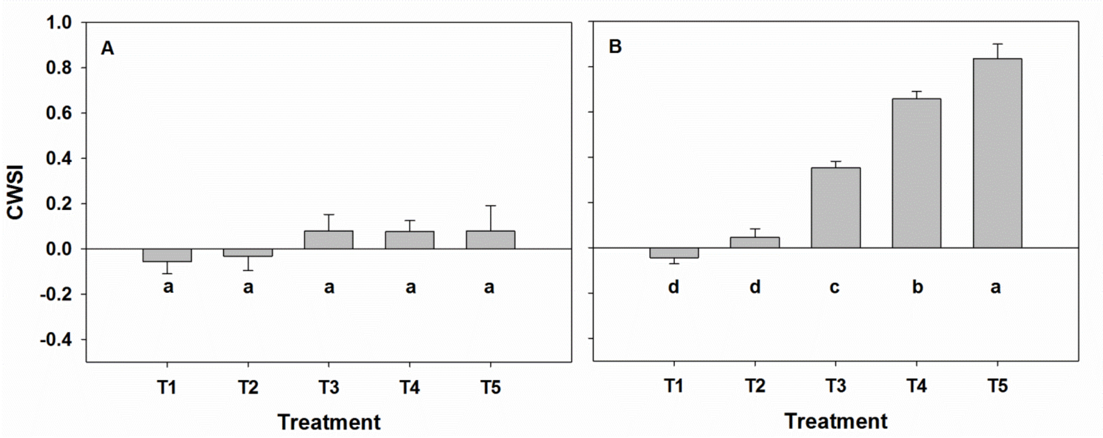

A
B
CWSI
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
a
a
a
a
a
d
d
c
b
a
T1
T2
T3
T4
T5
Treatment

Figure 3.8

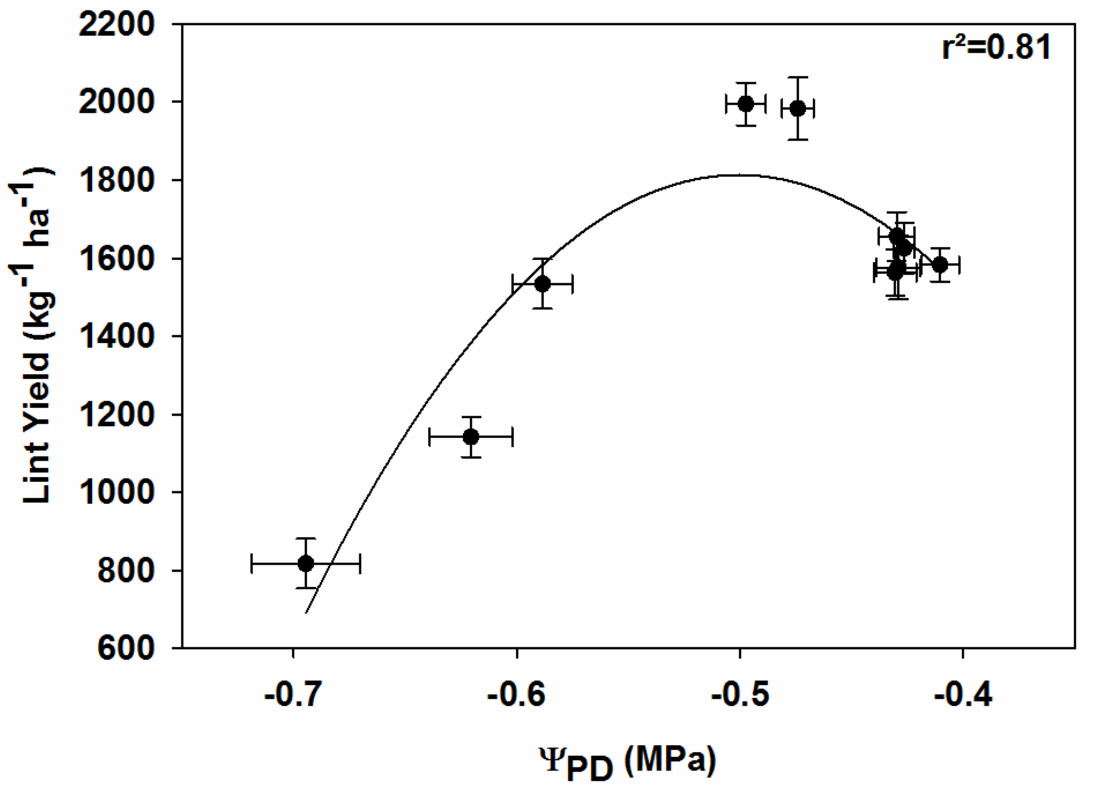

r²=0.81
2200
2000
1800
1600
1400
1200
1000
800
600
Lint Yield (kg^{-1} ha^{-1})
-0.7
-0.6
-0.5
-0.4
Ψ_{PD} (MPa)

Figure 3.9

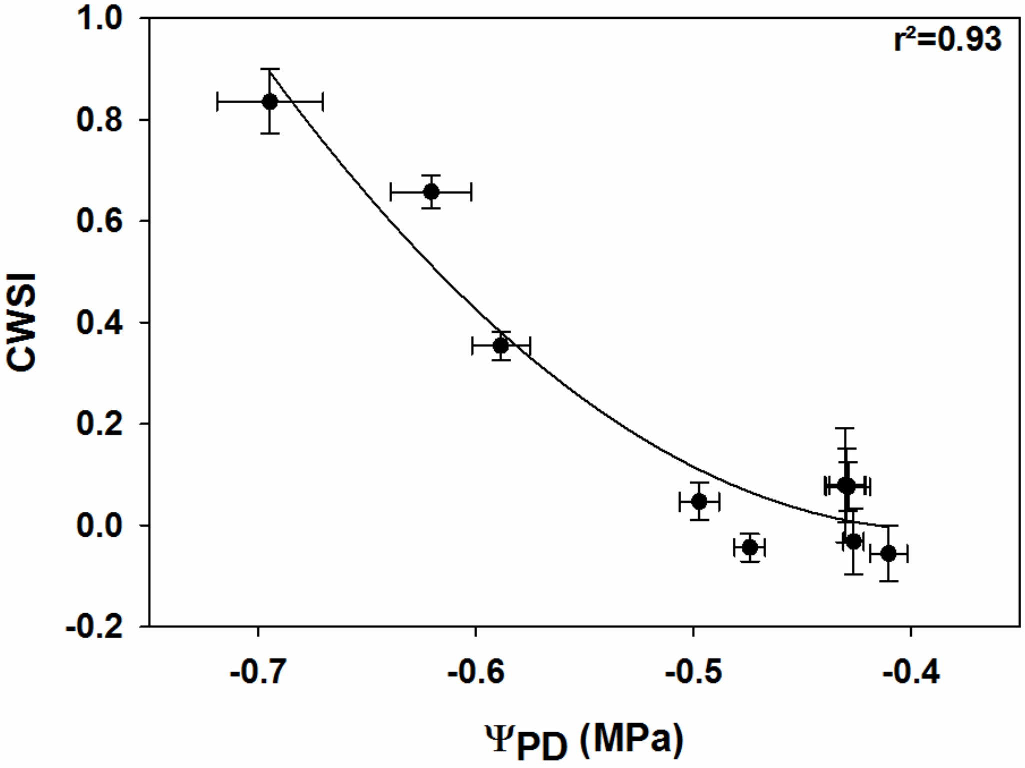


Figure 3.10

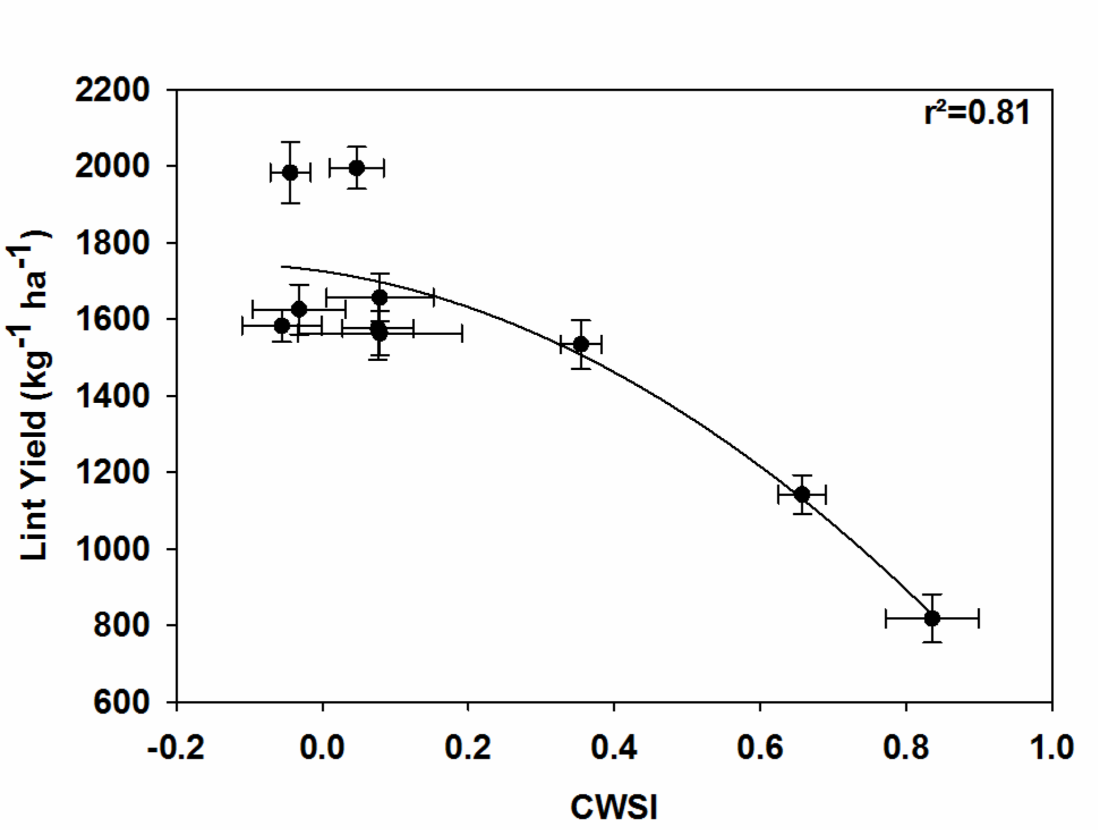

r²=0.81
Lint Yield (kg-1 ha-1)
2200
2000
1800
1600
1400
1200
1000
800
600
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
CWSI

Figure 3.11

CHAPTER 4

YOUNG COTTON LEAVES EXHIBIT EXCEPTIONAL PHOTOSYNTHETIC TOLERANCE TO THE COMBINED EFFECTS OF DROUGHT AND HIGH TEMPERATURE[3].

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[3]. To be submitted to Plant, Cell, & Environment

Abstract

To address the impact of leaf development on drought and heat tolerance, cotton (*G. hirsutum*) plants were exposed to five irrigation treatments during the 2014 season near Camilla, Georgia, and two leaf stages were sampled on three different dates. Measurements included leaf morphology and pigment characterizations, F_v/F_m response to temperature, predawn and midday water potential (Ψ_l), gas exchange, fluorescence, leaf temperature (T_{leaf}), and air temperature. Increased photosystem II thermotolerance was observed for young leaves sampled early in the growing season. Additionally, net photosynthesis (P_N) in young leaves was not negatively impacted by leaf temperatures well-above the 35 °C threshold known to limit P_N or by exposure to extreme drought. For example, in young leaves as leaf temperature increased from 31 to 37 °C, no decline in P_N was observed. In contrast, P_N in more mature leaves declined by 66% over the same temperature range. High leaf temperatures were a function of plant water status in mature leaves but not in young leaves. The substantial differences in heat and drought tolerance between two different stages of leaf development may provide opportunities to improve tolerance to heat and drought by regulating pre-existing genes within the same genotype.

Keyword index

Cotton, heat stress, photosynthesis, respiration, leaf expansion, drought, water deficit, thermotolerance.

Introduction

Drought induced declines in net photosynthesis (P_N) have been attributed to two general mechanisms: stomatal and non-stomatal limitations. Non-stomatal, drought-induced effects on photosystem II (PSII) include decreases in linear electron transport, maximum quantum yield (*i.e.*, damage to photosystem II), and actual quantum yield of electron flow through PSII (Pettigrew 2004; Ennahli & Earl 2005; Carmo-Silva et al. 2012). These decreases are, in part, associated with downregulation of the light reactions through processes such as increased non-photochemical quenching (Golding & Johnson 2003). As a result of decreased ATP and ATP synthase levels, drought can limit substrate regeneration for the carbon reactions (Tezara et al. 1999). Still, compelling arguments for a predominantly diffusional limitation (decreased CO_2 at the carboxylation site due to decreased stomatal or mesophyll conductance) under mild or moderate drought are more common (Hsiao 1973; Turner et al. 1986; Cornic & Fresneau 2002; Ennahli & Earl 2005; Zhang et al. 2011; Chastain et al. 2014). In addition, drought typically has little to no effect on primary photochemistry for field-grown plants (Kitao & Lei 2007; Massacci et al. 2008; Zhang et al. 2011; Snider et al. 2013; Chastain et al. 2014; Snider et al. 2014), and excess energy is dissipated through photorespiration (Cornic & Fresneau 2002; Ennahli & Earl 2005; Massacci et al. 2008; Chastain et al. 2014).

As drought progresses, stomatal closure leads to increased leaf temperatures as a result of diminished transpiration rates (Baker et al. 2007). In cotton, as leaf temperature increases above 35°C P_N begins to decline significantly (Law & Crafts-Brandner 1999; Crafts-Brandner & Salvucci 2000; Salvucci & Crafts-Brandner 2004;

Wise et al. 2004; Bibi et al. 2008). Heat-induced photosynthetic declines were originally attributed to the exceptional sensitivity of PSII (Berry & Björkman 1980); however, more recent research has shown that the maximum quantum yield of photosystem II is not appreciably affected at temperatures high enough to significantly inhibit photosynthesis (Law & Crafts-Brandner 1999; Crafts-Brandner & Salvucci 2000; Salvucci & Crafts-Brandner 2004; Snider et al. 2013). A number of studies have indicated that Rubisco activase may be the most heat-sensitive component of the photosynthetic apparatus (Feller et al. 1998; Crafts-Brandner & Salvucci 2000; Salvucci & Crafts-Brandner 2004). However, others have provided evidence that photosynthetic electron transport may be the weak link to moderately high leaf temperature due to disruptions in the thylakoid membrane at sites other than PSII (Schrader et al. 2004; Wise et al. 2004). In addition to the impact of moderately high temperature on photosynthesis proper, declines in P_N under high temperatures have been attributed, in part, to increased photorespiration (Berry & Björkman 1980; Jordan & Ogren 1984; Greer 2015), as well as an increase in dark respiration (Atkin & Tjoelker 2003; Greer 2015). However, as temperatures continue to increase (> 42.5°C), respiration ultimately decreases (Law & Crafts-Brandner 1999; Crafts-Brandner & Salvucci 2000; Salvucci & Crafts-Brandner 2004).

It is important to note that assessments of plant responses to abiotic stresses, such as drought and heat have focused primarily on fully expanded leaves or whole plants. Drought studies that address physiological responses during leaf ontogeny have predominantly characterized seasonal responses in tree species (Cavender-Bares & Bazzaz 2000; Winkel et al. 2001; Grassi & Magnani 2005). Though only a limited number of studies are available that address heat tolerance as a function of leaf ontogeny, young leaves have been found to be unaffected high temperature. Specifically, due to decreased stomatal functionality, young leaves typically exhibit elevated temperatures under field conditions relative to more mature leaves on the same plant (Snider et al., 2009a; Hall et al., 2014). Furthermore, young leaves, are able to withstand temperatures well above those shown to induce photosynthetic declines in more fully expanded leaves (Choinski & Gould 2010; Snider et al. 2010; Hall et al. 2014). All of the aforementioned studies have relied heavily on chlorophyll a fluorometry to detect photosynthetic heat tolerance and a few of those studies have attributed enhanced thermotolerance to higher saturated fatty acid levels in photosynthetic membranes (Choinski & Gould 2010; Hall et al. 2014). Of the previously mentioned studies, most were conducted on rain-fed, non-crop species. Where information is available for a field-grown crop of agronomic importance, (Hall et al. 2014), all data were obtained under an irrigation regime designed to limit yield loss (well-watered conditions).

Given the link between drought and high temperature stress, it seems likely that young leaves may also be more tolerant to the combined effects of drought and high temperature extremes. To date, no studies that we are aware of have investigated ontogenic differences in photosynthetic thermotolerance as affected by plant water status. In the current study, it was hypothesized that young *G. hirsutum* leaves would exhibit enhanced thermotolerance of primary photochemistry (as observed in previous studies) and greater tolerance of P_N to co-occurring drought and high leaf temperature relative to more mature leaves on the same plant.

Materials and methods

Plant Material

Experiments were conducted at C.M. Stripling Irrigation Research Park near Camilla, Georgia (31°16’55.5”N, 84°17’39.9”W) in 2014. The soil type at this location is classified as Lucy loamy sand (loamy, kaolinitic, thermic Arenic Kandiudults). Prior to planting, rows were strip tilled to a depth of 0.45 m and seeds of two, commercially-available *Gossypium hirsutum* cultivars [PHY 499 WRF (Dow AgroSciences), FM 1944 GLB2 (Bayer CropScience)] were sown on June 02, 2014 at a 0.91m inter-row spacing and at a rate of 11 seeds m^{-1}. Plots for each cultivar (n = 4) were 6 rows wide, 12.2 m long, and had 2.4 m bare-soil alleys. Supplemental irrigation was applied as needed to promote stand establishment prior to imposition of irrigation treatments at squaring (18.5 cm in 2014). Fertilization and pest management practices were conducted according to University of Georgia Cooperative Extension Service recommendations. Climactic data were provided by the Georgia Automated Environmental Monitoring Network (www.georgiaweather.net) weather station located at the study site. Field observations were conducted on July 10, July 26, and August 8, 2014, both during a predawn (0400-0600 h) and midday time window (1200-1500 h; hereafter referred to as predawn and midday, respectively).

Irrigation Regimes:

Beginning at squaring (defined as the phenological stage at which floral buds were first visible in every plot), five different irrigation treatments were initiated as follows. Dryland plots only received water via rainfall during the growing season, and well-watered plots received supplemental irrigation to meet weekly water requirements for cotton as defined using University of Georgia Cooperative Extension Service 'Checkbook' recommendations (Collins et al. 2014). Irrigation based on plant water status was accomplished by using three distinct predawn leaf water potentials (Ψ_{PD}) as irrigation triggers. Measurements were done three times per week with irrigation triggered when treatment averages reached or exceeded -0.5, -0.7, and -0.90 MPa. The amount of irrigation water applied following each trigger event was 1/3 of total weekly irrigation requirements according to the checkbook method. Checkbook, -0.5, -0.7, -0.90 MPa, and dryland are referred to as treatments 1-5 (T1-5), respectively. Irrigation was accomplished using subsurface drip tape (Netafim USA, Fresno CA) positioned approximately 0.3 m deep between alternating rows.

Leaf selection and characterization

For all sample dates, measurements were taken from nodes two and four (N2, N4, respectively), counted from the first newly unfurled leaf (node one, N1) towards the base of the plant. Following midday measurements, five leaves of each node were excised and placed in a sealed plastic bag containing moist paper towels and kept at ~20°C. Leaf thickness (L_T) was measured using an outside, screw-gauge micrometer. Chlorophyll a and b content were determined by placing 1.13 cm^2 of leaf disks in 5 mL of 95% ethanol at4 °C for 2 weeks, or until leaf disks were white in color (Dillenburg et al. 1995). The absorbance of a 300 µL aliquot of sample extract was measured at 649 and 665 nm using a 96-well microplate reader, and pigments were then estimated according to the equations of Knudson et al. (1977). Total chlorophyll content was expressed on a leaf area (µg cm^2) and a leaf volume basis (µg cm^{-3}). Individual leaf area (L_A) was measured using an LI-3100 leaf area meter (LI-COR, Lincoln, NE). Leaves were then placed in a forced-air dryer for 48 hours at 80°C before determining dry mass. Specific leaf weight (SLW) was expressed in g of dry weight per m^2.

Leaf water potential

To provide a measure of plant water status on each sample date, leaf water potential (Ψ_L) was measured predawn (Ψ_{PD}; 0400 to 0600 h) and midday (Ψ_{MD}; 1200 to 1400 h) on N4 leaves only. The leaves from node 2 were not measured because the petioles were too short. Briefly, Ψ_L measurements were made by excising leaves near the base of the petiole. Leaves were then immediately sealed with an adjustable compression gasket and placed in a sealed pressure chamber (Model 615, PMS Instruments, Albany, OR). Air pressure inside the leaf chamber was then increased at a rate of 0.1 MPa s^{-1} until water was visible at the cut surface of the petiole. Measurements were initiated within 5-10 s of the initial excision.

Chlorophyll fluorescence, leaf temperature, and gas exchange

Simultaneous gas exchange and fluorescence measurements for N2 and N4 leaves were conducted using a Portable Photosynthesis System (Model LI-6400, LI-COR, Lincoln NE) fitted with a Leaf Chamber Fluorometer (Model LI-6400-40, LI-

COR, Lincoln, NE). For each sample period, flow rate was set to 500 µmol s^{-1}. Block temperature was set to ambient air temperature within the plant canopy (determined by opening the leaf chamber and placing the leaf thermocouple in a shaded position in the canopy). Leaf temperature was determined using a type-T, fine-wire thermocouple in contact with the abaxial leaf surface during gas exchange measurements. Chamber CO_2 concentration was set at 400 µmol mol^{-1} for all measurements. Immediately prior to fluorescence measurements, once CO_2 exchange had reached a steady state (< 0.2 µmol m^{-2} s^{-1} fluctuation; 1-3 min), predawn respiration (R_n) values were logged. For determination of maximum quantum yield of electron flow through photosystem II (F_v/F_m), minimum fluorescence intensity (F_o) was measured in situ, during predawn hours, under the following settings: intensity = 1, rate = 0.25 kHz, filter = 1 Hz, gain = 10. To estimate maximal fluorescence intensity (F_m), a rectangular type, 0.8 s flash (target set to 7) at a rate of 20 kHz, was then initiated. F_v/F_m was estimated according to Maxwell and Johnson (2000) [$F_v/F_m = (F_m - F_o)/ F_m$]. Midday measurements were conducted under a photosynthetic photon flux density (*PPFD*) of 2000 µmol m^{-2} s^{-1} (90% red, 10% blue), which is considered to be above saturating light intensity for cotton (Constable & Rawson 1980; Krieg & Sung 1986; Ehleringer & Hammond 1987; Constable & Oosterhuis 2010; Wells 2011), provided by the fluorometer chamber LED light source. Immediately prior to fluorescence measurements, once steady state photosynthesis was achieved, all gas exchange data were logged. For determination of actual quantum yield of electron transport rate through PSII, light saturated steady state fluorescence (F_s) was estimated using the following settings: intensity = 5, rate = 20 kHz, filter = 1 Hz, gain = 10. To estimate maximal fluorescence intensity (Fm'), a rectangular type, 0.8 s flash (target set to 8) at a rate of 20 kHz, was applied. Subsequently, actual quantum yield of electron transfer through PSII was estimated according to Maxwell and Johnson (2000) [$\Phi_{PSII} = (F_m{}' - F_s)/ F_m{}'$]. Electron transport rate through photosystem II was estimated as: ETR = Φ_{PSII} x *PPFD* x 0.5 x 0.84, where 0.5 is based on excitation energy divided equally between photosystems I and II) and 0.84is a common leaf absorbance coefficient for C3plants)] (Why is this cited here? This is not the original source for the ETR equation or the 0.84 value).

Photosystem II thermotolerance assessment

To evaluate the temperature sensitivity of photosystem II in contrasting leaf stages of *G. hirsutum*, uppermost, N2 and N4 leaves were harvested on the same day that midday physiological measurements were conducted. To ensure that leaves were in the dark-adapted state, leaves were transported to the laboratory in plastic bags containing moist paper towels to prevent desiccation and were allowed to dark adapt for 24 h. Subsequently, 0.76 cm^2 leaf segments were excised from each leaf and placed on moist filter paper in contact with a thermogradient table described previously (Grey et al. 2011). Briefly, a table consisting of a solid aluminum block 2.4 m long by 0.9 m wide by 7.6 cm thick with a mass of 470 kg was utilized. A thermal gradient was first established by pumping a chilled or heated mixture of ethylene glycol:water (1:10) into the table at a rate of 3.8 L min-1, thus establishing a temperature gradient and providing a stable temperature at any given position along the width of the table. Using thermocouples (Insulated PR-T-24 wire, Omega

Engineering Inc, Stamford, CT) mounted to the undersurface of the aluminum table and a data logger (Graphtec Midi Logger, GRAPHTEC Corporation, 503-10 Shinano-cho, Totsuka-ku Yokohama, Japan) allowed temperatures to be continuously monitored immediately below each leaf sample throughout each incubation period. Each leaf segment was first incubated for 5 min at 30°C, generally accepted as the optimal temperature for net photosynthesis in cotton (Law & Crafts-Brandner 1999; Crafts-Brandner & Salvucci 2000; Salvucci & Crafts-Brandner 2004; Wise et al. 2004; Bibi et al. 2008). Following incubation, the rise in the chlorophyll a fluorescence transient under a saturating light flash was measured using the OS5p fluorometer, and the OJIP protocol, which is described in detail elsewhere (Strasser et al. 2000). Importantly, relative fluorescence intensity of the sample under a low intensity modulation light source (1µmol m^{-2} s^{-1}) prior to exposure to saturating light represents the O step (F_o) and the maximum fluorescence intensity, regardless of time required to reach maximal fluorescence, represents the P step (F_m). F_m and F_o were used to calculate maximum quantum yield of photosystem II (F_v/F_m). Following F_v/F_m measurement at 30°C, samples were sequentially incubated at 35, 40, 45, and 50°C and F_v/F_m was determined at each temperature. F_v/F_m versus temperature data for all leaves evaluated were fit to a third order polynomial, and coefficients of determination for temperature versus F_v/F_m curves of individual leaves were greater than 0.9 for all N2 and N4 leaves. The above-optimum temperature resulting in a 15% decline in F_v/F_m from the maximum observed value (T_{15}) was determined for each leaf as this is a standard method of assessing primary photochemical heat tolerance in a number of species (Froux et al. 2004; Snider et al. 2010; Snider et al. 2013). In a few instances, a 15% decline in F_v/F_m was not observed within the range of temperatures utilized, and T_{15} was extrapolated slightly beyond 50 °C using the regression equation obtained for a given leaf. Examples of T_{15} determinations within and beyond the range of data are given in Figure 4.1.

Experimental Design and Statistical Analysis

This study was originally constructed to evaluate the use of predawn leaf water potential as a means of scheduling irrigation over two years (manuscript in preparation). Plots were arranged in a split-plot randomized complete block design with irrigation being the whole-plot and cultivar being the sub-plot. Multiple cultivars were added to ensure that treatment responses can be related to upland cotton in general, rather than to a specific variety that may or may not be more tolerant to drought. For our current study; we were not interested in cultivar-specific effects and chose to focus on the physiological differences between leaf age classes and their response to drought. Consequently, cultivar was left out of the model, making this study a randomized complete block design. Data were analyzed by JMP Pro 10.0 (SAS, Cary, NC). Main effects on N4 and N2 leaves were analyzed by one-way mixed effects ANOVA using standard least squares, restricted maximum likelihood, according to a randomized complete block design. Blocks were treated as a random effect. Post-hoc differences were determined using Fisher's Least Significant Difference (alpha = 0.05). Differences between age classes were determined by means of a paired t-test within each treatment on a given sample date ($\alpha = 0.05$). Relationships amongst Ψ_{MD}, g_s, T_{Leaf}, $T_{Leaf} - T_{Air}$, LAVPD, and P_N were determined by second and third order polynomial regression (determined by best fit). All quadratic and cubic components of regressions shown in figures 4, 5, and 7 were significant ($P < 0.05$).

Results

Climatic Conditions and Irrigation

Maximum and minimum ambient air temperatures (from an on-site weather station) as well as precipitation are shown in Figure 4.2. Actual air temperatures at the canopy level (as measured by a type-T, fine wire thermocouple) were 33.5 ± 0.02, 32.2 ± 0.03, and 38.3 ± 0.03 for July 10, 26, and August 8, 2014, respectively. Irrigation amounts (Table 1) were 9.5, 24.2, 30.7, and 46.1 % lower than T1 for T2-5, respectively. During the period beginning June 24 and ending August 08 (a time frame encompassing all sample dates) total rainfall amounted to 3.3 cm, or 9.5% of total rainfall for the entire growing season.

Leaf Characteristics

Leaf surface area exhibited no response to irrigation treatment on July 10 and 26 (Table 3.2). On August 8, leaves for T3-5 were 13, 16, and 18 % smaller than T1 and 2 (P = 0.03; no difference detected between T1 and T2). Leaf thickness showed no effect of irrigation treatment for July 10 and August 8; however, on July 26, T3 leaves were slightly thicker (P=0.001; ~4.5 %) than T1 and T2, with no differences detected amongst other treatments. Specific leaf weight showed no response to irrigation treatment on July 10 and August 8; however, on July 26, T3 exhibited the highest SLW (P=0.04; 25 % greater than T1). T5 SLW was 24% higher than T1, with no differences detected between T2-5. Within a sample date, chlorophyll a, b and total chlorophylls (on a leaf area basis) exhibited similar responses to irrigation treatment. On July 10, T5 had higher chlorophyll contents than T2-4 (13, 16, and 14 % higher for chlorophyll a, b and total chlorophylls, respectively), with no differences detected between T1 and 5. On July 26, T3, 4, and 5 exhibited higher chlorophyll a, b and total chlorophylls than T1 and 2 (P=0.04, 0.01, and 0.004 for July 10, 26 and August 8, respectively). T4 was 18, 19, and 18% higher than T1 and 2 for chlorophyll a, b and total chlorophylls, respectively. T3 and 5 were not different from T1. For August 8, no effect of irrigation treatment was detected for chlorophyll b; however, similar trends for chlorophyll a and total chlorophylls were observed in response to irrigation treatment (P=0.005, 0.01, respectively). Specifically, T3-5 exhibited higher pigment contents than T1 and 2 (11 and 10%, respectively). No effect of irrigation treatment was observed for chlorophyll a/b ratio. No effect of irrigation treatment was observed for total chlorophylls per unit volume for July 26 and August 8; however, on July 10, T5 exhibited 20 % higher chlorophyll content than the average of T1-4.

N2 leaves had lower surface area than N4 leaves for all sample dates and irrigation treatments (Table 3.2). Specifically, N2 leaves were, on average, 76, 79, and 76 % smaller on July 10, 26, and August 8, respectively. For July 10 and 26, N2 leaves were, on average, 41 and 30 % thinner than N4 leaves. N2 leaves were also 26% thinner than N4 leaves in T3 on August 8. Specific leaf weights were 16, 21, 30, and 17 % lower for N2 leaves, relative to N4 leaves on July 10. Conversely, SLW was 54, 29, and 31 g m^{-2}, greater for N2 leaves in T1, 2, and 4, relative to N4 leaves on July 26. On August 8, SLW declined 34 and 13 % for N2, relative to N4 leaves in

T1 and 5, respectively. Chlorophylls on a surface area basis varied across leaf age class and treatment. Chlorophyll a was lower in N2 leaves for all treatments and sample dates, when compared to N4 leaves. On average, N2 leaves had 29, 39, and 27 % lower chlorophyll a contents, when compared to N4 leaves for July 10, 26, and August 8, respectively. Chlorophyll b was also lower for N2 leaves, for most treatments on July 10 and 26, when compared to N4 leaves. Specifically, for T1, 2, 3, and 5, chlorophyll a was 20, 14, 11, and 20 % lower in N2 leaves (and average of 16 %) on July 10. On July 26, N2 leaves were 29, 33, 24, 30, and 21 % lower in chlorophyll b content for T1-5, respectively. On August 8, N2 leaves were 25 and 20 % lower than N4 leaves in chlorophyll b for T1 and 2, respectively. Total chlorophylls (per unit surface area) were lower in N2 leaves for all treatments and sample dates when compared to N4 leaves. On July 10, total chlorophyll N2 to N4 differences ranged from ~20-30 % (25 % average). Similarly, on July 26, N2 leaves were ~31-40% lower than N4 leaves in total chlorophyll content (35% average). In T1 and T2, on August 8, N2 leaves had ~33 % lower chlorophyll contents, whereas T5 N2 leaf chlorophyll content was only 15 % lower than N4 leaves. Chlorophyll a/b ratio effects due to node of attachment were similar for all sample dates and treatments. Specifically, ratios were always ~16 % lower in N2 leaves, relative to N4 leaves. Early in the growing season, on July 10, N2 leaves were higher in total chlorophylls than N4 leaves, on a per unit volume basis. Specifically, T1-5 N2 leaves were 27, 35, 44, 35, and 13 % higher in total chlorophylls, relative to N4 leaves. Later in the season, on July 26, N4 to N2 differences in µg chlorophyll cm^{-3}were only detected in T1 and 2 (12 and 18% lower for N2 than for N4, respectively). For august

8, T1 showed 28 % lower total chlorophyll content for N2 leaves, relative to N4; however, no differences were observed between the two leaf stages in any other irrigation treatment.

Leaf Water Potential

Predawn leaf water potential (Ψ_{PD}) was significantly affected by irrigation treatment on each sample date (P = 0.01, 0.002, < 0.001, for July 10, July 26, and August 8, respectively). T5 had 14 % lower Ψ_{PD} than T1, 2, and 4 on July 10. T4 and 5 were 16 % lower than T1 and T2 on July 26. Similarly, T4-5 were 40 % lower than T1-2 on August 8 (Fig. 4.3, A, B, C). Irrigation treatment had a significant effect on Ψ_{MD} for each sample date (P=0.04, <0.0001, and <0.0001, for July 10, 26, and August 8, respectively). On July 10, no significant differences were observed for Ψ_{MD} for T1-4, whereas Ψ_{MD} for T5 was 18.6 % lower than for T1 plants. For Ψ_{MD} on July 26, T1 and 2 were similar, whereas T3-5 had 30.6, 28.6, and 38.3 % lower Ψ_{MD} than T1. On August 8, T3-5 had 45, 50.5, and 49.5 % lower Ψ_{MD} than T1, whereas T2 was not significantly different than T1 (Fig. 4.3, D, E, F).

Stomatal Conductance and Leaf Temperature

Midday leaf water potential of N4 showed a non-linear relationship with g_s on July 26 and August 8 for N4 leaves (r^2 = 0.67 and 0.85, respectively; Fig 3.4 B, C). N2 leaf g_s was also related with plant water status on August 8 (r^2=0.72; Fig 3.4 C). For the season as a whole, only N4 leaves exhibited a relationship between g_s and Ψ_{MD} (r^2=0.54; Fig. 4.4 D). Stomatal conductance was also related to leaf temperature and leaf to air temperature differential for N2 and N4 leaves on two of three sample dates ($r^2 \geq 0.90$; July 26, August 8; Fig. 4.5 B, C, and F, G, respectively). Stomatal conductance did not show a relationship with either T_{Leaf} or $T_{Leaf} - T_{Air}$ for N2 leaves on July 26, whereas in N4 leaves, a relationship was observed ($r^2 \geq 0.62$; Fig. 4.5 A, E) for both response variables. For all leaves (both N2 and N4) measured during the season combined, g_s accounted for 65 and 81 % of variation in T_{Leaf} and $T_{Leaf} - T_{Air}$ (third and second order polynomial, respectively; Fig. 4.5 D, H). Additionally, g_s at which N4 leaf temperatures equaled air temperatures ($g_{s,0}$) varied by sample date. Specifically, N4 $g_{s,0}$ values were 0.06, 0.34, 0.18, and 0.23 mol m^{-2} s^{-1} for July 10, 26, August 8, and for the season combined, respectively (Fig 3.5 E-H). Leaf to air vapor pressure deficit was also strongly related to g_s (Fig. 4.5 I-L). For both age classes, g_s accounted for > 95% of variability in leaf-to-air vapor pressure deficit (LAVPD) on July 26 and August 8. On August 8, no relationship greater than $r^2 = 0.5$ was detected for N2 leaves, whereas for N4 leaves, g_s accounted for 83% of the variability in LAVPD. For season long data and both age classes combined (Fig. 4.5 L), g_s accounted for 76 % of the variation in LAVPD.

The effect of irrigation treatment on g_s and T_{Leaf} are illustrated in Figure 4.6. For N4 and N2 leaves, no effect of irrigation treatment was observed for g_s, $T_{Leaf,}$ or $T_{Leaf} - T_{Air}$ on July 10. N2 leaves exhibited ~81 % lower g_s (Fig. 4.4 A) and were warmer (Fig. 4.6 D) than N4 leaves for all irrigation treatments. Node 2 leaves were also above air temperature for all irrigation treatments, whereas N4 leaves were cooler than ambient temperatures (Fig. 4.6 G) on the July 10 sample date.

On July 26, there was a significant effect of irrigation on g_s, $T_{Leaf,}$ and $T_{Leaf} - T_{Air}$ ($P < 0.001$ for each) for N4 and N2 leaves. N4 leaf conductances were highest (0.84 mol m^{-2} s^{-1}) in treatments 1 and 2. T3 and T4 were not statistically different from each other and were 70 % lower than T1 and 2. T5 had the lowest conductance (86 % lower than T1, 2; Fig. 4.4 B). Similarly, N2 leaf conductances were 52% lower in T3-5, compared to T1-2. Leaf temperatures (Fig. 4.5 B) for N4 and N2 leaves followed a similar trend to g_s. Specifically, T1 and T2 N4 leaves were coolest (~28.7 °C) on July 26; T3 and T4 were 5.3°C warmer than T1 and 2; T5 N4 leaves were 7.2°C warmer than T1-2 (Fig. 4.6 E). N4 leaves in T1and T2 were below air temperature, whereas all other treatments were warmer than ambient air temperature (Fig. 4.6 H) on July 26. Leaf temperature of N2 leaves were 2.1°C warmer for T3-5, when compared to T1-2 on July 26. Node 2 leaves in every treatment were warmer than air temperature on this date (Fig. 4.6 H).

On August 8, there was a significant effect of irrigation on g_s, $T_{Leaf,}$ and $T_{Leaf} - T_{Air}$ ($P < 0.001$ for each) for N4 and N2 leaves. For all parameters, treatment responses separated into three distinct groups. T1 and T2 N4 leaves had the highest g_s (0.85 mol m^{-2} s^{-1}), lowest leaf temperatures (31.7°C), and were well below ambient air temperatures (Fig. 4.6 C, F, I). T3 N4 g_s values were 78% lower than T1-2 and leaf temperatures were 6.5°C warmer than T1-2. T4 and T5 N4 leaves had 92% lower conductances, and were 8.9°C warmer than T1-2 (Fig. 4.6 C, F, I). Stomatal conductance for N2 leaves was highest in T1. For T2, N2 g_s decreased 24%, relative to T1. For T3-5, N2 leaf conductances were ~90% lower than T1. N2 leaf temperature and leaf to air temperature trends were similar to g_s. Specifically, leaves were coolest in T1, with T2 being 1.5°C warmer than T1 (also, T1 and 2 were well below ambient air temperatures). T3-5 were similar to each other; leaf temperatures were above air temperature and were 6.6°C warmer than T1. T1-2 N2 leaves had conductance values 46% below N4 leaves and were 3.1°C warmer than N4 leaves for T1 and T2 on August 8. Leaves of both age classes were below air temperature for these treatments. For T3, N2 leaves had conductances that were 49% lower than N4 leaves. N2 leaf temperature was slightly higher than N4 leaves in (<1%) in T3. N4 leaves were at or slightly above air temperature, whereas N2 leaves were all well above ambient temperature. No differences were observed between leaf developmental stages for T4 and T5 (Fig. 4.6 C, F, I).

Single-leaf CO_2 Exchange

Early in the growing season (July 10), leaf temperatures were similar within each age class and no significant relationship between leaf temperature and P_N was observed within a given leaf stage (Fig. 4.7 A). On July 26, as leaf temperature variation increased, leaf temperature accounted for 94 % of the variation in P_N for N4 leaves, whereas for N2 leaves, no relationship between leaf temperature and P_N was observed (Fig. 4.7 B). On the last sample date (August 8), N4 and N2 leaf temperature accounted for 97 and 83 % of the variation in P_N (Fig. 4.7 C), respectively. For combined season data, N4 leaf temperature was highly predictive of P_N, whereas no relationship was detected in N2 leaves.

No irrigation treatment effect on P_N, P_G, and R_D for N4 or N2 leaves was observed on July 10 (Fig. 4.8). For all treatments, N2 P_N and P_G rates were 75 and

52% lower than that of N4 leaves, whereas R_D rates were 53% higher in N2 leaves, relative to N4 leaves on July 10. T1 and T2 N4 leaves had higher photosynthetic rates than T3-5 on July 26 (41 and 34 %, for P_N and P_G, respectively). No effect of irrigation was observed in N2 leaves on this date for P_N and P_G; however, N4 to N2 % declines ranged from 72 to 38 % for P_N and 60 to 24 %, for P_G, respectively. On July 26, R_D % increases for N2, relative to N4 leaves ranged from 53 to 24 % for T1-5, respectively. On August 8, T1-2 N4 photosynthetic rates were highest. T3 N4 photosynthetic rates were 52 and 44 % lower than T1-2, for P_N and P_G, respectively. T4-5 N4 photosynthetic rates were the lowest and were 82 and 70 % lower than T1-2, for P_N and P_G, respectively. For N2 leaves on August 8, T1 had the highest P_N and P_G values. T2 and T3-5 P_N rates were 29 and 70% lower than T1, respectively. Similarly, P_G rates for N2 leaves showed a strong response to irrigation treatment. Specifically, T1 had the highest rates, followed by T2 (an 18% decrease). T4-5 were similar to each other, and were ~60% lower than T1. R_D rates were not significantly different between N2 and N4 leaves in treatments 1, 3, and 4 on August 8. In T2, N2 respiration was 36 % higher than for N4, whereas in T5, R_D rates were 23 % lower for N2 leaves relative to N4 leaves (Fig. 4.8).

Chlorophyll Fluorescence

No effect of irrigation treatment was observed in N4 or N2 leaves for F_v/F_m, Φ_{PSII}, or ETR on July 10 or 26 (Fig. 4.9); however, F_v/F_m in T3-5 N4 leaves was slightly higher than T1-2 (1.2 %; P=0.006). Similarly, N4 F_v/F_m values were marginally higher in T3-5, compared to T1-2 (~2 %; P<0.001) on August 8. This marginal increase in F_v/F_m was also observed in N2 leaves for July 26 and August 8 for T3-5, relative to T1-2. N4 Φ_{PSII} values for T1-3 were 27 % higher than T4-5 on August 8. Electron transport rates of N4 leaves separated into two distinct groups (T1-3 and T4-5; $P<0.0001$). Specifically, T4-5 ETR were 27 % lower than T1-3. No effect of irrigation treatment was found for N2 leaf Φ_{PSII} or ETR for July 26 or August 8. N2 leaves had a ~2.6 % lower F_v/F_m than N4 leaves on all sample dates. For Φ_{PSII} and ETR on July 26, N4 to N2 decreases were similar across all irrigation treatments (52 and 43 %, for Φ_{PSII} and ETR, respectively). August 8 N4 to N2 percent decreases were similar for both Φ_{PSII} and ETR. Specifically, decreases were greatest within T2 (48 % for both parameters); a 31% difference in Φ_{PSII} and ETR was observed between N2 and N4 leaves in in T5.

Photosystem II thermotolerance assessment

Maximum quantum yield of electron transport through photosystem II (F_v/F_m) response to incubation temperature is illustrated in Figure 4.10. Trends were similar for both age classes from 30 to 45°C, although small significant differences were observed (2 % or less). On July 10 and August 8, F_v/F_m at 50°C was distinctly lower in N4 than in N2 leaves in all treatments. No significant differences between age classes were observed at 50°C for July 26. For all sample dates, within each age class, no effect of irrigation treatment was observed for T_{15}. Similar to the trends reported above, T_{15} differences were observed between age classes for all irrigation treatments on July 10 (Fig. 4.11). Differences were greatest in T5 (2.3°C higher in N2 than in N4) and lowest in T2 (0.8°C higher in N2 than in N4). No differences between leaf ages were observed on July 26 for any irrigation treatment. On August 8, N2 T_{15} values were higher than for N4 leaves in all irrigation treatments, save T4?. Differences were greatest in T2 (4.2°C difference in T_{15}). T1, T3, and T5 N2 leaf T_{15} values were 2.9, 3.2, and 2.8°C higher than that of N4 leaves, within each treatment.

Discussion

TheN2 leaves were more photosynthetically tolerant to the combined effects of drought and high temperature than N4 leaves. In addition to increased heat tolerance of primary photochemistry for young leaves sampled early in the growing season (as measured by chlorophyll *a* fluorescence; Fig 3.10, 3.11), for the first time with field-grown cotton, we demonstrate that N2 leaf P_N is not negatively impacted by leaf temperatures well-above the 35 °C threshold known to (Fig. 4.7A, B), inhibit P_N in fully-expanded leaves (Law & Crafts-Brandner 1999; Crafts-Brandner & Salvucci 2000; Salvucci & Crafts-Brandner 2004; Wise et al. 2004; Bibi et al. 2008).

For example, early in the growing season (July 10), T_{15} values for N2 leaves were 0.8 – 2.3°C higher than for N4 leaves (Fig. 4.11, A). This effect was not seen on July 26, similar to a report by Hall et al. (2014). This loss of a difference between leaf expansion stages appears to be due to a ~ 1.3°C increase in N4 T_{15} values, with little to no change in N2 leaves. These effects of leaf development and sample time are particularly evident in the temperature response curves shown in Figure 4.10. Specifically, F_v/F_m at 50°C was 31 to 66% greater in N2 leaves, when compared to N4 leaves (July 10; Fig. 4.10 A-E). On July 26, N2 and N4 leaves did not differ in F_v/F_m at 50°C (Fig. 4.10 F-J). Late in the growing season (August 8), PSII was again more heat tolerant in N2, relative to N4 leaves, primarily due to increased T_{15} in N2 leaves (Fig. 4.11C). T_{15} values were unresponsive to irrigation treatment (Fig. 4.11), indicating that PSII heat tolerance may be inherent to young leaves in general, rather than the result of drought induced temperature extremes. These findings are comparable to those of Hall et al. (2014) who observed that young cotton leaves had an optimal range (of light adapted primary photochemistry; Φ_{PSII}) from 30 to 40°C, whereas the optimum temperature range of more expanded leaves was from 25-35°C, when sampled early in the growing season. Later in the growing season, fully expanded leaf thermal optima shifted to a higher temperature range (30-40°C). Although one could argue that the increase in heat tolerance for mature leaves later during the growing season may be indicative of an acclimation response to higher summer temperatures (Snider et al. 2013), it should be noted that plants were exposed to similar ambient temperature conditions prior to sampling on July 10 and July 26 (Fig. 4.1). Thus, the increase in heat tolerance of fully expanded leaves from July 10 to July 26 may be dependent upon the stage of plant development. In support of this, Snider et al. (2014) demonstrated similar seasonal variation in heat tolerance for fully expanded leaves of field-grown *G. hirsutum* despite exposure to optimal temperature conditions and water availability throughout the season.

On the first two sample dates (July 10 and August 8) and combined data for all three sample dates , leaf temperature was a poor predictor of P_N for N2 leaves (Fig. 4.7 A, B, D). Specifically, over a T_{Leaf} range of 31 to 37°C, no relationship with P_N could be determined (Fig. 4.7 B) In contrast, N4 leaf P_N responded negatively as leaf temperature increased for July 26 and August 10, as well as for data combined from all sample dates (Fig. 4.7 B, C, D). Specifically, on July 26, as T_{Leaf} increased from 31 to 37°C, N4 P_N declined by 66% (Fig. 4.7B). These N4 P_N declines were strongly associated with irrigation treatment, whereas N2 leaves were not affected (Fig. 4.8 B, E). As drought severity increased CO_2 exchange became more similar between leaf age classes. Specifically, on July 26, T1-5 N4 to N2 % declines ranged from 72 to 38 % for PN and 60 to 24 %, for PG, respectively. While P_N was generally low (~ 10 µmol m^{-2} s^{-1}) for N2 leaves, this lack of a negative response under drought conditions suggest N2 leaves are more drought tolerant or buffered from water deficit conditions than N4 leaves on the same plant.

Low rainfall (Table 1; Fig. 4.2) coupled with our plant-based approach to irrigation scheduling resulted in increasing separation in Ψ_L (Fig. 4.3) amongst treatments as the season progressed. Early in the growing season (July 10) T1-4 Ψ_L were similar, whereas T5 exhibited marginally higher Ψ_L values (Fig. 4.3 A, D). On this date, Ψ_{PD} values were well above those shown to negatively affect leaf metabolism in cotton (Chastain et al. 2014; Snider et al. 2014). Consequently, leaves from all irrigation treatments responded similarly in terms of g_s, T_{leaf}, P_N, P_G, R_D, in situ primary photochemistry, and PSII heat tolerance, within a leaf age class. As the season progressed, T1-2 maintained similar water potentials, whereas, in T3-5, water stress became more severe (Fig. 4.3 B, C, E, F). For N4 leaves, Ψ_{MD} wasa strong predictor of g_s for July 26 and August 8 (Fig. 4.4 B, C), indicating that irrigation treatments were driving T_{Leaf} differences. In contrast, N2 leaves maintained low g_s, (< 0.3 mol m^{-2} s^{-1}) and high T_{Leaf} (greater than air temperature) values for July 10 and 26 (Fig. 6A, B, D, E). Interestingly, g_s was shown to be a very strong predictor of

T_{Leaf}, T_{Leaf} - T_{Air}, as well as LAVPD for all leaves during the entire growing season, regardless of mainstem node of attachment (Fig 3.5 D, H, L), indicating that T_{leaf} for N2 leaves responded to g_s in a similar as N4 leaves, albeit over a narrower range.

N4 leaf gas exchange trends in response to drought were in agreement with other reports on drought stress effects in field-grown cotton (Hsiao 1973; Turner et al. 1986; Ennahli & Earl 2005; Zhang et al. 2011; Chastain et al. 2014). Specifically, when separation among irrigation treatments was observed (in terms of predawn and midday leaf water potential; Fig. 4.3), g_s, P_N, and P_G decreased in treatments with lower water status (Fig. 4.6 B; Fig. 4.8 B, E). In addition, R_D was shown to increase in response to drought (Fig. 4.8 I). One inherent limitation in the current study is that respiration rates were calculated based on a single Q_{10} value for all treatments. Recent work indicates that Q_{10} can vary widely depending upon prior growth conditions (Atkin & Tjoelker 2003), so interpretation of the respiration data provided herein should be treated with caution. However, it was not logistically feasible to determine Q_{10} for every treatment in the current study; therefore, the method described in Valentini et al. (1995) represented the most feasible approach to characterize R_D for a study of this size.

No biologically relevant effect of irrigation treatment was seen for in-situ chlorophyll fluorescence parameters for July 10 and 26 for either age class, which is in agreement with multiple other studies (Kitao & Lei 2007; Massacci et al. 2008; Zhang et al. 2011; Snider et al. 2013; Chastain et al. 2014; Snider et al. 2014), strengthening the argument that primary photochemistry is not a limitation to P_N under drought. In contrast, N2 leaves were relatively unresponsive to irrigation treatment on July 26. With the exception of an ~52% decrease in g_s and a 16-20 % increase in T_{Leaf} for N2, relative to N4 leaves on July 26, N2 leaf physiology remained stable across irrigation treatments. Specifically, no treatment effect was observed for P_N, P_G, or R_D on July 26 for N2 leaves, and in situ primary photochemistry was also unaffected on any biologically relevant level.

Our late season sample date (August 8) had multiple interesting responses in conflict with what we observed earlier in the growing season. In particular, Ψ_{MD} showed a strong relationship with g_s on this date for N2 leaves (Fig. 4.4 C). In addition, T_{Leaf} and P_N were also strongly related for N2 leaves (Fig. 4.7 C). For previous sample dates, these relationships were not observed. Interestingly, light-adapted, in situ primary photochemistry responded negatively as drought stress increased (Fig. 4.9 C, F, I). Similar declines have been reported for cotton (Pettigrew 2004; Ennahli & Earl 2005; Carmo-Silva et al. 2012); however, most reports indicate little to no response of these parameters to drought stress for field-grown cotton (Kitao & Lei 2007; Massacci et al. 2008; Zhang et al. 2011; Snider et al. 2013; Chastain et al. 2014; Snider et al. 2014). We propose that decreased leaf expansion rates and rate of mainstem node addition (Krieg & Sung 1986) was likely a confounding factor in assessing cotton's developmental response to drought and likely confounds the effects observed in other studies assessing the impact of drought on physiological processes. Specifically, when investigating the physiological effects of drought on a leaf area basis (P_N for example), researchers typically select the uppermost, fully expanded leaf for measurements (Snider et al. 2013; Chastain et al. 2014; Snider et al. 2014). As stated previously, for the period beginning June 24 and ending August 08 (a time frame encompassing all sample dates), total rainfall amounted to 3.3 cm, or 9.5% of total rainfall for the entire growing season. This had a pronounced effect on growth. Loss of turgor in drought stressed plants and the resultant effect on cell expansion (Hsiao 1973) can introduce error when attempting to make comparisons across irrigation treatments. For instance, on August 8, N2 to N4 leaf characteristic differences were seen less often in T3-5, when compared to dates earlier in the growing season. Specifically, leaf thickness, specific leaf weight, chlorophyll b, and total chlorophylls per unit leaf volume were more similar between age classes (Table 3.2). Of particular importance is the fact that T3 to T5 exhibited a decline in the rate of mainstem node addition from July 22 through August 10 (data not shown). Specifically, T3-5 plants were ~ 25 % shorter, and had ~ 20 % fewer mainstem nodes (data not shown) on August 8, 2014. Thus, the decline in ETR observed under drought conditions may have also been a function of leaf age since cotton leaves are known reach peak P_N at ~19 days after unfurling (Constable & Rawson 1980). Thus, leaves exposed to chronic drought stress and sampled on August 8, differed in leaf morphological characteristics and likely age, relative to leaves that were sampled earlier in the season.

Conclusion

In our current study, we showed that young cotton leaves are more tolerant, both in primary photochemistry and carbon reactions to the combined effects of drought and high temperature that are common under field conditions. The drastic differences in heat and drought tolerance between the two different stages of leaf development on the same plant is promising in that, researchers may be able to improve overall tolerance to heat and drought by regulating pre-existing genes within the same genotype. Furthermore, the increase in heat tolerance of more expanded leaves as the season progressed may offer additional evidence of a plant growth stage-dependent alteration in photosystem II thermotolerance. Finally, this study emphasizes the fact that long-term drought can alter plant growth such that single-leaf physiological response to drought may be confounded with differences in leaf age and characteristics at the time of sampling, indicating that care should be taken when attempting to interpret single-leaf responses of plants exposed to chronic drought stress.

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Figure Captions

Figure 4.1 Maximum quantum yield of photosystem II (F_v/F_m) versus incubation temperature for individual leaves of *Gossypium hirsutum* cv. PHY 499 on July 26, 2014. T_{15} represents the temperature causing a 15% decline from the maximum observed F_v/F_m value and is represented by an open circle in each graph. In both graphs, closed circles represent measured F_v/F_m values after a five minute incubation time at each temperature. In B, T_{15} was extrapolated to a 15% decline in F_v/F_m using third-order polynomial regression.

Figure 4.2 Daily maximum (T_{max}) and minimum (T_{min}) air temperatures, as well as daily precipitation throughout the 2014 growing season for Stripling Irrigation Research Park near Camilla, Georgia. Sample dates are shown as downward-facing arrows.

Figure 4.3 Predawn (Ψ_{PD}) and Midday (Ψ_{MD}) leaf water potential for five different irrigation treatments (T1 through T5) by treatment for July 10 (Left), July 26 (Center), and August 8, 2014 (Right), for *Gossypium hirsutum* grown near Camilla, GA. Bars sharing a letter are not different (LSD; $P < 0.05$). Bars represent means ± SE (n = 8).

Figure 4.4 Stomatal conductance (g_s) versus midday leaf water potential (Ψ_{MD} of N4 leaves) for N4 (closed circles) and N2 (open circles) leaves on July 10 (A), July 26 (B), and August 8, 2014 (C) for *Gossypium hirsutum* grown near Camilla. Panel D represents all sample dates combined. Regressions with coefficients of determination below 0.5 are not shown. Each point represents a replicate plot (N = 80 for A-C; N = 240 for D).

Figure 4.5 Stomatal conductance (g_s) versus midday leaf temperature (T_{leaf}), leaf-to-air temperature differential ($T_{leaf} – T_{air}$), and leaf to air vapor pressure deficit (LAVPD) for N4 (closed circles) and N2 (open circles) leaves on July 10 (A-I), July 26 (B-J), and August 8, 2014 (C-K) for *Gossypium hirsutum* grown near Camilla. Panels D through H represent all sample dates combined. Regressions with coefficients of determination below 0.5 are not shown. Each point represents a replicate plot (N = 80 for left three columns; N = 240 for far right column).

Figure 4.6 Stomatal conductance to water vapor (g_s; A, B, C), leaf surface temperature (T_{leaf}; D, E, F), and leaf to air temperature differential ($T_{leaf} – T_{air}$; G, H, I) for N4 (black bars) and N2 (grey bars) leaves on July 10 (Left), July 26 (Center), and August 8, 2014 (Right) for *Gossypium hirsutum* grown near Camilla. Within a given treatment and date, differences between N4 and N2 (Paired t-test, $P < 0.05$) are denoted with asterisks. Data are means ± SE (n = 8).

Figure 4.7 Net photosynthesis (P_N) response to leaf temperature for N4 (closed circles) and N2 (open circles) leaves on July 10 (A), July 26 (B), and August 8, 2014 (C) for *Gossypium hirsutum* grown near Camilla. Panel D represents all sample dates combined. Regressions with coefficients of determination below 0.5 are not shown. Each point represents a replicate plot (N = 80 for A-C; N = 240 for D).

Figure 4.8 Net photosynthesis (P_N; A, B, C), gross photosynthesis (P_G; D, E, F), and midday dark respiration (R_D; H, I, J) for N4 (black bars) and N2 (grey bars) leaves on July 10 (Left), July 26 (Center), and August 8, 2014 (Right) for *Gossypium hirsutum* grown near Camilla. Within a given treatment and date, differences between N4 and N2 leaves (Paired t-test, $P < 0.05$) are denoted with asterisks. Data are means ± SE (n = 8).

Figure 4.9 Maximum (A, B, C) and actual (D, E, F) quantum yield of electron transport through photosystem II, and electron transport rate through photosystem II (G, H, I) for N4 (black bars) and N2 (grey bars) leaves on July 10 (Left), July 26 (Center), and August 8, 2014 (Right) for *Gossypium hirsutum* grown near Camilla. Within a given treatment and date, statistical differences between N4 and N2 (Paired t-test, $P < 0.05$) are denoted with asterisks. Data are means ± SE (n = 8).

Figure 4.10 The response of maximum quantum yield of photosystem II (F_v/F_m) to leaf temperature for *Gossypium hirsutum* grown near Camilla in 2014. Columns 1 (far left) to 5 (far right) represent irrigation treatments 1 to 5, respectively. Rows 1-3 represent July 10, July 26, and August 8, respectively. Within each treatment and temperature, significant differences between N4 (black circles) and N2 (open circles) leaves are indicated by asterisks (paired T-test, $P < 0.05$). Data are means ± SE ($n = 8$).

Figure 4.11 T_{15} values for all irrigation treatments on July 10 (A), July 26 (B), and August 8, 2014 (C) for *Gossypium hirsutum* grown near Camilla. Within each treatment, significant differences between N4 (black bars) and N2 (grey bars) leaves are indicated by asterisks (paired T-test, $P < 0.05$). Data are means ± SE ($n = 8$). T_{15} is the above-optimum temperature causing a 15% decline in F_v/F_m relative to the optimum temperature (30°C).

Tables

Table 4.1. 2014 season-long irrigation amount, rainfall, and total water received by each irrigation treatment for *Gossypium hirsutum* grown near Camilla, Georgia.

Treatment	Irrigation (cm)	Rainfall (cm)	Total water (cm)
T1	29.9	34.9	64.8
T2	23.7	34.9	58.6
T3	14.2	34.9	49.1
T4	10.0	34.9	44.9
T5	0	34.9	34.9

Table 4.2. Leaf area, leaf thickness, specific leaf weight, chlorophyll a, b, and total chlorophyll per unit leaf area, chlorophyll a/b ratio, and total chlorophyll per unit leaf volume for *Gossypium hirsutum* grown near Camilla, Georgia in 2014. Asterisks denote statistically significant differences (paired t-test, $P < 0.05$) between leaf nodes of attachment within a given date and treatment. Data are means ± SE ($n = 8$).

Date	Treatment	Age	Leaf Area (cm2)	Thickness (mm)	SLW (g/m2)	Chlorophyll a (µg/cm2)	Chlorophyll b (µg/cm2)	Chlorophyll (µg/cm2)	Chlorophyll a/b	Chlorophyll (µg/cm^3)
7/10/2014	T1	N4	*152.96 ± 4.98	*0.26 ± 0.01	*54.50 ± 1.68	*24.82 ± 0.78	*9.99 ± 0.38	*34.80 ± 1.14	*2.49 ± 0.05	*137.32 ± 7.03
		N2	33.92 ± 3.20	0.14 ± 0.00	45.70 ± 4.02	16.71 ± 0.81	8.01 ± 0.46	24.72 ± 1.24	2.10 ± 0.06	174.27 ± 7.72
	T2	N4	*172.38 ± 8.92	*0.27 ± 0.01	*55.68 ± 4.56	*23.83 ± 0.94	*9.63 ± 0.40	*33.47 ± 1.30	*2.48 ± 0.05	*127.88 ± 7.45
		N2	37.61 ± 3.98	0.15 ± 0.01	43.99 ± 2.70	16.65 ± 0.66	8.23 ± 0.20	24.87 ± 0.79	2.03 ± 0.07	172.99 ± 14.68
	T3	N4	*160.88 ± 9.69	*0.25 ± 0.01	*62.38 ± 3.58	*23.13 ± 0.40	*9.27 ± 0.25	*32.40 ± 0.63	*2.50 ± 0.04	*130.78 ± 5.35
		N2	40.97 ± 3.39	0.14 ± 0.00	43.56 ± 2.65	17.08 ± 0.73	8.23 ± 0.40	25.31 ± 1.11	2.08 ± 0.04	188.93 ± 12.76
	T4	N4	*147.78 ± 9.09	*0.24 ± 0.01	58.84 ± 2.51	*21.96 ± 0.52	9.22 ± 0.36	*31.18 ± 0.73	*2.40 ± 0.08	*128.93 ± 4.38
		N2	37.85 ± 2.58	0.15 ± 0.00	58.35 ± 14.2	16.86 ± 0.96	8.35 ± 0.45	25.21 ± 1.39	2.02 ± 0.05	174.95 ± 12.79
	T5	N4	*150.25 ± 4.33	*0.23 ± 0.01	*57.89 ± 2.25	*26.54 ± 1.30	*11.21 ± 0.71	*37.75 ± 2.01	*2.39 ± 0.05	*164.18 ± 12.73
		N2	35.22 ± 3.09	0.15 ± 0.01	47.51 ± 2.92	18.43 ± 0.92	8.93 ± 0.49	27.36 ± 1.40	2.07 ± 0.03	186.89 ± 13.56
7/26/2014	T1	N4	*158.00 ± 10.3	*0.19 ± 0.01	*49.93 ± 2.25	*20.92 ± 0.84	*8.77 ± 0.31	*29.69 ± 1.09	*2.39 ± 0.06	*155.12 ± 6.33
		N2	29.25 ± 1.58	0.14 ± 0.01	77.36 ± 7.21	12.45 ± 0.14	6.22 ± 0.29	18.67 ± 0.38	2.02 ± 0.07	136.44 ± 7.01
	T2	N4	*145.38 ± 3.94	*0.18 ± 0.00	*55.66 ± 1.82	*21.74 ± 1.11	*8.99 ± 0.56	*30.73 ± 1.65	*2.43 ± 0.05	*172.20 ± 9.66
		N2	30.38 ± 1.35	0.13 ± 0.00	71.93 ± 5.50	12.15 ± 0.63	6.01 ± 0.44	18.16 ± 1.04	2.04 ± 0.06	140.97 ± 9.29
	T3	N4	*133.13 ± 3.93	*0.22 ± 0.01	66.77 ± 5.22	*24.30 ± 1.31	*10.06 ± 0.63	*34.36 ± 1.93	*2.43 ± 0.04	155.90 ± 11.71
		N2	29.50 ± 1.21	0.15 ± 0.00	77.72 ± 4.73	15.65 ± 0.88	7.69 ± 0.52	23.34 ± 1.39	2.05 ± 0.04	161.57 ± 10.76
	T4	N4	*138.38 ± 5.12	*0.21 ± 0.01	*59.55 ± 2.34	*25.95 ± 1.12	*10.99 ± 0.65	*36.93 ± 1.75	*2.38 ± 0.05	173.98 ± 11.29
		N2	31.00 ± 1.61	0.15 ± 0.00	77.75 ± 6.91	15.76 ± 0.48	7.72 ± 0.31	23.49 ± 0.74	2.05 ± 0.06	162.62 ± 7.34
	T5	N4	*136.38 ± 8.42	*0.21 ± 0.01	65.53 ± 6.47	*25.72 ± 0.74	*10.76 ± 0.37	*36.48 ± 1.10	*2.39 ± 0.03	172.93 ± 5.21
		N2	29.88 ± 1.83	0.14 ± 0.00	83.35 ± 7.62	16.70 ± 0.90	8.51 ± 0.51	25.22 ± 1.40	1.97 ± 0.03	186.83 ± 12.11
8/8/2014	T1	N4	*142.63 ± 8.25	0.19 ± 0.02	*73.56 ± 3.94	*28.43 ± 1.01	*12.79 ± 0.54	*41.21 ± 1.44	*2.23 ± 0.06	*229.20 ± 20.09
		N2	33.75 ± 1.59	0.17 ± 0.02	48.25 ± 5.74	17.15 ± 0.42	9.54 ± 0.20	26.69 ± 0.53	1.80 ± 0.05	165.81 ± 13.53
	T2	N4	*144.00 ± 7.63	0.20 ± 0.02	69.92 ± 1.84	*28.67 ± 0.79	*12.65 ± 0.51	*41.32 ± 1.25	*2.28 ± 0.05	223.88 ± 20.18
		N2	28.75 ± 1.98	0.18 ± 0.02	62.45 ± 6.52	18.53 ± 0.84	10.07 ± 0.39	28.61 ± 1.09	1.85 ± 0.08	163.60 ± 13.52
	T3	N4	*124.43 ± 8.97	*0.23 ± 0.01	69.19 ± 3.19	*32.41 ± 0.52	13.78 ± 0.46	*46.18 ± 0.92	*2.36 ± 0.05	182.14 ± 29.67
		N2	29.13 ± 2.14	0.17 ± 0.01	69.93 ± 6.34	24.52 ± 0.90	12.53 ± 0.63	37.05 ± 1.49	1.97 ± 0.05	221.46 ± 17.04
	T4	N4	*120.38 ± 4.44	0.20 ± 0.01	68.64 ± 2.38	*31.68 ± 1.27	13.86 ± 0.60	*45.54 ± 1.80	*2.29 ± 0.06	244.21 ± 24.62
		N2	29.38 ± 1.91	0.18 ± 0.01	70.51 ± 4.01	27.07 ± 0.83	14.21 ± 0.60	41.29 ± 1.34	1.91 ± 0.05	232.19 ± 18.00
	T5	N4	*117.00 ± 6.76	0.21 ± 0.02	*70.56 ± 1.92	*32.40 ± 1.03	14.33 ± 0.44	*46.73 ± 1.34	*2.27 ± 0.06	231.83 ± 19.28
		N2	31.13 ± 2.04	0.17 ± 0.01	61.31 ± 6.06	26.25 ± 1.69	13.74 ± 0.44	39.99 ± 2.06	1.90 ± 0.08	244.23 ± 21.16

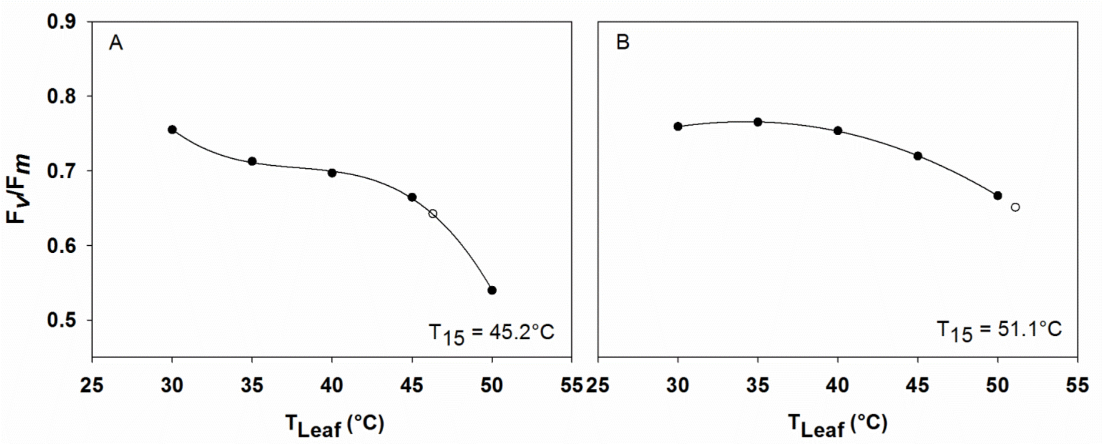
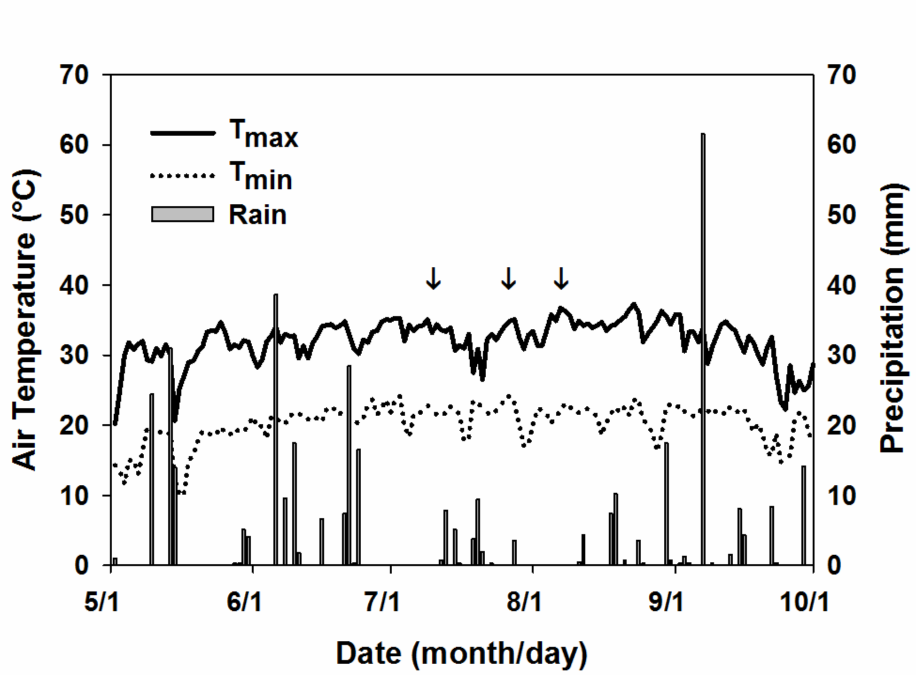

0.9
0.8
0.7
0.6
0.5
F_v/F_m
A
B
T_{15} = 45.2°C
T_{15} = 51.1°C
25
30
35
40
45
50
55
T_{Leaf} (°C)

Figure 4.1



70
60
50
40
30
20
10
0
Air Temperature (°C)
Tmax
Tmin
Rain
↓
↓
↓
70
60
50
40
30
20
10
0
Precipitation (mm)
5/1
6/1
7/1
8/1
9/1
10/1
Date (month/day)

Figure 4.2

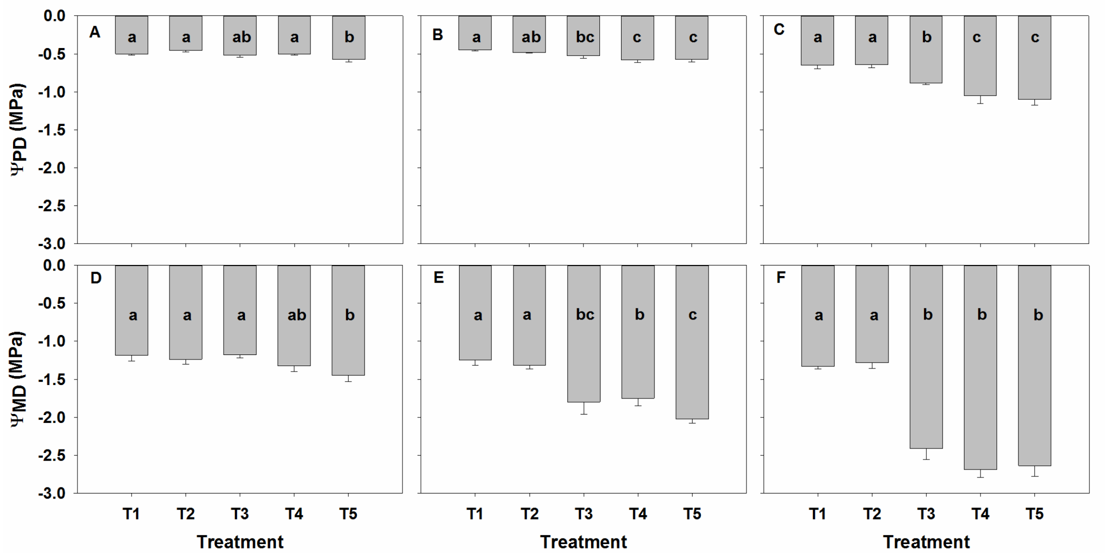

A
B
C
D
E
F
ΨPD (MPa)
ΨMD (MPa)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
a a ab a b
a ab bc c c
a a b c c
a a a ab b
a a bc b c
a a b b b
T1
T2
T3
T4
T5
Treatment

Figure 4.3

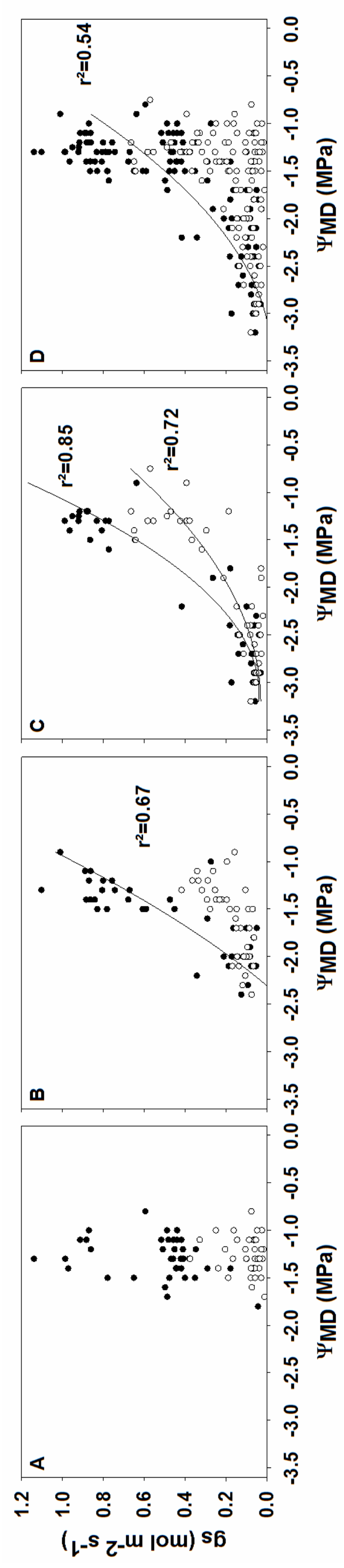

A
B
C
D
r²=0.67
r²=0.85
r²=0.72
r²=0.54
gs (mol m-2 s-1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-3.5
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
ΨMD (MPa)

Figure 4.4

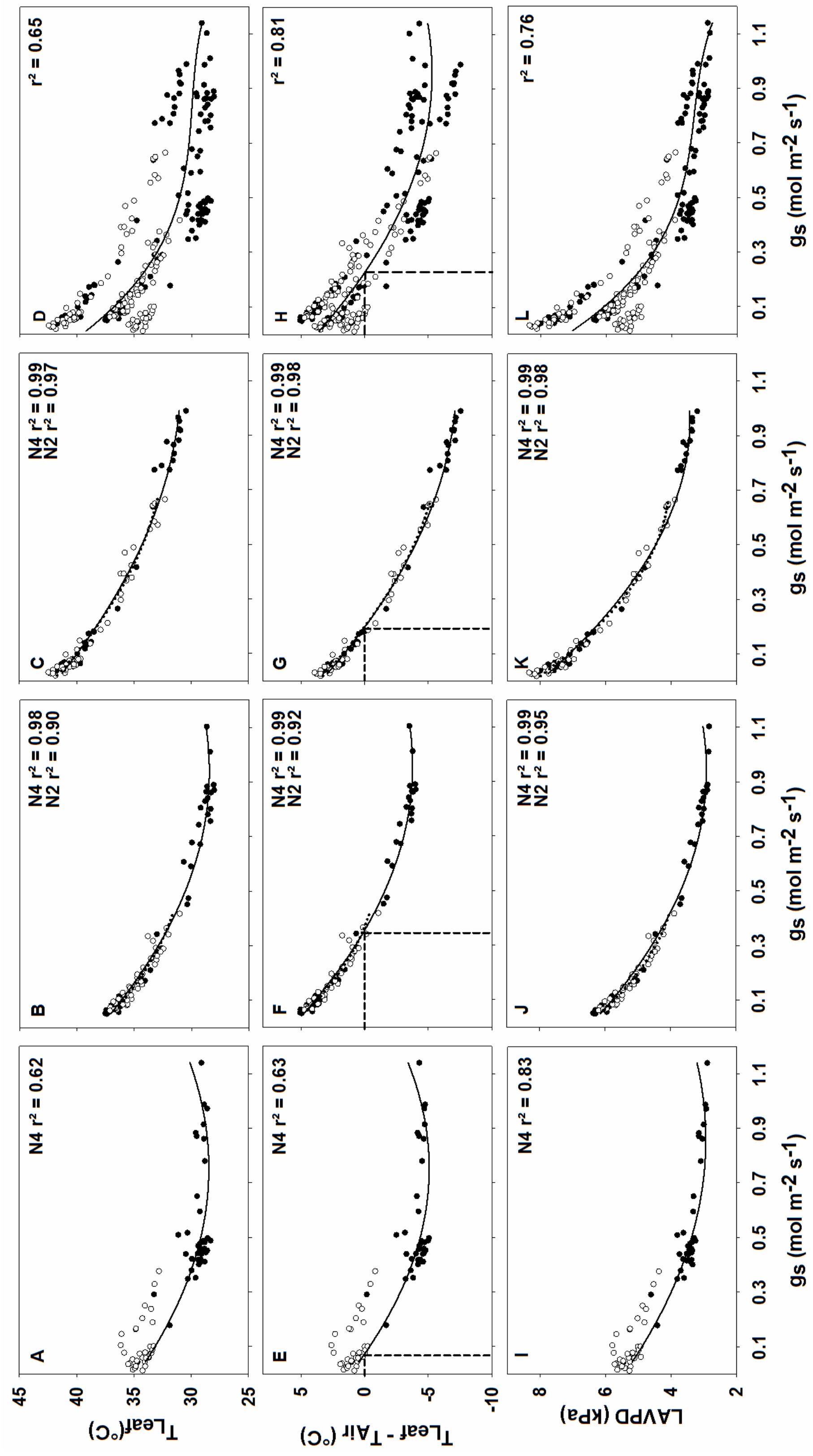

A
B
C
D
E
F
G
H
I
J
K
L
N4 r² = 0.62
N4 r² = 0.98
N2 r² = 0.90
N4 r² = 0.99
N2 r² = 0.97
r² = 0.65
N4 r² = 0.63
N4 r² = 0.99
N2 r² = 0.92
N4 r² = 0.99
N2 r² = 0.98
r² = 0.81
N4 r² = 0.83
N4 r² = 0.99
N2 r² = 0.95
N4 r² = 0.99
N2 r² = 0.98
r² = 0.76
TLeaf(°C)
45
40
35
30
25
TLeaf - TAir (°C)
5
0
-5
-10
LAVPD (kPa)
8
6
4
2
0.1
0.3
0.5
0.7
0.9
1.1
gs (mol m-2 s-1)

Figure 4.5

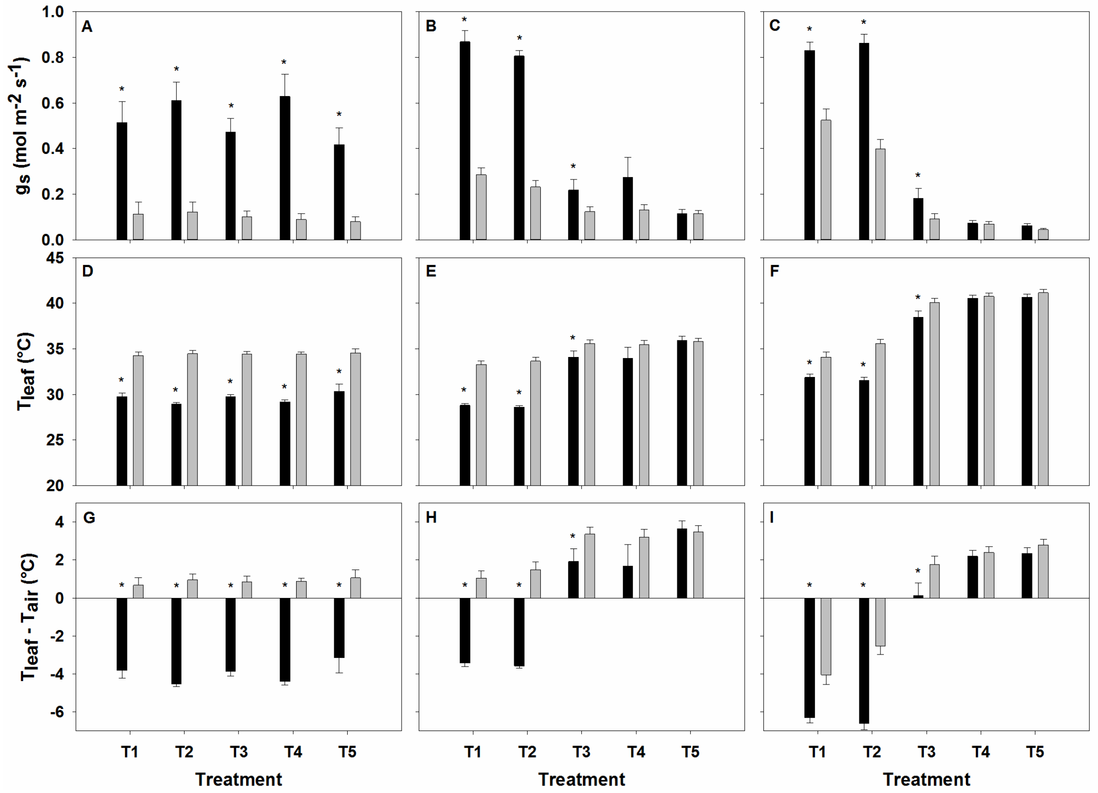

A
B
C
D
E
F
G
H
I
g_s (mol m^{-2} s^{-1})
T_{leaf} (°C)
T_{leaf} - T_{air} (°C)
1.0
0.8
0.6
0.4
0.2
0.0
45
40
35
30
25
20
4
2
0
-2
-4
-6
T1
T2
T3
T4
T5
Treatment
*

Figure 4.6

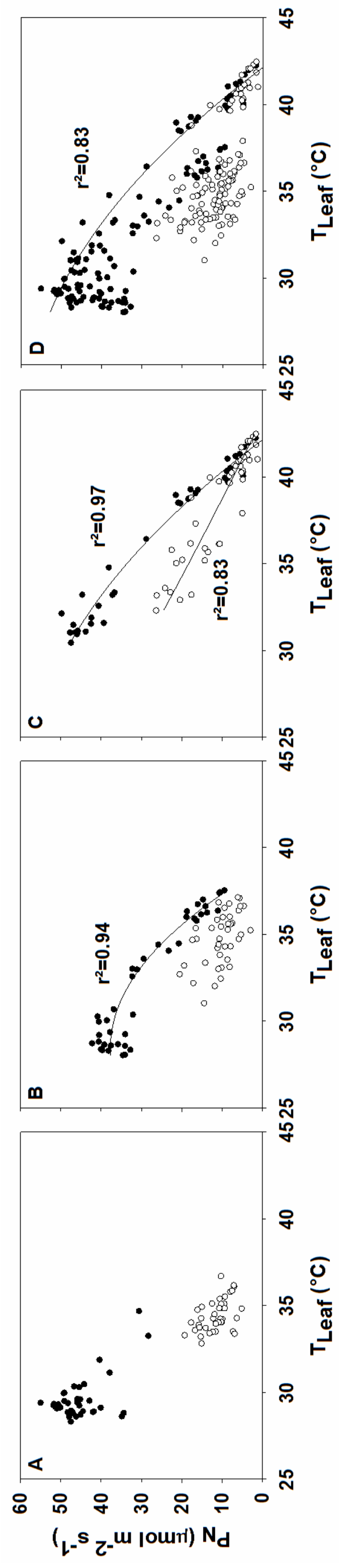

A
B
C
D
P_N (µmol m^{-2} s^{-1})
60
50
40
30
20
10
0
25
30
35
40
45
T_{Leaf} (°C)
r²=0.94
r²=0.97
r²=0.83
r²=0.83

Figure 4.7

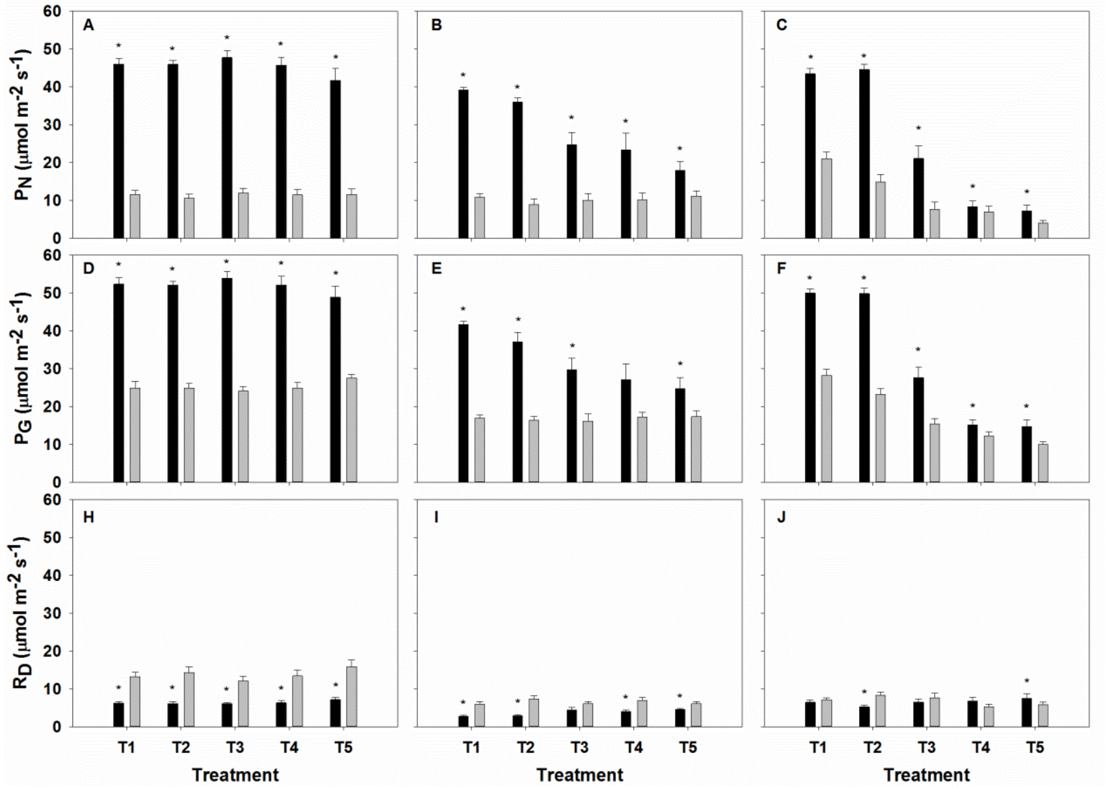

A
B
C
D
E
F
H
I
J
P_N (μmol m^{-2} s^{-1})
P_G (μmol m^{-2} s^{-1})
R_D (μmol m^{-2} s^{-1})
0
10
20
30
40
50
60
T1
T2
T3
T4
T5
Treatment
*

Figure 4.8

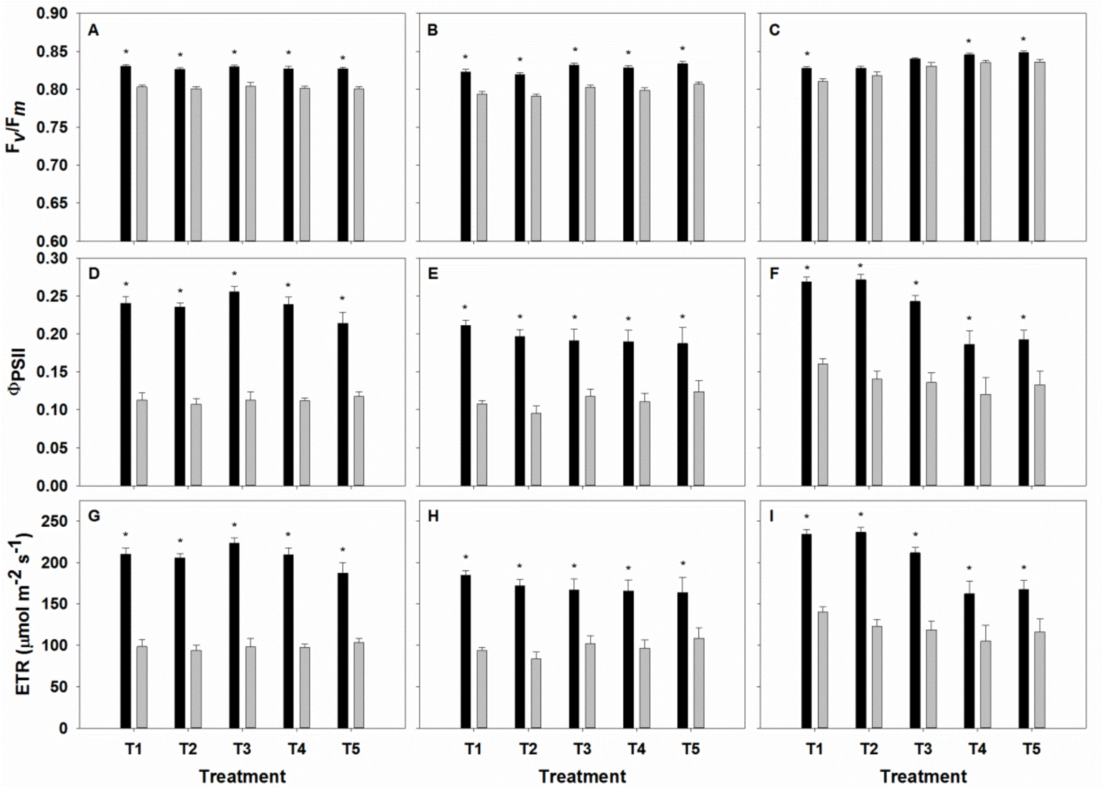

A
B
C
D
E
F
G
H
I
F_v/F_m
0.90
0.85
0.80
0.75
0.70
0.65
0.60
ΦPSII
0.30
0.25
0.20
0.15
0.10
0.05
0.00
ETR (μmol m^{-2} s^{-1})
250
200
150
100
50
0
*
T1
T2
T3
T4
T5
Treatment

Figure 4.9

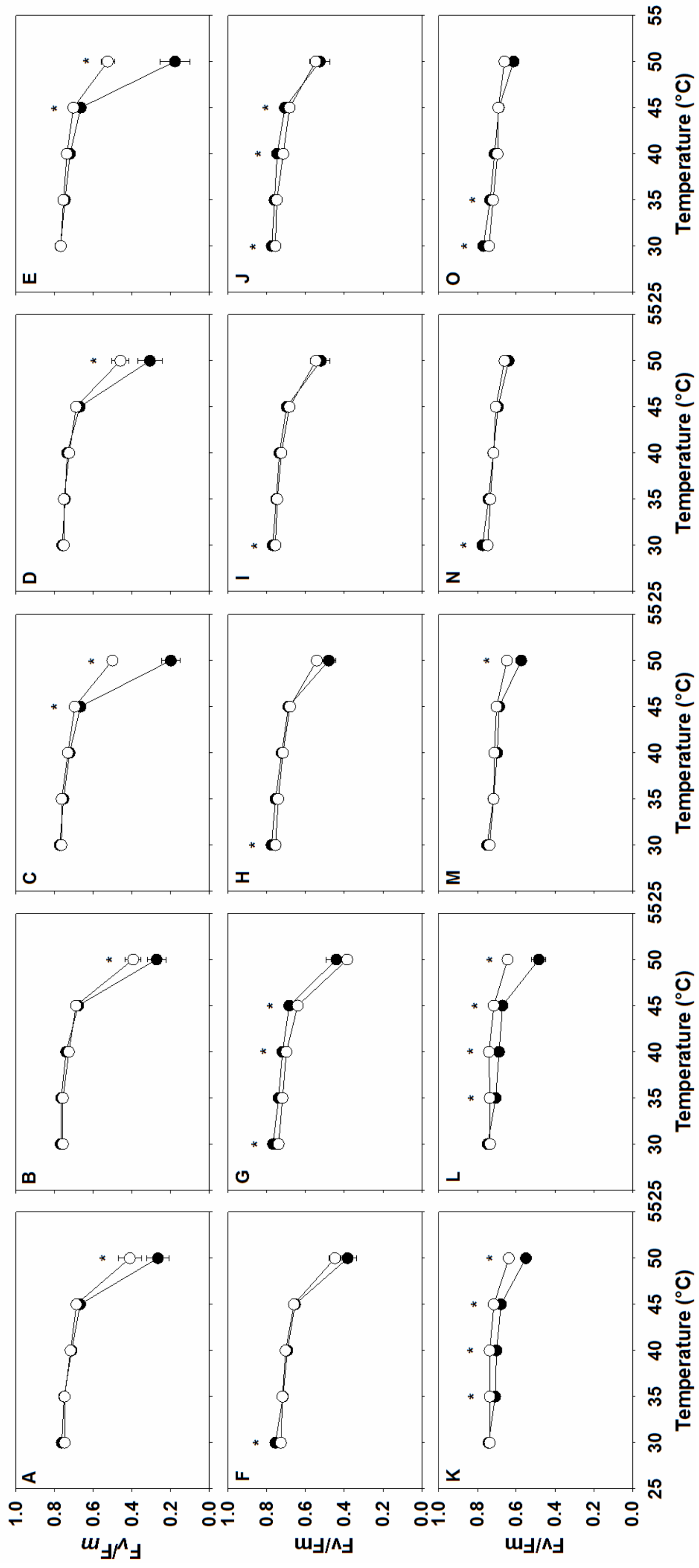

A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
Fv/Fm
1.0
0.8
0.6
0.4
0.2
0.0
Temperature (°C)
25
30
35
40
45
50
55
*

Figure 4.10

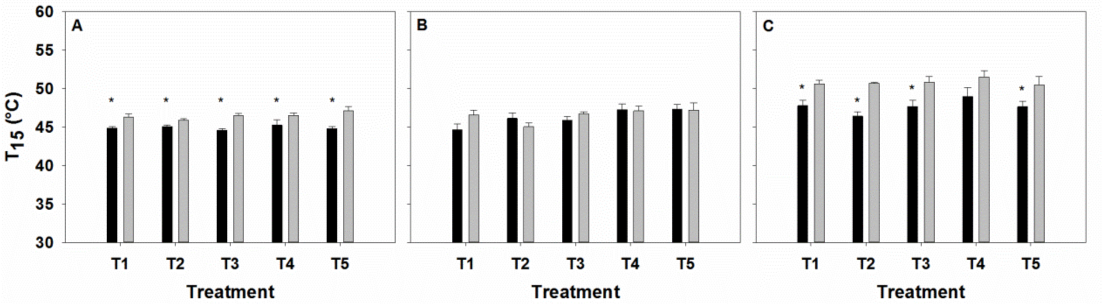


Figure 4.11

CHAPTER 5

CONCLUSION

In the current dissertation, three experiments were conducted under field conditions to assess 1) the underlying limitations to net photosynthesis under drought, 2) the potential for plant-based irrigation scheduling to improve water productivity in Georgia cotton production, and 3) the impact of leaf development on drought and heat tolerance.

For experiment 1, Ψ_{PD} ranged from -0.31 to -0.95 MPa, and Ψ_{MD} ranged from -1.02 to -2.67 MPa, depending upon irrigation regime and sample date during the 2012 and 2013 growing seasons. *G. hirsutum* responded to water deficit by decreasing stomatal conductance, increasing photorespiration, and increasing the ratio of dark respiration to gross photosynthesis, thereby limiting P_N and decreasing lint yield (lint yield declines observed during the 2012 growing season only). Conversely, even extreme water deficit, causing a 54% decline in P_N, did not negatively affect actual quantum yield, maximum quantum yield, or photosynthetic electron transport. It was concluded that P_N was primarily limited in drought-stressed *G. hirsutum* by decreased stomatal conductance, along with increases in respiratory and photorespiratory carbon losses, not inhibition or down-regulation of electron transport through photosystem II. It was further concluded that Ψ_{PD} is a reliable indicator of drought stress and the need for irrigation in field-grown cotton.

For experiment 2, we found that scheduling irrigation based on Ψ_{PD} produced similar yields to current practices, while decreasing overall water use from 7-31 %, depending upon rainfall levels and treatment utilized. When the cotton crop was exposed to drought (2014), using a -0.5 MPa Ψ_{PD} threshold produced the highest water productivity (40.7 kg ha^{-1} cm^{-1}), produced the highest yields (1995.2 kg ha^{-1}), and did not negatively impact fiber quality relative to traditional practices. In addition, decreased fiber yield in drought-stressed treatments was primarily due to decreased bolls m^{-2} (as much as a 43 % decline in severely stressed treatments). Using a well-watered baseline developed in 2013, we calculated a crop water stress index (CWSI) that exhibited a very strong, non-linear relationship with Ψ_{PD} values between ~-0.4 and -0.7 MPa ($r^2 = 0.81$). A strong, non-linear relationship was seen between CWSI and lint yield ($r^2 = 0.81$). Ψ_{PD} appears to be an effective means of determining the need for irrigation in cotton, and in the current study, yield and water productivity were maximized at a season-long average Ψ_{PD} threshold of -0.5 MPa.

For experiment 3, increased photosystem II thermotolerance was observed for young leaves sampled early in the growing season (as has been shown previously). Additionally, net photosynthesis (P_N) in young leaves was not negatively impacted by leaf temperatures well-above the 35 °C threshold known to limit P_N or by exposure to extreme drought. For example, in young leaves as leaf temperature increased from 31 to 37°C, no decline in P_N was observed. In contrast, P_N in more mature leaves declined by 66% over the same temperature range. High leaf temperatures were a function of plant water status in mature leaves but not in young leaves. The substantial differences in heat and drought tolerance between two different stages of leaf development may provide opportunities to improve tolerance to heat and drought by regulating pre-existing genes within the same genotype.