Improving water and plant growth regulator (PGR) management practices in cotton: Cultivar physiological responses and PGR rates

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) is grown in an increasingly unpredictable climate and often under rainfed conditions. Without irrigation to alleviate common drought conditions, cultivar selection becomes a primary tool in mitigating the negative effects of water-deficit stress. Cultivars can differ in water use under water-limited environments, which can affect crop performance. Moreover, cotton management must involve balancing vegetative and reproductive growth through the use of plant growth regulators (PGR) due to the plant's perennial growth pattern. The efficacy of PGRs are dependent on many variables including environmental conditions, irrigation, and genetics (cultivar). In 2020 and 2021, a series of growth chamber, greenhouse, and field studies were conducted to identify drought tolerance traits as well as physiological responses to variable rates of PGR among a variety of cotton cultivars. In the greenhouse, cultivar transpiration rates (TR) were measured in response to progressive soil drying. In growth chambers, cultivar TR and shoot hydraulic conductance (K_{shoot}) were measured in response to increasing vapor pressure deficit (VPD) and temperature. To establish a connection between the performance of the cotton cultivars under both progressive soil drying and high VPD environments, an extreme water-deficit stress field evaluation was performed in 2020. In a second field study in 2021, cultivar's stomatal conductance (g_s), plant height, wilting, relative water content (RWC), specific leaf area (SLA), and yield were measured in response to varying irrigation and PGR amounts. In the greenhouse, genotypic variation among cultivars contributed to differences in TR decline in response to soil. In the growth chamber, only one cultivar, PHY 400 W3FE, had expressed limited TR in response to

increasing VPD (TR_{lim}). Furthermore, cultivars differed significantly in their K_{shoot} response to high VPD at 38° [degree] C and correlations were measured between K_{shoot} and a variety of drought tolerance parameters. In the field studies, cultivars demonstrated their significant variation in physiological response to an extreme water-deficit stress as well as PGR application rates under varying irrigation amounts. The results of these studies provide a better understanding of the impact of cultivar selection as well as PGR rate response under varying water regimes in field-scale cotton production.

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CHAPTER I
INTRODUCTION

Review of Literature

Background

Cotton (*Gossypium hirsutum* L.) is an economically important crop grown for its natural fiber that is used globally in the textile market. Cotton is grown in the southern region of the United States where it is exposed to high temperature and evaporative demand throughout the growing season. The increasing variability in climate is threatening cotton production through more frequent temperature extremes, more significant and frequent soil water deficits, and greater irregularities in rainfall patterns (Zafar et al., 2018).

Approximately 64% of US and 88% of Tennessee cotton production is dryland, with no irrigation applied (USDA, 2019; UTIA, 2021). This leads cotton production to be especially susceptible to changes in climate, including high temperature and sustained periods of drought. This susceptibility was shown in 2011, when drought in Texas caused 55% of cotton fields to be abandoned and a subsequent financial loss of roughly $2.2 billion (NASS, 2012).

When irrigation is not available to alleviate drought stress, growers are often reliant on cultivar selection as the primary tool in mitigating the negative effects of drought. In response to ongoing changes in environmental conditions, the discovery and development of cotton cultivars that produce stable yield under variable climatic conditions, such as drought stress and high evaporative demand, can protect growers from economic losses and allow for the continued production of fiber for a growing population (Ullah et al., 2017).

The current trend in plant physiology research has involved a "genocentric" approach to evaluate plant response to the environment (Sinclair and Purcell, 2005). The over-emphasis of research to identify and understand gene regulation of complex physiological processes such as drought tolerance has led to disconnects between practical research contributing to improved plant production (Passioura, 2020). The approach of starting at the molecular level does not often account for the epigenetic response plants demonstrate when faced with environmental stress. The demonstrated existence of a genetic trait gives no assurance to the performance of that plant in a particular environment (Sinclair, 2011; Passioura, 2020). Abiotic stresses such as drought affect a plant throughout all levels of organization (e.g. cells, tissues, organs), and involves many feedbacks and feedforwards (Passioura, 2020). Therefore, the evaluation of complex traits should be completed under applied conditions that are relevant for real-world plant production.

Sinclair (2011) offers a 'top-down' approach as an avenue for bridging the disconnect between fundamental and practical plant physiology research. This approach starts with a whole-crop perspective to consider how the crop community may perform in the field across a range of environments. Observations on performance of plant physiology under stress is the starting point for research, rather than the pursuit of predicting the genetic role in whole plant physiological responses (Sinclair, 2011). This perspective on translational research has guided the majority of the methodology within this project.

Plant response to vapor pressure deficit

Atmospheric vapor pressure deficit (VPD) is a ratio between the two known values of the actual water vapor content of the atmosphere and the amount capable of existing at saturation under the same conditions (Anderson, 1936). From a plant perspective, VPD is the difference between vapor pressure within the leaf air space versus the vapor pressure of the outside air (Sinclair et al., 2017). If the difference in vapor pressure between leaf and air is large (>3 kPa), water vapor will rapidly diffuse out of a leaf and transpirational demand on a plant will be high (Sinclair et al., 2005; 2017) (Fig. 1; all tables and figures are located in the appendix after each chapter). Recent climate model projections indicate average VPD will increase roughly 20% over the next 50 years, which will increase crop water demand and potentially limit crop growth rate and productivity (Lobell et al., 2014). To exacerbate this issue, Lobell et al. (2014) found evidence that drought sensitivity in maize, particularly sensitivity to high VPD, has steadily increased over the past 18 years.

Stomata are the barrier between the atmospheric air and the inner air space of a leaf and are responsible for controlling water status within a plant while also allowing for gas exchange needed for photosynthesis (Lendzian, 2006). When stomata are open, water vapor within the leaf air space of the mesophyll is exchanged for atmospheric carbon dioxide. Diffusion of water vapor out of the leaf is the driving force behind transpiration (TR) and is regulated by stomatal opening and closing. In turn, the stomatal aperture is matched closely to the hydraulic flow of water from roots into leaves to avoid leaf desiccation (Attia et al., 2015). Therefore, plants that have the ability to close their

stomata early under high VPD conditions (VPD breakpoint) will limit transpiration (TR_{lim}), leading to a reduction in water use and conservation of soil water content (Shekoofa et al., 2013; Shekoofa and Sinclair, 2018, Sheldon et al., 2021; Sarkar et al., 2022). It is suggested that under extreme drought or high VPD conditions, restriction of stomatal conductance might increase photosynthetic return per unit of transpiration (Gholipoor et al., 2010; Carpentieri-Pipolo et al., 2012; Gaffney et al., 2015; Shekoofa et al., 2021; Sarkar et al., 2022). Sarkar et al. (2022) suggested that advanced mapping populations can reveal quantitative trait loci (QTLs) for drought-tolerance traits under complicated genetic control to enhance the TR_{lim} trait in a recombinant inbred line (RIL) soybean population. Validation of identified QTLs will be useful in molecular breeding of these favorable and informative QTL alleles for a superior cultivar with the ability to produce stable yield under water limiting conditions.

Genotypic variation in TR_{lim} response to high VPD has been measured among several crop species including: sorghum (*Sorghum bicolor*) (Gholipoor et al., 2010), maize (*Zea mays*) (Gholipoor et al., 2013; Shekoofa et al., 2016; Jafarikouhini et al., 2022), cowpea (*Vigna unguiculata*) (Belko et al., 2013), soybean (*Glycine max*) (Fletcher et al., 2007; Purdom et al., 2021; Sarkar et al., 2022), hemp (*Cannabis sativa* L.) (Sheldon et al., 2021), chickpea (*Cicer arietinum*) (Sivasakthi et al., 2020), and cotton (Devi and Reddy, 2018; Shekoofa et al., 2020). However, little research has investigated how the demonstration of TR_{lim} among cotton cultivars in a controlled environment translates to field conditions.

Plant response to soil drying

The impact of drought on plant growth is complex and involves feedback between stomata activity, plant hydraulic movement, and soil drying that work in network and parallel (Hayat et al., 2020). The physiological response to soil drying can lead to drought-induced loss of plant hydraulic conductance (K_{plant}), stomatal conductance (g_s), carbon assimilation, and plant productivity (Sperry and Love, 2015). Therefore, adequate soil water is imperative for the maintenance of plant physiological functions and yield performance.

To maintain adequate soil water, a plant must be able to adjust the transpiration rate (TR) in response to environmental conditions such as soil drying (Ray and Sinclair, 1997; King et al., 2009; Shekoofa et al., 2013; Shekoofa and Sinclair, 2018; Sadok et al., 2021). A common approach to documenting transpiration response to drying soil is to plot plant normalized transpiration rate (NTR) versus fraction of transpirable soil water (FTSW) through a soil drying cycle in a greenhouse setting with potted plants. Normalized transpiration rate is the daily TR of a given plant during progressive soil drying divided by the mean TR during the well-watered stage. Fraction of transpirable soil water is an expression of the available soil water remaining within a given pot (Ray and Sinclair, 1998). These graphs commonly show two phases in which TR is essentially equal to the rate of well-watered plants at high soil water content, and NTR decreases at low soil water content. This approach allows quantification of possible differences among genotypes in the FTSW at which the reduction in TR (i.e. breakpoint) occurs between the two phases (Beseli et al., 2020)

The earlier in the soil drying cycle a plant is able to limit its TR, the more soil water will be conserved, leading to an increase in water availability when the plant is under high water demand for yield production (Sinclair and Ludlow, 1985; Sinclair, 2012; Sinclair et al., 2018; Devi and Reddy, 2018; Purdom et al., 2021; 2022). Crop simulations of sorghum (Sinclair et al., 2005) and soybean (Sinclair et al., 2010) showed that reduced TR early in the growing season under water-deficit stress could result in yield increase of 75 to 80%. Therefore, the discovery of cotton cultivars with the ability to limit TR in response to soil drying could lead to yield increases given the majority of cotton production is under rainfed conditions and often experiences periods of drought throughout a growing season (NASS, 2019).

Plant hydraulic conductance

Hydraulic conductance through a plant shoot must work in conjunction with water loss from transpiration to avoid leaf desiccation (Meinzer and Grantz, 1990; Attia et al., 2015). A low plant hydraulic conductance would require stomatal closure to match the reduced water flux supplying the leaves (Choudhary et al., 2013; Sinclair, 2017). It has been hypothesized that under conditions of high VPD, plants with low hydraulic conductivity would be especially prone to stomatal closure to maintain leaf water potential (Bunce, 2006).

Choudhary et al. (2013) measured hydraulic limitation in two sorghum genotypes and demonstrated a decrease in leaf hydraulic conductance was associated with conservative water use by the whole plant during soil drying and under high atmospheric VPD. Additionally, the demonstration of TR_{lim} under high VPD in a drought tolerant

soybean genotype was found to likely be the result of restricted hydraulic conductance within the plant leaves (Sinclair, 2017). When cotton plants were exposed to a high temperature (38°C), the loss of TR_{lim} was measured in several cultivars that had previously showed TR_{lim} under a cooler temperature of 32°C (Shekoofa et al., 2020). The loss of a VPD breakpoint with a relatively small increase in temperature suggests that TR_{lim} may be a temperature-sensitive process which appears to be highly associated with changes in plant hydraulic conductance (Pradham et al., 2019). Under high temperatures, cultivars without the ability to restrict hydraulic conductance would allow water to readily replenish leaves, resulting in open stomata and unrestricted transpiration with increasing VPD (Sadok and Sinclair, 2010; Pradham et al., 2019; Li et al., 2019; Sarkar et al., 2022). Sarkar et al. (2022) reported that the TR response among soybean genotypes at 32°C did not necessarily predict expression of the trait at 37°C. At the higher temperature, the expression of TR_{lim} trait reduced to about 43, 10, and 0% for slow, moderate, and high wilting genotypes, respectively. This could be because the rest of the genotypes that expressed the TR_{lim} (i.e., VPD breakpoint) and low leaf hydraulic conductance at 32°C started to increase TR under 37°C to disperse excess temperature and heat.

Investigation into cotton cultivar difference in ability to restrict hydraulic conductance under varying temperature and VPD is therefore warranted. To date, the majority of research done on hydraulic conductance in row crops has focused heavily on leaf hydraulic conductance. A better understanding of the response of hydraulic

conductance in plant shoots to environmental changes is needed, given that plant stem and leaf function in conjunction for water transport.

Plant growth regulators and cotton

Appropriate plant architecture under varying water statuses, temperatures, and evaporative demands is important for cotton yield improvement (Biles and Cothren, 2001). Plant growth regulators (PGRs) are chemicals used to alter the growth of a plant or plant part (Gaba, 2005). The PGR mepiquat chloride (MC) is globally used in cotton plants structural manipulation to avoid excess vegetative growth and subsequent yield loss (Wang et al., 2014). Overall, MC benefits cotton production by helping to balance vegetative versus reproductive growth (Fig. 2) (Cook and Kennedy, 2000; Gwathmey and Clement, 2010).

Cotton plant response to application of MC is highly dependent on temperature and water availability. High temperatures and low soil water availability can decrease the assimilation and translocation of MC within a plant, leading to a reduction in efficacy (Rosolem et al., 2013). Therefore, appropriate timing and rate of MC application is essential when it comes to cotton management. However, making rate or timing decisions can be difficult because cultivar, irrigation, soil water content, growth stage, and nitrogen availability all impact response (Reddy et al., 1992; Rosolem et al., 2013; Zhao et al., 2017; Baio et al., 2018). With the use of irrigation for cotton production increasing in the Mid-South of the USA (Vories and Evett, 2014), questions have been raised about how MC management should differ under irrigation, especially with new cotton cultivars that are characterized by ‘aggressive’ growth. Therefore, a better characterization of the

response of modern cotton cultivars to varying levels of MC under different irrigation treatments will benefit cotton producers by allowing for more informed crop management decisions.

Objectives

This project seeks to evaluate the performance and physiological differences of select cotton cultivars under induced crop water stresses in a greenhouse, controlled environments, and field settings to improve water management in cotton production. The discovery of cotton cultivars that show drought tolerant traits will enable growers to make informed decisions on cultivar selection for drought stress mitigation. This project will also evaluate the response of four cotton cultivars to passive, moderate, and aggressive MC regimes under well-watered and rainfed treatments to determine the best management practices for plant growth regulation under different environments. The specific goals of this research were to:

1. Screen cotton cultivars for drought tolerance traits under an extreme water-deficit stress (rainout shelter) in a field. We hypothesized that differences in physiological parameters among cotton cultivars under an extreme water-deficit stress would be due to genotypic variation in drought tolerance traits.
2. Measure shoot hydraulic conductance rates in a variety of cotton cultivars under varying temperature and VPD levels in controlled environments. We hypothesized that the variation in cultivar VPD breakpoints and NTR slopes would be due to differences in their hydraulic conductance response under variable temperature and VPDs.

3. Investigate the effects of varying rates of MC on cotton plant height, yield, and other physiological parameters under two irrigation regimes (i.e., rainfed and well-watered). We hypothesized that potential differences in yield would be due to the interaction of MC and amount of irrigation applied.

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Appendix

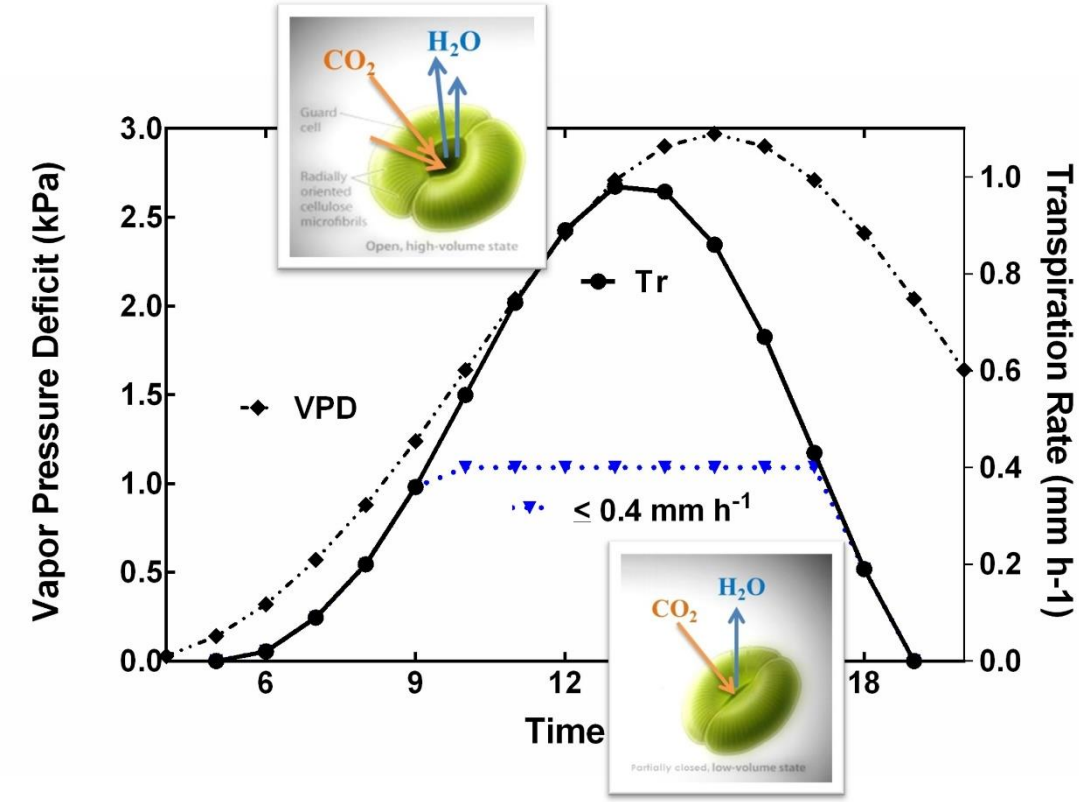


Figure 1. Transpiration response (TR) to increasing vapor pressure (VPD) over time of two limited transpiration (TR_{lim}) cultivars (dotted line with triangles) and one cultivar without TR_{lim} (solid line with black circles).



Figure 2. Cotton plants treated with mepiquat chloride (MC) are shorter in height (left) than ones not treated with MC (right).

CHAPTER II
SCREENING COTTON CULTIVARS UNDER INDUCED WATER DEFICIT STRESS IN CONTROLLED ENVIRONMENTS AND FIELD SETTINGS: EXPRESSION OF DROUGHT TOLERANCE TRAITS

A version of this chapter was originally submitted for publication by Kurt T. Wedegaertner, Avat Shekoofa, Kendall Sheldon, Joaquín Simón and Tyson B. Raper:

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Abstract

Cotton (*Gossypium hirsutum* L.) is often grown in climates of intermittent drought conditions. Plants that limit transpiration rates (TRs) when initially exposed to water-deficit stress will preserve water for use later during critical growth stages. Two traits resulting in conservative TRs are TR limitations under soil drying and high vapor pressure deficit (VPD, >2.5 kPa). The objective of this study was to assess the performance of four contrasting cotton cultivars and their TRs under induced water stresses. Three studies were conducted to test: (i) the early stomatal closure under soil drying in a greenhouse, (ii) the TR to varying VPD levels in a growth chamber, and (iii) the stomatal conductance (g_s), wilting score, specific leaf area (SLA), relative water content (RWC), and yield in an extreme field environment. Significant differences in fraction of transpirable soil water threshold (FTSW threshold) were detected among cultivars in the greenhouse. The FTSW threshold among cultivars ranged from 0.29 to 0.39. Under varying VPD levels, only PHY 400 W3FE expressed a limited TR (TR_{lim}) with increasing VPD at 1.6 kPa. In the field study, differences in g_s, wilting score, RWC, SLA, and lint yield were observed among cultivars within the water limited treatments (i.e., rainout and rainfed). PHY 400 W3FE had the lowest wilting score compared to other cultivars. Under the rainout treatment, PHY 400 W3FE yielded 37% higher than PHY 500 W3FE. Results indicate a trend in water saving potential among cotton cultivars, given the differences in their TR sensitivity to water-deficit stress conditions.

Introduction

Cotton (*Gossypium hirsutum* L.) is an economically important crop grown to produce natural fiber for the global textile industry. In 2020, the United States grew cotton on 4.8 million hectares and produced nearly 4.3 million metric tons of lint (USDA, 2020a) valuing approximately 7 billion USD (lint plus seed) (USDA, 2020b). One of the most limiting factors in cotton production is water, which impacts cotton plants by ultimately reducing lint yield and negatively impacting fiber quality (Parida et al. 2007; Devi and Reddy, 2018). Increasing variability in the world climate is threatening cotton production globally due to more frequent temperature extremes, more significant and frequent soil water deficits, and greater irregularities in rainfall patterns (Zafar et al. 2018). Additionally, 64% of U.S. cotton production is rainfed (NASS, 2019), leaving the majority of cotton acreage vulnerable to a changing climate. Therefore, the discovery of water-conserving traits in plants is valuable in reducing exposure to drought, resulting in crop yield loss (Devi and Reddy, 2018; Shekoofa et al. 2020; Broughton et al. 2021).

Drought tolerance in plants is broadly comprised of two steps: stress sensing/signaling followed by the activation of various parallel stress responses, including physiological, molecular, and biochemical mechanisms (Mahmood et al. 2020; Kuromori et al. 2022). One particularly beneficial physiological response to drought is a plant's ability to partially close its stomata early in the soil drying process, or when exposed to high vapor pressure deficit (VPD) conditions (Sinclair, 2017). Increased VPD results in increased atmospheric demand for water through evapotranspiration (Penman, 1948). The early stomatal closure limits the plants rate of transpiration and conserves soil

water (Shekoofa et al. 2013; Shekoofa and Sinclair, 2018). This early-season conservation of soil water allows for more water to be available to a plant later in the season when it is critically needed for boll development and fiber production (Devi and Reddy, 2018; Shekoofa et al. 2020). Therefore, cultivars that demonstrate stomatal closure under one or both of these environmental conditions will have an advantage in continuing physiological and metabolic functions further when high temperatures and droughts are critical (Pradhan, Shekoofa, and Sinclair 2019; Sadok, Lopez, and Smith 2021; Shekoofa et al. 2021). Genotypic variation of limitation in transpiration rate (TR) response to high VPD and water-deficit stress have been observed in several crop species including soybean (*Glycine max*) (Fletcher, Sinclair, and Allen 2007; Purdom et al. 2021), peanut (*Arachis hypogaea*) (Devi, Sinclair, and Vadez 2010; Pradhan, Shekoofa, and Sinclair 2019), sorghum (*Sorghum bicolor*) (Gholipoor et al. 2010), and maize (*Zea mays*) (Shekoofa et al. 2016; Devi and Reddy, 2020b). Additional studies have observed limited TR through stomatal control with progressive soil drying in a variety of crop species (Liu et al. 2005; Devi et al. 2009; Gholipoor et al. 2013; Manandhar et al. 2017; Devi and Reddy, 2020b).With potentially warmer temperatures, changes in rainfall distribution and altered VPD in future climates (Kirschbaum 2004), it is important to understand the potential impact of VPD on leaf-level physiology of crops, which will lead to a broader understanding of crop responses to projected future climates (Broughton et al. 2021).

The objective of this study was to evaluate the performance of early stomatal closure in selected newly released and modern cotton cultivars under progressive soil

drying in a greenhouse, high VPD conditions in a growth chamber, and extreme water-deficit stress in a field.

Materials and Methods

Greenhouse study: Transpiration rate responses under progressive soil dry down

Four newly released and modern cotton cultivars: 'PHY 400 W3FE', 'PHY 360 W3FE', 'PHY 500 W3FE, and 'PHY 332 W3FE' (Phytogen Cottonseed, Corteva AgroScience, Indianapolis, IN) were planted in the greenhouse at the West Tennessee Research and Education Center (WTREC) in Jackson, Tennessee. Four seeds of each cultivar were sown at a depth of 2.5 cm in a soil mix composed of 50% sand and 50% Lexington silt loam (fine-silty, mixed, active, thermic Ultic Hapludalf) in 3.8-liter pots. Plants were thinned to one plant per pot, ten days after planting (DAP). Fifteen DAP, all pots were fertilized with Miracle-Gro Water Soluble All Purpose Plant Food containing 24-8-16 ($N–P_2O_5–K_2O$). Plants were grown in a greenhouse under natural light supplemented with 600 W high-pressure sodium lamps (P. L. Light Systems, Lincoln, ON, Canada) to maintain 600-650 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the plant level, in a 15 h day and 9 h night schedule. Air temperature and relative humidity were recorded every 5 minutes with an EL-USB-TC-LCD humidity/temperature digital data logger (Lascar Electronics, Erie, PA). Temperature was regulated at 35°C day/26°C night with 58% humidity.

When plants developed 3 to 4 true leaves (approximately 28-30 DAP) the soil drying experiment was initiated. On the afternoon before initiation of the soil drying

cycle, pots were over-watered until dripping and allowed to drain overnight. Pots were then placed into two double-bagged 15-liter (43 cm × 46 cm × 13.9 µm) white colored plastic bags and secured at the base of the plant with plastic twist ties to prevent evaporation from the soil, following the method described by Shekoofa et al. (2013). A small plastic tube was inserted adjacent to the plant stem to facilitate daily watering. Each pot was weighed after bagging and the weight was recorded as the initial pot weight. Then, pots were weighed daily between 1100 and 1300 h to obtain gravimetric water loss through transpiration. Daily transpiration rate of each plant was measured for three days by calculating the difference in weight of each pot on consecutive days. Plants were then assigned to one of two treatments: Water-deficit stressed (WS) or well-watered (WW). Within each cultivar, four plants were assigned to the water-deficit stressed treatment, and three were assigned to the well-watered treatment, which served as a reference for calculating the normalized transpiration rate (NTR).

After daily weighing of plants, between 1100 to 1300 h, water was added to maintain the WW plants within 200 g below the initial pot weight by replacing daily the amount of water lost from the pot to the 200 g deficit level. Soil water was allowed to gradually transpire at a rate of no greater than 100 g day^{-1} from the WS plants to simulate a prolonged development of water-deficit stress. Water was only added to the WS plants if daily water loss exceeded 100 g day^{-1}.

The transpiration data were analyzed by a procedure previously described by Shekoofa et al. (2013), in which daily TR data underwent two normalizations. The first normalization minimized the influence of large variations in daily TR, by dividing the TR

of each WS plant by the average daily TR for the WW plants within each cultivar. The daily transpiration rate was normalized again to account for differences such as plant size. This second normalization was done for each plant by dividing the daily transpiration rate by the mean transpiration rate of that same plant during the first 3 days of the experiment when the soil was still near pot-capacity. This rate was termed as the normalized transpiration rate (NTR) and its value during the high water content/wet phase of the dry-down cycle for each plant was, therefore by definition, centered on a value of 1.0. Collection of data for each pot continued until the plant dried to a NTR ≤0.1, which was defined as the endpoint of transpirable soil water. The water content of each drying pot was based on the total transpirable soil water, which was the difference in weight between the initial and final weight of the pot. The FTSW was calculated as the difference between daily weight and final weight, divided by the total transpirable water using the following equation (Ray and Sinclair, 1998; Shekoofa et al. 2013).

$$\text{FTSW} = (\text{daily weight} - \text{final weight})/(\text{initial weight} - \text{final weight})$$

Growth chamber study: Transpiration rate responses under high evaporative demand

The same four cotton cultivars tested in the greenhouse study (soil drying experiment) were germinated from seeds and grown under a well-watered condition in a greenhouse at WTREC. Plants were grown in pots constructed from polyvinyl chloride pipe 10 cm diameter and 25 cm tall. The bottom of each pot was fitted with a flat end cap with a small hole drilled to allow drainage of any excess water. The top of the pot was affixed with a toilet flange to allow for attachment of a VPD chamber. The pots were filled with commercially available potting mixes. Miracle-Gro potting mix containing a

21:11:13 N–P_2O_5–K_2O fertilizer was used. Four seeds were planted per pot; 10 DAP, each pot was thinned to one plant. Plants were grown in a greenhouse where natural light was supplemented with 600 W high-pressure sodium lamps (P. L. Light Systems, Lincoln, ON, Canada) to maintain 600–650 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the plant level, in a 15 h day and 9 h night schedule. Air temperature and relative humidity were recorded every 5 minutes with an EL-USB-TC-LCD humidity/temperature digital data logger (Lascar Electronics, Erie, PA). Plants were kept in the greenhouse for 30 days at a temperature of 33/23°C (day/night) with a humidity of 60%. After 30 days**,** they were moved into a walk-in growth chamber set to a temperature of 32°C. The temperature was kept at a constant 32°C comparable to the greenhouse environment. Three days later, TR responses under varying VPD levels were initiated.

The evening before the initiation of TR measurements, pots were over-watered until dripping, and aluminum foil was placed over each pot's soil surface to prevent soil water evaporation. The next morning, the above-ground portion of each pot was enclosed in a 21-liter clear food container (Cambro Manufacturing, Huntington Beach, CA) by attaching the container to the previously installed toilet flange. Each container was equipped with a 12-V, 76-mm-diameter computer box fan (Masscool, Walnut, CA) to continuously stir the air inside the chamber. Additionally, a hole was drilled in the side-wall of each container to allow an EL-USB-1-LCD humidity/temperature digital data logger (MicroDaq, Contoocook, NH) to be fitted into the chamber to monitor the chamber environmental condition. Twelve containers were constructed to allow for three replications of each cultivar to be measured simultaneously. The TR response to VPD

levels was monitored in a system similar to that described by Fletcher, Sinclair, and Allen (2007).

Three different levels of VPD were achieved using air flowmeters and controlling the air sources with either dehumidified or ambient air delivered to the chamber through a plastic tube inserted through the top of the chamber. The temperature in the growth chamber was maintained at a constant 32°C with a light intensity of 660 µmol photosynthetically active radiation (PAR) $m^{-2} \cdot s^{-1}$. Plants were exposed first to low VPD (0.5-1.5 kPa), then medium VPD (1.5-2.5 kPa), and finally high VPD (2.5-3.5 kPa). At each level of VPD, plants were first given 30 to 45 minutes to acclimate to the environment, then all pots were weighed individually on a balance with a resolution of 0.1 g to establish the initial weight. Plants were kept under each VPD level for 60 minutes, after which a final weight was taken of each individual pot. Transpiration rate was calculated by subtracting the initial pot weight from the final pot weight. This process was repeated for each VPD level. Measurements for all 12 plants were made on two consecutive days. After completing measurements on the first day, the pots were over watered and allowed to drain overnight. After finalizing the second day of measurements, leaves were harvested and the total plant leaf area was measured using a LI-3100 leaf area meter (Li-Cor Inc., Lincoln, NE). This enabled the calculation of TR as an expression of water loss divided by plant leaf area.

Field study: Transpiration rate responses under an extreme environment

Four cotton cultivars used in the controlled environmental studies representing different transpiration rate responses were planted in a field at the WTREC on 11 May

2020. Cotton seeds were direct seeded at a depth of 2.5 cm in Lexington silt loam (fine-silty, mixed, active, thermic Ultic Hapludalf). The study was conducted in a split plot design with three different water treatments: (1) irrigated: plants were watered with above-ground drip tape when a soil matric potential value of -75 to -80 (kPa) was detected by a ZL6-Meter logger using TEROS 21 soil matric potential sensors (METER Group Inc., Pullman, WA) at depth: 45 cm; (2) rainfed: plants only received rainfall with no extra water supplement; and (3) water-deficit stress (rainout): plants were covered with a portable rainout shelter in their early reproductive stage (60 DAP).

Main plots received one of the three water treatments. Subplots were planted with one of the four cultivars in two rows, 96 cm row spacing, and 4.6 m long, considering 1.5 m space between each water treatment. Four replicates of each subplot treatment were conducted within each of the three main plot water treatments. The water-deficit stress was induced by moving a plastic-covered, portable shelter with open ends over the cotton plots to eliminate precipitation when canopy coverage reached 80% (early square stage). This was determined with visual assessment and achieved at 60 DAP. The shelter was framed with aluminum and covered with 0.15 mm thick polyethylene (Atlas Manufacturing, Alapaha, GA). The sides of the rainout shelter were equipped with curtains so that air could freely circulate through the shelters. The side curtains were rolled down during rainy days. The shelter was removed before harvesting cotton in early November 2020.

Cotton plants received 262.6, 477.1, and 540.6 mm of water respectively under water-deficit stress, rainfed, and irrigated treatments (Table 1). The research field was

soil-sampled, and fertilizer and lime were applied according to the current University of Tennessee Soil and Plant Pest Center recommendations (UTIA, 2017). The cotton crop was managed according to University of Tennessee recommendations for growth regulator and pesticide applications. (UTIA, 2021a; 2021b).

Physiological parameters and yield

Stomatal conductance (g_s, mol·H_2O·m^{-2}·s^{-1}) and photosynthesis (P, μmol CO_2·m^{-2}·s^{-1}) rates were recorded in each subplot under each water treatment using a LiCor 6400-XT (Li-Cor Inc., Lincoln, NE) between 1000 and 1400 h. Measurements were taken on the two uppermost fully expanded leaves on an individual plant in each subplot. A 6 cm^2 square of the cotton leaf was enclosed in the instrument chamber and allowed to equilibrate to the chamber's environmental condition for 60 s before a measurement was taken. The leaf section within the LiCor chamber was exposed to 36°C and 2000 μmol PAR·m^{-2}·s^{-1} using a 6400-01 light source. The flow rate was 500 μmol s^{-1} and CO_2 concentration was maintained at 400 μmol CO_2·mol^{-1} air. These settings were intended to replicate the ambient VPD conditions the plant would experience during a typical midday (~ 3–4 kPa). Measurements from each leaf within each subplot were averaged for analysis. LiCor 6400-XT measurements were taken 84, 93, 105, and 121 DAP in 2020. EL-USB-TC-LCD humidity/temperature digital data loggers (Lascar Electronics, Erie, PA) were also installed in the field to monitor and measure the cotton canopy temperature, relative humidity, and vapor pressure deficit (VPD) during growing seasons (Table 2).

Wilting score data were taken under the rainfed treatment at 90, 106, 120, and 127 DAP and under the rainout treatment at 90, 92, 105, 106, 120, and 127 DAP, respectively. The wilting scoring scale ranged from 0-5, 0 = no wilting, 1 = a few leaves wilted, 2 = half of leaves wilted, 3 = most leaves wilted, 4 = plant severely wilted, 5 = plant dead. In addition, leaf relative water content (RWC) and specific leaf area (SLA) measurements were taken, following the procedure described by Gonzalez and Gonzalez-Vilar (2001) and demonstrated in Sheldon et al. (2021). Leaf samples for RWC and SLA were taken on sunny days between 1100 and 1400 h when the ambient VPD and temperature were up >3.5 kPa and 36°C. Irrigated and rainfed plots were sampled on: 70, 84, and 106 DAP and the rainout plots on 70, 84, 92, 105, and 120 DAP.

At the end of October, seedcotton and lint were harvested from each plot by a John Deere 9900 two-row picker (John Deere, Moline, IL) outfitted with an in-basket, catch-and-weigh system. Each plot was subsequently harvested, weighed, and subsampled for turnout and fiber quality. Subsamples from each plot were then air-dried, bulked by cultivar, and weighed prior to ginning. Samples were ginned at the University of Tennessee Cotton MicroGin located at the West Tennessee Research and Education Center in Jackson, TN.

Statistical analysis

Data collected within the greenhouse study were analyzed using a two linear-segment regression analysis (Prism 8.0, GraphPad Software Inc., San Diego, CA). This regression analysis generated the FTSW threshold for the initiation in the decline in NTR.

The 95% confidence interval of the threshold for each cultivar was used to assess statistical differences among cultivars (Sinclair et al. 2018).

Within the growth chamber study, data from all plants of a cultivar within each VPD level (low, medium, and high) were combined to perform a two-segment linear regression (Prism 8.0, GraphPad Software Inc., San Diego, CA) for TR vs. VPD. If significantly different ($p < 0.05$) slopes for two segments were not obtained for a cultivar, a simple linear regression was applied to all the data (Shekoofa et al. 2013).

For physiological parameters and lint yield taken under the field study, data were analyzed using mean separation procedure, Tukey's HSD test. RWC data were analyzed for significant difference using a two tailed t-test (JMP, version Pro 15, SAS Institute Inc., Cary, NC).

Results and Discussion

Greenhouse study

The results indicated significant differences in the rate of FTSW threshold among cotton cultivars ranging from 0.29 to 0.39 (Fig. 3). Cultivars PHY 332 W3FE and PHY 400 W3FE had FTSW threshold values of 0.39 and 0.36, respectively which were significantly higher than cultivar PHY 360 W3FE with a FTSW threshold of 0.29 (Fig. 3). The difference in FTSW threshold observed in PHY 400 W3FE and PHY 332 W3FE indicate their potential for soil water conservation through stomatal closure early in the progressive soil drying process, whereas PHY 360 and PHY 500 W3FE had late stomatal closure and lower instance of water saving. Devi and Reddy (2020a) found that 15 cotton

cultivars expressed FTSW threshold of 0.35 to 0.60 and suggested the observed variability is related to adjustments in the leaf water potential and ability of some cultivars to maintain hydraulic conductivity under progressive soil drying. The early soil water conservation demonstrated in PHY 400 W3FE led to its average day to the endpoint of drying down cycle (NTR ≤0.1) to be the highest compared to the other three cultivars, in particular PHY 500 W3FE (Table 3). Devi and Reddy (2020a) indicated that the FTSW threshold for the decline of transpiration upon imposition of water-deficit stress should increase if the imposition of water-deficit stress took place in conditions of higher evaporative demand. The ability to limit transpiration sooner under water-deficit conditions as observed by PHY 332 W3FE and PHY 400 W3FE will allow these cultivars to conserve more soil moisture which will improve drought tolerance.

Growth chamber study

Out of four tested cotton cultivars only one, PHY 400 W3FE, expressed the limited transpiration trait (TR_{lim}), or VPD breakpoint at 32°C, with VPD breakpoint of 1.6 kPa and $R^2 = 0.77$ (Fig. 4d). The three remaining cultivars had linear TR responses to high evaporative demand (i.e., VPD >2.5 kPa) meaning they transpired water linearly with no potential of water saving earlier during growth (Fig. 4 a-c). The growth chamber results are compatible with the greenhouse study in which PHY 400 W3FE demonstrated early stomatal closure under progressive soil drying (Fig. 3). Genotypic variation in expression of TR_{lim} and VPD breakpoint among cotton cultivars have been observed by Devi and Reddy (2018) in which eight of 17 cultivars expressed TR_{lim} as VPD increased. These results differ from Shekoofa et al. (2020) in which all of the 11 tested cotton

cultivars expressed TR_{lim} at 32°C. Devi and Sinclair (2011) offered the hypothesis that a reduction of aquaporins could be responsible for the observed TR_{lim} under high VPD conditions in some cultivars. A reduced population of aquaporins would restrict the amount of water able to move through a plant during high evaporative demand, causing stomata to close to maintain leaf water potential and avoid desiccation. A key consequence of limiting TR in response to increasing VPD is the conservation of soil moisture for use later in the season when drought conditions are more likely (Shekoofa et al. 216; Devi and Reddy, 2018). Crop model simulations across a variety of crop species have found that under rainfed, water-deficit stress conditions, the expression of TR_{lim} could improve yield by 75% (Sinclair, 2005; Sinclair et al. 2010). Therefore, the discovery of cotton cultivars with this trait is of importance considering the majority of cotton production is under rainfed environments (NASS, 2019).

Field study

Stomatal conductance

Under the rainout treatment, cultivars PHY 332 W3FE, PHY 400 W3FE, and PHY 360 W3FE had lower g_s (0.11, 0.16, and 0.23,) and photosynthesis (11.89, 12.21, and 14.96) values than PHY 500 W3FE (0.37 and 20.71) when averaged across all sampling dates (Table 4). Late in the season under the rainout treatment (105, 120 DAP) PHY 500 W3FE had significantly high g_s and photosynthesis values at the 0.5 alpha level when compared to the other three cultivars. As water-deficit stress became severe, PHY 500 W3FE showed no ability to limit transpiration through stomatal closure, which was also demonstrated under high VPD and soil drying conditions in the controlled

environment studies. This varies from Devi and Reddy (2018), who found that cotton genotypes with a linear VPD- TR response lowered their gs and photosynthesis at a VPD of 3.3 kPa in a controlled environment. Increased gs for PHY 500 W3FE was observed in this study when VPD exceeded 6.0 kPa in a field environment (Table 2). Suggesting that at very high VPD levels, which are possible in the field, some cultivars may be unable to limit TR. Devi and Reddy (2018) concluded their study with a statement that future work is needed for the confirmation of the limited TR in the field in a wide range of VPD. The other possibility might be the stability of temperature in the chamber study versus the field study (Shekoofa et al. 2016; 2020).

Furthermore, early season measurement (84 DAP) of g_s for cultivar PHY 400 W3FE showed a larger difference when comparing rainout (i.e., water-deficit stress) and irrigated (i.e., well-watered) treatments than cultivar PHY 500 W3FE (Table 5). Indicating that cultivar PHY 400 W3FE was better able to adjust to the water-deficit stress condition early through stomatal closure than PHY 500 W3FE. A reduced g_s earlier under drought conditions have been shown to improve yield production in soybean (Purdom et al. 2021) and peanut growth and yield stability (Sinclair et al. 2018). Additionally, Li et al. (2019) showed that early stomatal closure in response to soil drying played a critical role in protecting the integrity of xylem water transport in cotton, which in turn led to observed increases in pre-dawn leaf water potential.

Wilting score

Differences in wilting score were observed among the cultivars and water treatments (Fig. 5). Cultivar PHY 400 W3FE had the lowest wilting scores early in the

season (90 DAP) as well as at the end of the season (127 DAP) under rainfed (0.75 and 0.12) and rainout treatments (1.37 and 3.17) respectively. When wilting scores were averaged for each, rainout and rainfed treatments, PHY 400 W3FE had the lowest score under both water treatments and cultivar PHY 500 W3FE had the highest, 0.71, 2.68 and 1.37, 3.37, respectively. The lower wilt scores observed by PHY 400 W3FE throughout the growing season under both rainout and rainfed water treatments suggest field confirmation of observed drought tolerance traits under the controlled environment studies. It has been suggested that observation of slow-wilting is an expression of soil water conservation due to plant-imposed limitation on maximum TR (Fletcher, Sinclair, and Allen 2017; Shekoofa et al. 2016; Sinclair et al. 2018; Purdom et al. 2021). Shekoofa et al. (2020) reported that the field observations of delayed wilting and higher leaf water potential (LWP) in pre-dawn were consistent with the expression of the TR_{lim} trait to conserve soil water for four out of eleven cotton cultivars.

Specific leaf area and leaf relative water content

Significant differences in specific leaf area (SLA) were observed under the water-deficit stress treatment (Fig. 6). When averaged across all sampling dates, cultivar PHY 400 W3FE had significantly lower SLA when compared to cultivar PHY 360 W3FE, with SLA values of 142.2 cm^2/g and 166.5 cm^2/g respectively. Lower SLA under water-deficit stress conditions may have advantage for drought tolerant varieties by decreasing the surface to volume ratio which reduces leaf temperature, limiting the need for transpirational cooling (Rosbakh, Römermann, and Poschlod 2015). Marron et al. (2003) showed a decrease in SLA to be associated with thickening of the cell wall which may

help increase the ability of a leaf to accommodate variations in leaf water potential during successive drying cycles. Additionally, Shekoofa et al. (2015) showed a significant negative correlation between water use efficiency coefficient (WUE_k) and SLA across six peanut genotypes, suggesting that lower SLA under water-deficit stress could increase drought tolerance in plants.

Temporal differences in relative water content (RWC) were observed among cultivars under rainfed and rainout water treatments (Table 6). Under the rainfed treatment, PHY 332 W3FE, PHY 360 W3FE, and PHY 400 W3FE had significant increases in RWC between sampling dates of 70 DAP and 106 DAP (15.86, 14.17, 12.27%, respectively), while PHY 500 W3FE showed no significant increase in RWC between the two measurements (8.95%) (Table 6). Under the rainout treatment, PHY 332 W3FE, PHY 360 W3FE, and PHY 400 W3FE showed no significant reductions in RWC between sampling dates of 84 DAP and 120 DAP (-4.11, -1.27, -4.01%, respectively) while PHY 500 W3FE had a significant reduction in RWC between the two measurements (-17.56%). All p-values were calculated at the 0.5 alpha level. Under water-deficit stress conditions, the difference in reduction of RWC indicates a cultivar's ability to maintain leaf water status for support of important physiological functions such as photosynthesis, and temperature maintenance (Nautiyal, Rachaputi, and Joshi 2002). Therefore, it has been hypothesized that cultivars which demonstrate the ability to maintain RWC as soil drying progresses will have a distinct advantage in carbohydrate production which could lead to yield improvement. Lafitte (2002) showed that rice grain yield had significant positive correlations with RWC measured during reproductive stage

water-deficit stress in the dry season. However, the correlation only accounted for one-quarter of the observed variation in yield suggesting that other factors are at least as important as RWC in determining yield response. Meaning that RWC measurements should be complied with other parameters such as SLA, g_s, and wilt score to more accurately represent how cultivars respond to water-deficit stress and the impact on yield, especially in field settings.

Yield

Overall, cotton lint yield for plots under irrigation and rainfed were significantly higher than rainout (Fig. 7). Under each water treatment cultivar PHY 400 W3FE had the highest lint yield (Fig. 8). Under extreme water-deficit stress, rainout, cultivars PHY 400 W3FE, PHY 360 W3FE, and PHY 332 W3FE yielded significantly higher than PHY 500 W3FE (Fig. 8). Under the rainout treatment, cultivar PHY 400 W3FE yielded 37% higher than PHY 500 W3FE (Fig. 8). Yield results from this study suggest that under conditions of water-deficit stress in the field, expression of traits such as: reduction of g_s, SLA, and wilt score and increased RWC, will conserve soil moisture and likely contributed to this difference in yield. Sinclair et al. (2014) showed through crop yield simulations that the greatest yield increases for cultivars that expressed TR_{lim} trait, were in drier years.

Conclusion

The expression of drought tolerance traits trended similarly under all three studies and varying water-deficit environments. Cultivar PHY 400 W3FE, which expressed

drought tolerance traits in greenhouse and growth chamber studies had greater yield performance under an extreme water-deficit stress condition in the field study. On the contrary, PHY 500 W3FE which did not express TR_{lim} under high VPD and had a relatively low FTSW threshold under progressive soil drying had significantly reduced yield under an extreme water-deficit stress condition. Consequently, this observed stability of early stomatal closure and limited TR from controlled environments to field shows the potential for future cultivar screening of drought tolerance traits through similar methods. Findings from these studies reflect dynamic interactions between cultivars and environmental conditions.

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Appendix

Table 1. Total rainfall (mm) and irrigation received (mm) for each month and water treatment (2020).

		Rainfall (mm)
Month	**May**	99.0
	June	56.9
	July	106.7
	August	107.9
	September	78.7
	October	27.9
	Total rainfall (May-October)	477.1

		Irrigation (mm)	**Total Rainfall + Irrigation** (mm)
Water Treatment	**Irrigated**	63.5	540.6
	Rainfed	0.0	477.1
	Rainout	0.0	262.6*

* Includes the rainfall amount for May through July only.

Table 2. Maximum air temperature, photosynthetically active radiation (PAR), and ambient vapor pressure deficit (VPD) observed on each day between 1000 and 1500 h during field measurements.

Day of Measurement	**DAP**	**Water Treatment**	**Max. Temp** (C)^{o}	**Max. PAR** ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	**Max. VPD** (kPa)
3 Aug, 2020	84	Irrigated	30.5	2036	0.98
3 Aug, 2020	84	Rainfed	36.5	1948	2.65
3 Aug, 2020	84	Rainout	41.0	1513	4.65
11 Aug, 2020	93	Rainout	43.5	1547	4.86
24 Aug, 2020	105	Rainout	46.5	1615	6.14
8 Sep, 2020	121	Rainout	46.0	1388	6.28

Table 3. Average day to the endpoint of drying down cycle, determined as [Normalized transpiration rate (NTR ≤0.1)] among cotton cultivars.

Cotton cultivar	Day to endpoint of drying down
PHY 332 W3FE	10.0
PHY 400 W3FE	10.8
PHY 500 W3FE	9.6
PHY 360 W3FE	10.0

Table 4. Average stomatal conductance and photosynthesis values for each cotton cultivar under rainout treatment from three separate days after planting (DAP). Cultivars are ordered in ascending values. Cultivars followed by the same letter within each measurement on each DAP are not significantly different based on the 95% Confidence Interval (CI).

DAP	Cotton cultivar	Stomatal conductance (mol $H_2O \cdot m^{-2} \cdot s^{-1}$)	Photosynthesis (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)
84	**PHY 332 W3FE**	0.29 ns	27.68 ns
	PHY 400 W3FE	0.45	30.26
	PHY 500 W3FE	0.57	33.08
	PHY 360 W3FE	0.64	35.15
93	**PHY 332 W3FE**	0.04 ns	7.29 ns
	PHY 360 W3FE	0.06	8.64
	PHY 400 W3FE	0.21	20.19
	PHY 500 W3FE	0.48	18.64
105	**PHY 400 W3FE**	0.03 a	3.74 a
	PHY 332 W3FE	0.04 a	5.24 a
	PHY 360 W3FE	0.06 a	7.36 ab
	PHY 500 W3FE	0.27 b	14.50 b
121	**PHY 360 W3FE**	0.01 a	2.37 a
	PHY 332 W3FE	0.01 a	2.76 a
	PHY 400 W3FE	0.02 a	2.64 a
	PHY 500 W3FE	0.28 b	14.57 b

ns = Not significant ($p<0.05$)

Table 5. Difference in stomatal conductance (g_s) rates between cultivars PHY 400 W3FE and PHY 500 W3FE under rainout and irrigated treatments, measurements were taken on the same day early in the growing season 84 days after planting (DAP) under both treatments.

	Stomatal conductance (g_s, mol $H_2O \cdot m^{-2} \cdot s^{-1}$)		
Cotton cultivar	**Irrigated**	**Rainout**	**Difference**
PHY 400 W3FE	0.61	0.45	0.16
PHY 500 W3FE	0.51	0.57	0.06

Table 6. Temporal difference in relative water content (RWC) among cultivars in rainfed and rainout water treatments early in the growing season [70, 84 days after planting (DAP)] and late in the growing season (106, 120 DAP), respectively. P-value shows significance of difference of RWC between DAP. (Two tailed t-test, confidence interval = 0.95).

Rainfed	**Relative water content (RWC)**			
Cotton cultivar	**70 DAP**	**106 DAP**	**Difference**	**P-value**
PHY 332 W3FE	62.93	78.79	15.86	0.001
PHY 360 W3FE	63.99	78.16	14.17	0.004
PHY 400 W3FE	67.08	79.35	12.27	0.001
PHY 500 W3FE	64.12	73.07	8.95	0.241
Rainout				
Cotton cultivar	**84 DAP**	**120 DAP**	**Difference**	**P-value**
PHY 360 W3FE	70.69	69.42	-1.27	0.737
PHY 400 W3FE	73.44	69.43	-4.01	0.439
PHY 332 W3FE	75.50	71.39	-4.11	0.147
PHY 500 W3FE	74.16	56.60	-17.56	0.009

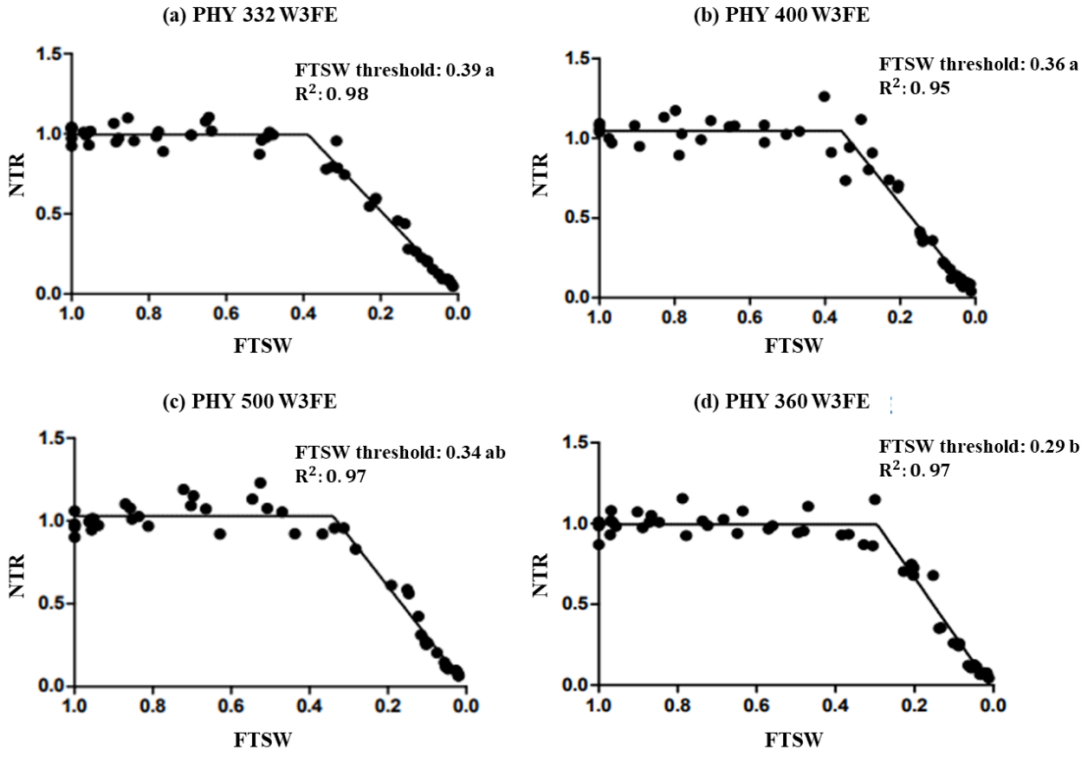


Figure 3. Threshold of fraction of transpirable soil water (FTSW) threshold for initiation of decline in normalized transpiration rate (NTR) as determined by the two-segment, linear-regression analysis. Thresholds accompanied by different letters are significantly different between cultivars based on the 95% confidence interval of the FTSW threshold.

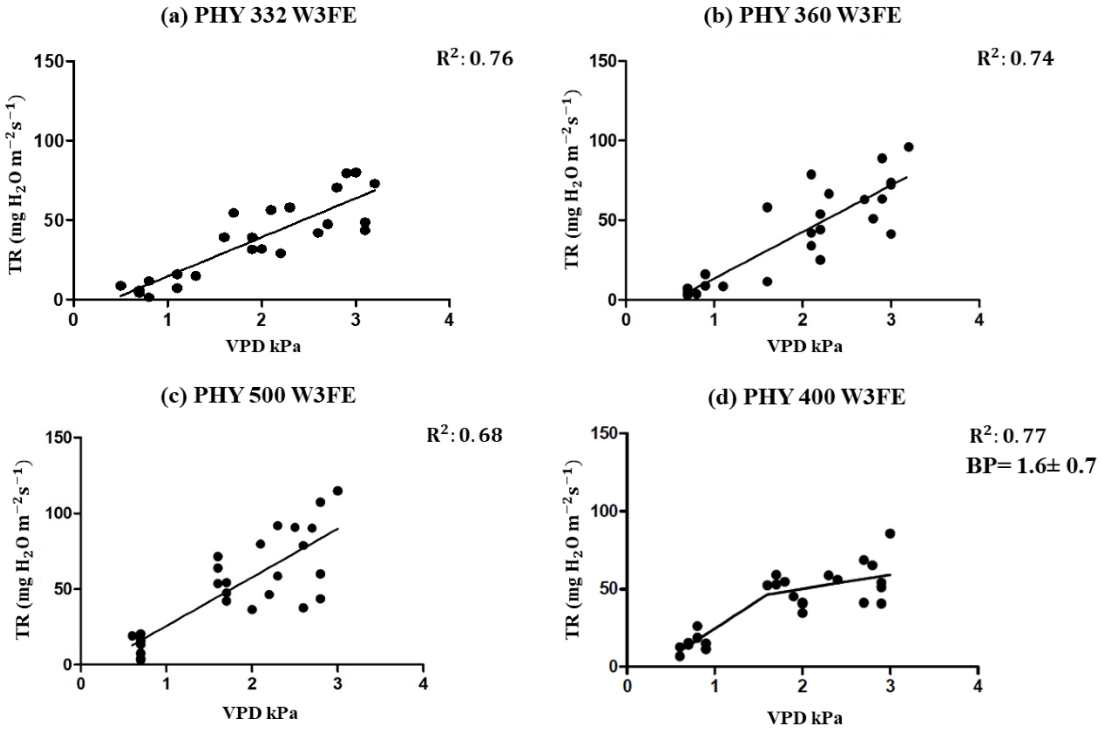


Figure 4. Transpiration response of four cotton cultivars [(a)PHY 332 W3FE (b) PHY 360 W3FE (c) PHY 500 W3FE (d) PHY 400 W3FE] to vapor pressure deficit (VPD) (kPa) under 32°C in a walk-in growth chamber. Results from two-segment linear regression include Breakpoint (BP) (X_0) ±SE, and R^2.

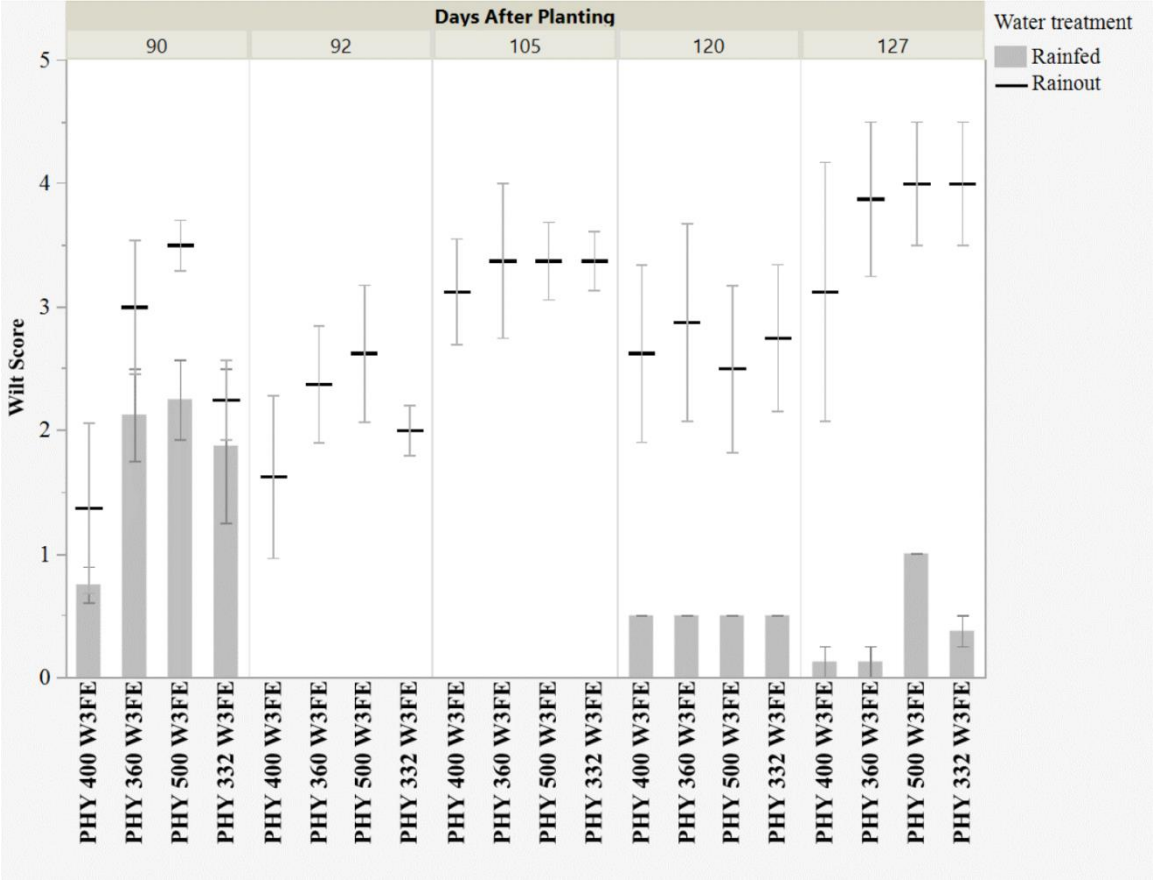


Figure 5. Each cultivar's average wilt scores for each day of measurement under rainfed (vertical bar) and rainout (horizontal line) treatments. Error bars represent the standard error of the mean.

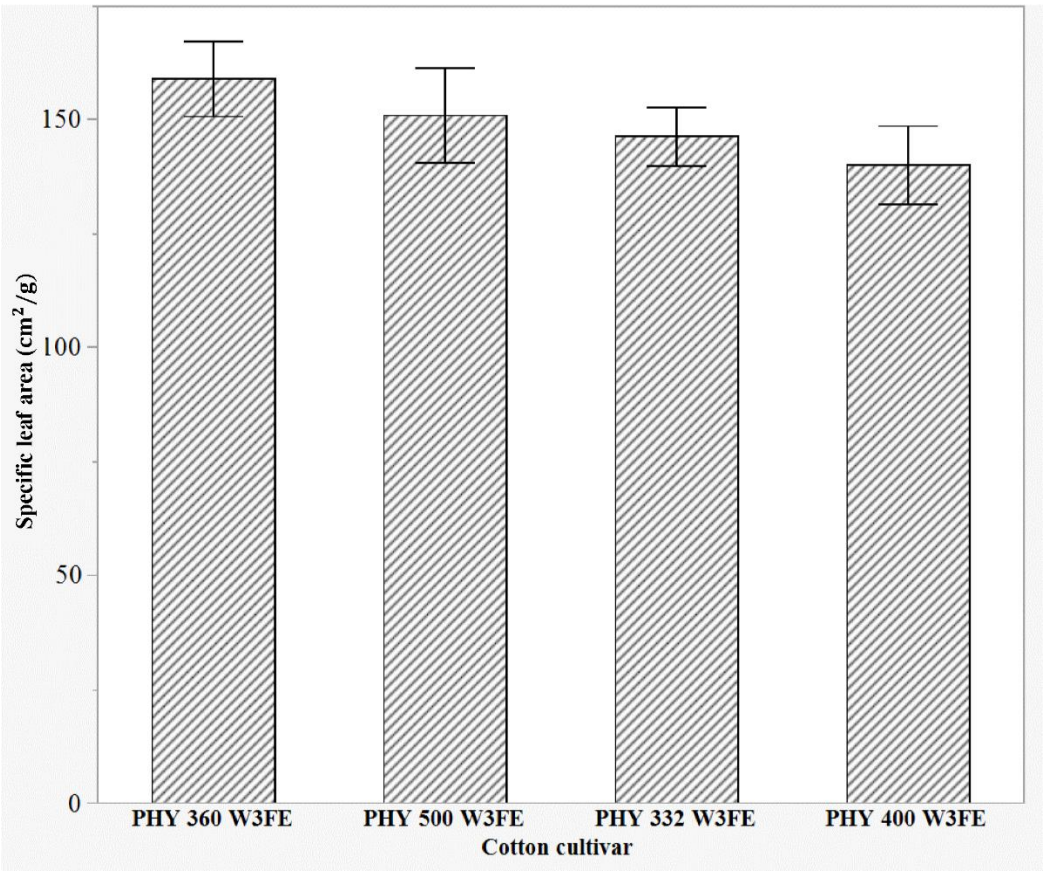


Figure 6. Specific leaf area (cm^2/g) for each cultivar under rainout treatment. Error bars represent standard error of the mean.

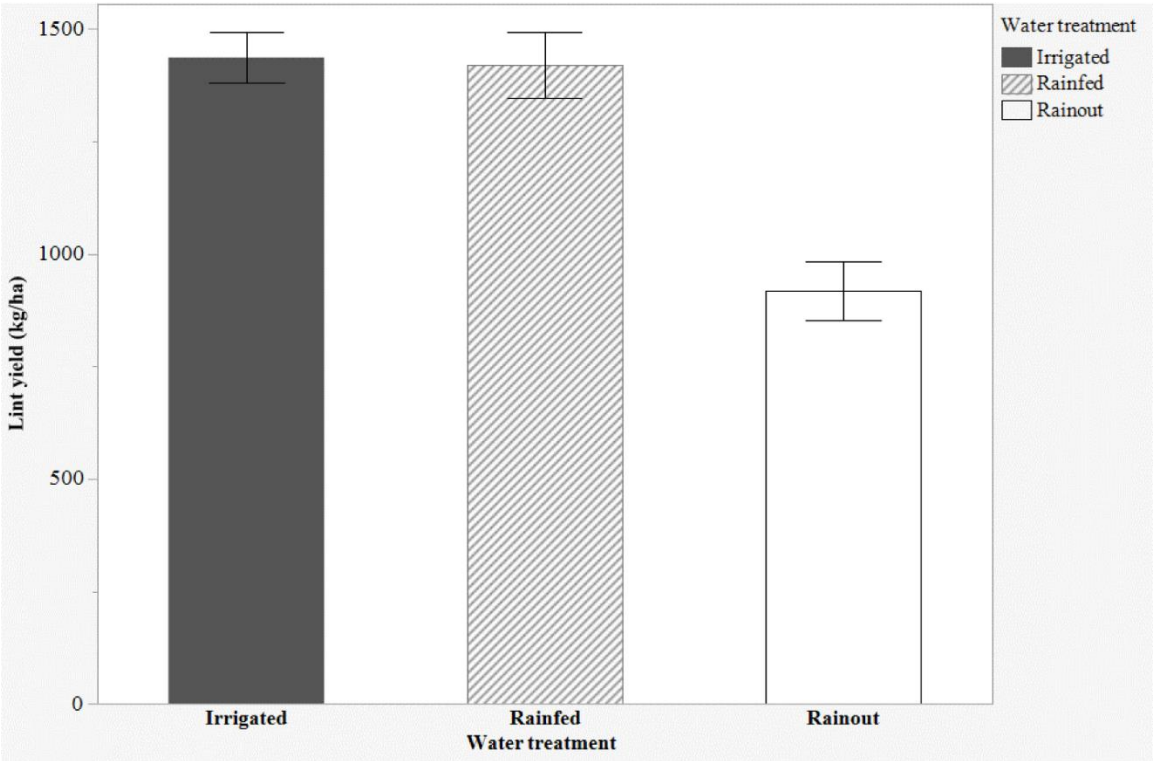


Figure 7. Cotton lint yield (kg/ha) under three water treatments. Error bars represent standard error of the mean.

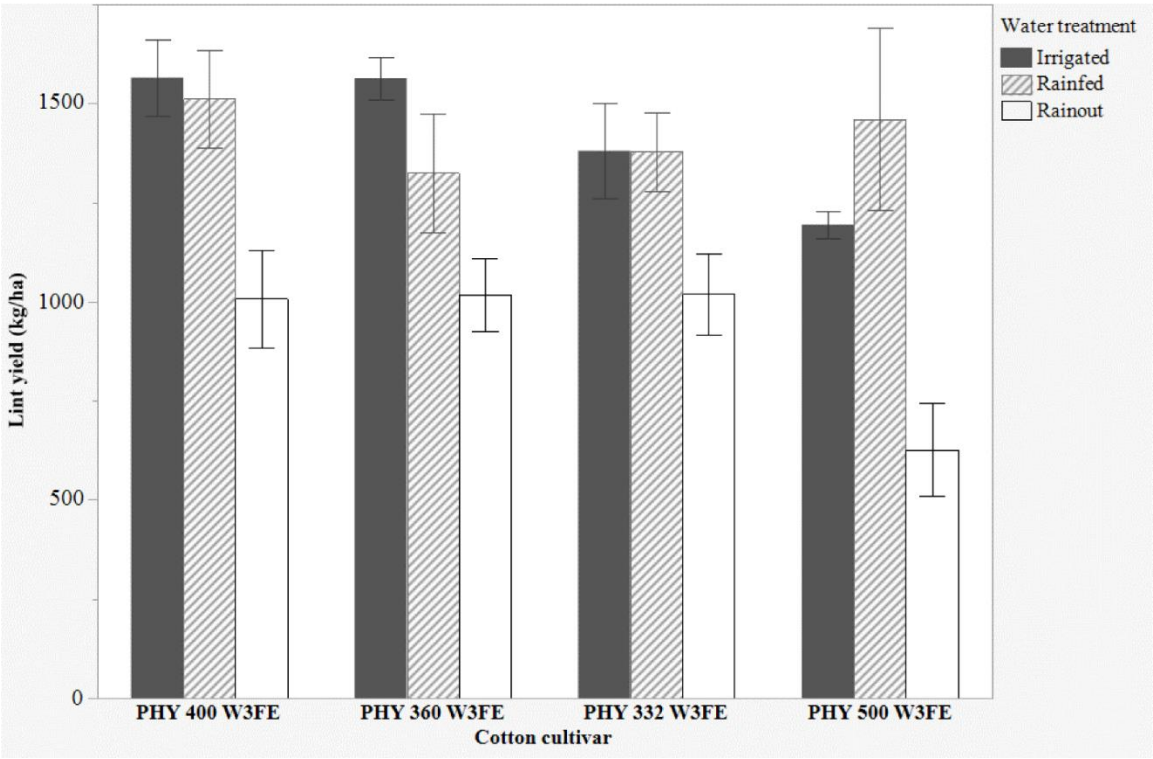


Figure 8. Cotton lint yield (kg/ha) for each cotton cultivar under three water treatments: Irrigated, rainfed, and rainout. Error bars represent standard error of the mean.

CHAPTER III
COTTON STOMATAL CLOSURE UNDER VARYING TEMPERATURE AND VAPOR PRESSURE DEFICIT, CORRELATION WITH THE HYDRAULIC CONDUCTANCE TRAIT

A version of this chapter was originally submitted for publication by Kurt T. Wedegaertner, Avat Shekoofa, Sam Purdom, Kellie Walters, Lori Duncan and Tyson B. Raper:

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Author Contribution
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Kellie Walters: Writing-review & editing.
Lori Duncan: Writing-review & editing.
Tyson B. Raper: Writing-review & editing.

Abstract

Cotton (*Gossypium hirsutum* L.) is often grown in locations characterized by high atmospheric evaporative demand. It has been hypothesized that plants which resist hydraulic flow under high evaporative demand will limit water use and conserve soil water. Therefore, a series of controlled environment experiments were conducted to assess if drought tolerance traits under progressive soil drying and high evaporative demand among ten cotton cultivars relate to differences in shoot hydraulic conductance (K_{shoot}) under two different temperature and vapor pressure deficit (VPD) environments (i.e., 38ºC, >3 kPa and 32ºC, 1-1.5 kPa). Plants were exposed to each environment for 90 min after which K_{shoot} was measured using a hydraulic conductance flow meter (HCFM). Physiological parameters measured included leaf area, stomatal conductance (g_s), and water use efficiency coefficient (WUE_k). The results were also compared with a previous study which measured the transpiration rate (TR) response of the same cotton cultivars under increasing VPD. Differences were observed in K_{shoot} among cultivars under the 38ºC, >3 kPa but not the 32ºC, 1-1.5 kPa environment. Under the 38ºC, >3 kPa environment, correlations were found between K_{shoot}, VPD breakpoint, fraction transpirable soil water (FTSW) threshold, WUE_k, and leaf area. Results indicate that once soil dried to a point when there was a decrease in TR, there was a coincident decrease in K_{shoot}. Results show that the ability of some cotton cultivars to restrict water loss under high evaporative demand and soil drying through early stomatal closure is associated with the cultivars' K_{shoot}.

Introduction

Plant hydraulic conductance (K_{plant}) is a measure of the efficiency of water movement through a particular plant organ such as roots, leaves, or shoots and is defined as the flow rate per unit pressure driving force (Sperry, 2000; Judd et al., 2016). A variety of plant physiological properties including water potential, stomatal conductance (g_s, $mmol \cdot m^{-2} \cdot s^{-1}$), and overall plant growth are related to K_{plant} (Cochard et. al, 1997; Cochard et. al, 2000; Sperry, 2000; Franks et. al, 2007; Brodribb et. al, 2010). Reduced K_{plant} under high atmospheric vapor pressure deficit (VPD) conditions could be used as an approach to decrease the rate of soil water use by restricting plant water loss (Sinclair et al., 2005; Choudhary et al., 2013). Investigation into potential differences among cotton cultivars' ability to limit hydraulic conductance under periods of high evaporative demand and temperature is therefore warranted.

The ability of crop plants to limit transpiration under high ambient VPD has been described extensively (Gholipoor et al., 2010; Shekoofa et al., 2013; Sinclair et al., 2017; Sheldon et al., 2021). This limited transpiration trait (TR_{lim}) is expressed as a VPD breakpoint, above which the TR reaches a plateau or begins to decrease in response to increasing VPD. A recent study on transpiration response to increasing VPD among different cotton cultivars demonstrated that at a high temperature (38ºC), some cotton cultivars lose their ability to limit transpiration under increasing VPD conditions (Shekoofa et al., 2020). The study suggested that the loss of the VPD breakpoint [i.e., limited transpiration (TR_{lim}) rate] at higher temperature may indicate an abrupt increase in K_{plant}. The loss of a VPD breakpoint with a relatively small increase in temperature

suggests that TR_{lim} may be a temperature-sensitive process which appears to be highly associated with changes in K_{plant} (Pradhan et al., 2019). Under high temperatures, cultivars without the ability to restrict hydraulic conductance would allow water to readily replenish leaves, resulting in open stomata and unrestricted transpiration with increasing VPD (Pradhan et al., 2019; Li et al., 2019; Sadok and Sinclair, 2010; Sarkar et al., 2022). Observations of the TR_{lim} trait and low leaf hydraulic conductance (K_{leaf}) [i.e., decrease in transpiration rate (TR)] at 32℃ do not necessarily predict expression of the trait at higher temperatures (Sarkar et al., 2022). By increasing temperature from 32 to 37℃, the expression of TR_{lim} trait was reduced to ~43, 10, and 0% for slow, moderate, and high wilting soybean genotypes, respectively. This could be because the remaining genotypes which expressed TR_{lim} (i.e., VPD breakpoint) and low leaf hydraulic conductance at 32℃ started to increase TR under 37℃ to disperse excess temperature and heat.

In 2013, Choudhary et al. demonstrated that two sorghum genotypes differed significantly in K_{leaf}. Additionally, this reduction in K_{leaf} was associated with conservative water use by the whole plant during soil drying and under high atmospheric VPD. Across a range of species and environmental conditions, hydraulic conductance resistance levels have been linked to maximum rates of g_s (i.e. gas exchange) (Sack and Frole 2006; Brodribb et al., 2007). Therefore, it is of interest to investigate the hydraulic conductance of cotton cultivars under varying VPD and temperature environments, assessing the possible involvement of hydraulic conductance resistance during periods of high evaporative demand and progressive soil drying.

To date, most hydraulic conductance research in row crops has focused on K_{leaf}. Therefore, this study objective was to assess the shoot hydraulic conductance (K_{shoot}) of ten cotton cultivars, under varying VPD and temperature to gain insight into possible connections to previously observed drought tolerance trait responses to water-deficit stress.

Materials and Methods

Plant materials and experiments setup

During July and September 2020, a set of two experiments were performed under controlled environment conditions at the West Tennessee Research and Education Center (WTREC). Ten cotton cultivars were selected based on their observed TR responses to high VPD and temperature (Shekoofa et al., 2020). Commercial cultivars selected include: 'Stoneville (ST) 4949 GLT' and 'ST 6182 GLT' (Stoneville Cottonseed, BASF Corp., Research Triangle Park, NC); 'DeltaPine (DP) 1612 B2XF' (Deltapine Cottonseed, Bayer CropScience, St. Louis, MO); and 'Phytogen (PHY) 330 W3FE', 'PHY 340 W3FE' and 'PHY 490 W3FE' (Phytogen Cottonseed, Corteva Agroscience, Indianapolis, IN). Breeding lines selected include: 'DP 393' (Bowman, Gutierrez, Percy, Calhoun and May, 2006) 'Arkot 9704' (Bourland and Jones, 2009), 'UA107' (Bourland and Jones, 2018a) and 'Arkot 0705' (Bourland and Jones, 2018b).

Growth chamber study

In each experiment, all cultivars were replicated four times using a randomized complete block design. An experimental unit consisted of a 3.7-liter pot filled with a soil

mix composed of fifty percent sand and fifty percent Lexington silt loam (fine-silty, mixed, active, thermic Ultic Hapludalf). Each pot was planted with six seeds at a depth of 2.5 cm. Pots were thinned to one plant ten days after planting (DAP). Fifteen DAP, all pots were fertilized with Miracle-Gro Water Soluble All Purpose Plant Food (Marysville, OH) containing 24-8-16 ($N–P_2O_5–K_2O$). Plants were grown under greenhouse conditions (14 hours of light/10 hours of dark, average day/night temperature 33/23 ± 4/3℃, average humidity 58 ± 12%). Air temperature and relative humidity were recorded every 5 minutes with a humidity/temperature digital data logger (Lascar Electronics, Erie, PA). Natural light was supplemented with 600 W high-pressure sodium lamps (P. L. Light Systems, Lincoln, ON, Canada) to maintain 600 to 650 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the plant level. Plants were maintained in a well-watered condition, receiving approximately 250 $ml \cdot day^{-1}$ (i.e., pot capacity) during the initial pre-treatment period, until they had developed 4-6 true leaves.

In each experiment, the evening before hydraulic conductance measurements, pots were over-watered until dripping, and aluminum foil was placed over the soil surface and around the plant stem to prevent soil evaporation. The next morning, plants were placed in a growth chamber (Conviron MTR30, Winnipeg, Manitoba, CA). Plants within each experiment were then exposed to a different environmental condition. In Experiment 1, conducted during July, 2020, plants were exposed to 38℃ and 50% humidity, VPD >3 kPa (high temperature, high VPD). In Experiment 2, conducted during September, 2020, plants were exposed to 32℃ and 80% humidity, VPD 1 to 1.5 kPa (low temperature, low VPD). Light intensity for both experiments were 500 to 550 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Air

temperature and relative humidity inside the growth chamber were recorded as previously described and light intensity was measured using a photosynthetically active radiation (PAR) meter (Quantum PAR, Lifees, Ontario, CA).

Plants were allowed 45 minutes to acclimate to the condition. Then, all pots were individually weighed on a balance with a resolution of 0.1 g to establish a beginning weight. Plants were kept inside the growth chamber under the same environmental conditions for 90 more minutes after which a final weight was measured. Transpiration rate was calculated for each plant by subtracting the final pot weight after exposure to growth chamber conditions from the initial pot weight for each set of experiments.

After recording final weight, g_s and photosynthetic rates (Pn) were measured individually for each plant using a portable photosynthesis machine (LiCor 6400-XT, Li-Cor, Lincoln, NE). The measurements for g_s and Pn were taken on two separated fully expanded leaves, with three measurements per leaf.

Since TR and Pn were measured, it was possible to calculate the water use efficiency coefficient (WUE_k, Pa) which is an instantaneous calculation of Pn and TR based on individual leaflets as well as the ambient VPD (Shekoofa et al., 2015). This measurement gives insight to the ratio of plant productivity to water loss (Sinclair et al., 1984).

equation 1:

$$WUE_k = (Pn \times VPD)/TR$$

A hydraulic conductance flow meter (HCFM, Dynamax, Inc., Houston, TX) was calibrated (Tyree et al., 1995) and used to measure K_{shoot} within cotton plants. The

HCFM measures hydraulic conductivity by perfusing water into an excised shoot or root while recording water flow (F, $kg \cdot H_2O \cdot s^{-1}$) and pressure (P, kPa). The hydraulic architecture of a whole shoot can be represented by a resistance diagram which plots the F into a plant shoot versus the applied P. The resulting slope between these two variables is K_{shoot} (Tyree et al., 1995). Each measurement required about 90 seconds. The ability to rapidly measure K_{shoot} with the HCFM allows for the screening of a large number of cultivars for their ability to limit hydraulic conductance during periods of high evaporative demands.

Immediately after measuring g_s and Pn using the Licor 6400 XT, the stem was cut 2 to 3-cm above the soil surface. The cut end of the stem was submerged in deionized water where it was cut again 1-cm above the original cut. The HCFM coupling was then attached to the cut stem under water to establish a water-tight seal. The plant with the attached HCFM coupling was then taken out of the water and placed horizontally on a flat surface. Degassed deionized water was then forced to flow through the stem system under steadily increasing pressure, ~5 $kPa \cdot s^{-1}$ until the supplying pressure reached 550 kPa (Fig. 9). The pressure was then released and a second measurement was taken, without the removal of the HCFM coupling between measurements (Vandeleur, 2008). The second measurement was used to calculate K_{shoot}. A previous shoot pressurization is necessary to fully saturate vessel elements and avoid an overestimation of K_{shoot} (Tyree et al., 1995; Bogeat-Triboulot et al., 2002). Shoot hydraulic conductance was calculated from the slope of the linear section on the plot of F versus P (Bogeat-Triboulot et al., 2002).

equation 2:

$$K_{shoot} = \Delta F/\Delta P$$

The initial flow rate was rapid and not linear due to the compression of air bubbles within the HCFM machine as well as within the xylem of the cotton shoot. However, as pressure increases, the contribution of bubble compression to the overall flow rate of water declines. Therefore, Tyree et al. (1995) proposed the slope after 200 kPa is a good representation for the actual hydraulic conductance. In both experiments, the linear regression was calculated after P = 100 kPa, due to the liner relationship that began at this P.

After the LiCor 6400 XT and HCFM measurements were conducted, physiological measurements taken on each plant included total leaf area using a leaf area meter (LI-3100, Li-Cor, Lincoln, NE) and dry leaf weight. Leaves were separated for each plant and placed in individual paper bags which were dried at 60℃ for 72 hours before weighing.

Greenhouse study

A progressive soil drying experiment was carried out at West Tennessee Research and Education Center (WTREC) in Jackson, TN involving eight of the ten cotton cultivars tested in the growth chamber study during July and September 2020. Four seeds of each cultivar were sown as described previously in 3.7-liter pots, thinned, and fertilized as described previously. Plants were grown in a greenhouse under natural light supplemented with 600 W high-pressure sodium lamps (P. L. Light Systems, Lincoln, ON, Canada) to maintain 600-650 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the plant level, with a 15-h

photoperiod. Air temperature and relative humidity were recorded as previously described. Temperature was regulated at an average of 35 ± 3°C day/26 ± 4°C night with 58 ± 14% humidity.

When plants developed 4 true leaves (approximately 28 to 30 DAP), the soil drying experiment was initiated. The pots were fully watered the evening before the experiment began. The following morning, each pot was enclosed in two double-bagged 15-liter (43 cm x 46 cm x 13.9 μm) plastic bags (Walmart, Bentonville, AR) with the bag opening bunched around the base of the stem and secured with a twist tie to prevent evaporation from the soil, following the method described by Shekoofa et al. (2013). An 8-mm-diameter × 80-mm-long tube was inserted adjacent to the plant stem to facilitate daily watering. Each pot was weighed after bagging and the weight was recorded as the initial pot weight. Then, pots were weighed daily between 1100 and 1300 h to obtain gravimetric water loss through transpiration.

Daily TR of each plant was measured for three days by calculating the difference in weight of each pot on consecutive days. Plants were then assigned to one of two treatments: Water-deficit stressed (WDS) or well watered (WW). Within each cultivar, four plants were assigned to the WDS treatment, and three were assigned to the WW treatment, which served as a reference for calculating the normalized transpiration rate (NTR).

Pots were weighed daily between 1100 to 1300 h. After weighing, water was added to maintain the WW plants by replacing the amount of water lost from the pot to the 200-g deficit level. Soil water was allowed to gradually transpire at a rate of no

greater than 100 g·day^{-1} from the WDS plants to simulate a prolonged development of water-deficit stress. Water was only added to the WDS plants if daily water loss exceeded 100 g·day^{-1}.

The transpiration data were analyzed by a procedure previously described by Shekoofa et al. (2013), in which daily TR data underwent two normalizations. The first normalization minimized the influence of large variations in daily TR, by dividing the TR of each WDS plant by the average daily TR for the WW plants within each cultivar. The daily TR was normalized again to account for differences in plant size. This second normalization was done for each plant by dividing the daily TR by the mean TR of that same plant during the first 3 days of the experiment when the soil was still near pot capacity. This describes the calculation of NTR and its value during the high-water content/wet phase of the dry-down cycle for each plant was, by definition, centered on a value of 1.0. Collection of data for each pot continued until the plant dried to a NTR ≤ 0.1, which was defined as the endpoint of transpirable soil water. The water content of each drying pot was based on the total transpirable soil water, which was the difference in weight between the initial and final weight of the pot. The fraction of transpirable soil water (FTSW) was calculated as the difference between daily weight and final weight, divided by the total transpirable water using the following equation (Ray and Sinclair, 1998; Shekoofa et al., 2013)

equation 3:

$$\text{FTSW} = (\text{daily weight} - \text{final weight})/(\text{initial weight} - \text{final weight})$$

Statistical analysis

Growth chamber study

To compare the HCFM results among the cultivars, the F from each cultivar's replication was averaged and plotted against the average P increase from all measurements in each of the two experiments. Fig. 10 represents specific cultivar's average change in water flow over the total average increase in pressure for each experiment.

Water flow and P increase were calculated and recorded using the HCFM computer program (HCFM-Gen3, Dynamax, Houston, TX). Shoot hydraulic conductance values were calculated through linear regression analyses by the HCFM computer program. Shoot hydraulic conductance was measured after P = 100 kPa. Physiological parameters were analyzed with a mixed model ANOVA and mean separation conducted with Tukey's HSD ($p = 0.05$). (JMP, version Pro 15, SAS Institute Inc., Cary, NC). Correlations were derived using bivariate analysis and linear analysis in JMP.

Greenhouse study

The relationship between NTR and FTSW was analyzed with a two-segment linear regression using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA). This software determined the FTSW threshold between the two segments where the NTR decrease was initiated. The FTSW value of the threshold where the two linear segments intersected was the critical statistic for comparing cultivars.

Results and Discussion

Shoot hydraulic conductance (K_{shoot}) among cultivars

In Experiment 1 from the growth chamber study, (38ºC, VPD >3 kPa) significant differences were observed for K_{shoot} among cotton cultivars (Table 7). Cultivar 'ST 6182 GLT' had the highest K_{shoot}, while 'DP 393 B2XF', 'Arkot 9704', 'DP 1612 B2XF', 'Arkot 0705', and 'UA 107' had lower K_{shoot} values (p value ≤ 0.05) (Table 7). The average K_{shoot} among cultivars ranged from 6.80×10^{-9} to 3.10×10^{-9} $kg \cdot H_2O \cdot s^{-1} \cdot MPa^{-1}$ (Table 7).

In Experiment 2 from the growth chamber study, (32ºC, VPD = 1 to 1.5 kPa) no significant differences were observed in K_{shoot} among all tested cotton cultivars at the 0.05 α level (Table 7). Average K_{shoot} among cultivars ranged from 1.97×10^{-9} to 1.23×10^{-9} $kg \cdot H_2O \cdot s^{-1} \cdot MPa^{-1}$ (Table 7). The results of this study follow a similar trend to Shekoofa et al. (2020) which tested the TR of the same cultivars included in this study to increasing VPD levels under low (32ºC) and high (38ºC) temperature stress. Shekoofa et al. (2020) indicated that all cultivars responded similarly under 32ºC by demonstrating the TR_{lim} trait and reducing TR as VPD increased past a certain point (1.4-2.6 kPa, VPD). In our study, all cultivars demonstrated similar K_{shoot} under 32ºC.

However, Shekoofa et al. (2020) reported that when exposed to increasing VPD levels under 38ºC, cultivars 'ST 6182 GLT', 'Arkot 9704', and 'Arkot 0705' lost their ability to limit transpiration and demonstrated a liner relationship of TR to increasing VPD. In our study, cultivars differed significantly in their K_{shoot} response under 38ºC. Cultivar 'ST 6182 GLT' had the highest K_{shoot} at 38ºC, suggesting that the loss of TR_{lim} is

associated with an increase in K_{shoot} at high temperatures. This result is consistent with the hypothesis that a low VPD breakpoint results from a low hydraulic conductance in the plant, which is expected to result in a reduced ability to move water as rapidly through the plant to the stomata (Choudhary et al., 2014).

Shoot hydraulic conductance (K_{shoot}) and stomatal conductance (g_s)

In Experiments 1 and 2, significant differences were observed for the g_s rate among cultivars (Table 8). A negative correlation was found between K_{shoot} rates under high evaporative demand and the change in g_s among cultivars between Experiment 1 and 2 ($R^2 = 0.37$, $p = 0.05$) (Fig. 11). This indicates that cultivars that demonstrated a greater reduction in g_s in response to a high VPD environment had lower K_{shoot} values in Experiment 1.

A larger difference in g_s between a high VPD environment (Experiment 1) and a low VPD environment (Experiment 2) suggests that a cultivar is better able to regulate g_s in response to the environmental changes. It has been hypothesized that a plant with lower K_{shoot} would transport less water through the shoot to supply leaves. With a limited water supply, a plant under high evaporative demand would be required to close stomata to conserve leaf water potential and avoid leaf desiccation (Bunce, 2006; Choudhary et al., 2013; Sinclair et al., 2017). The results of this experiment are in support of this hypothesis and suggest that a low K_{shoot} acts as a "hydraulic regulator", limiting stomatal activity under high evaporative demand.

Shoot hydraulic conductance (K_{shoot}) and VPD breakpoint (i.e., TR_{lim})

When plotting K_{shoot} values under high VPD in Experiment 1 against a cultivar's VPD breakpoint from Shekoofa et al. (2020), a positive correlation was established between K_{shoot} and VPD breakpoints ($R^2 = 0.39$, $p = 0.05$) (Fig. 12a). This finding indicates that cultivars that limited their TR response earlier under increasing VPD levels had lower K_{shoot} values under a high evaporative demand environment. These results are in agreement with those of Choudhary et al. (2014), who found a positive correlation between plant hydraulic conductance (K_{plant}) and VPD breakpoint among 12 maize hybrids ($R^2 = 0.30$).

Choudhary et al. (2014) considered the R^2 value for the correlation between plant hydraulic conductance (K_{plant}) and VPD breakpoint to be low and proposed that K_{plant} does not focus on a specific segment of the hydraulic pathway and does not segregate between root and shoot limitations. Similarities between R^2 reported by Chaudhary et al. (2014) and the present study suggests that segregation of root hydraulic conductance (K_{root}) is necessary to develop a better understanding of K_{plant} relationship with VPD levels. Overall, the positive correlation in this study further supports the hypothesis that low K_{shoot} lessens the ability to transport water through the plant to the stomata under high evaporative demand, which causes a low VPD breakpoint (Choudhary et al., 2014).

Shoot hydraulic conductance (K_{shoot}) and water use efficiency coefficient (WUE_k)

In Experiment 1, a negative correlation was found between K_{shoot} and WUE_k ($R^2 = 0.25$, $p = 0.14$) (Fig. 12b). This correlation indicates that under high evaporative demand, cultivars which limited K_{shoot} had greater WUE_k. This result is consistent with Sinclair et

al. (2008) which observed a low K_{leaf} to be associated with higher water use efficiency, limited TR, and increased water conservation in a "slow wilting" soybean cultivar. The observed "slow wilting" phenotype is thought to be indicative of several desirable drought-tolerance traits (Purdom et al., 2021). These results suggest the limitation of K_{shoot} under high evaporative demand could be directly related to stomatal associated traits under high VPD and water-deficit stress conditions. The restriction in K_{shoot} observed in this study will limit TR, conserving soil water earlier during plants growth for extended use throughout a growing season. Because stomatal regulation in cotton can be genotype-specific (Devi and Reddy, 2018), a comparison of traits associated with water relations across genotypes is important.

Shoot hydraulic conductance (K_{shoot}) and fraction of transpirable soil water (FTSW) threshold

Cultivars' K_{shoot} values from Experiment 1 were plotted against the FTSW results from the progressive soil drying experiment in the greenhouse. A negative correlation was found between K_{shoot} and FTSW threshold among cultivars ($R^2 = 0.32$, $p = 0.13$) (Fig. 12c). This finding indicates that cultivars that limited TR earlier in the soil drying process, had lower K_{shoot} under a high evaporative demand. Choudhary and Sinclair (2013) hypothesized that low K_{plant} means that decreases in TR will occur at a higher FTSW in the soil drying cycle. However, their results showed the opposite; a significant positive correlation between K_{plant} and the FTSW threshold was measured, suggesting low plant conductance was associated with a low FTSW threshold. They suggested the measured positive correlation may be a result of conductance measurements being made

on well-watered plants under no imposed stresses. In our study, K_{shoot} measurements were made on plants subjected to high evaporative stress (Experiment 1), which is likely the reason a negative correlation was measured.

Moreover, Li et al. (2016) conducted a study that measured changes in hydraulic conductivity of the soil-plant system (K_{total}) with decreasing soil volumetric water content. Their results showed a reduction in K_{total} was influential in the response of TR to soil drying, suggesting a more restrictive hydraulic conductivity in response to soil drying would impact FTSW threshold as a consequence of a limited TR**.**

Shoot hydraulic conductance (K_{shoot}) and normalized transpiration rate (NTR)

A positive correlation among cultivars was found between K_{shoot} values from Experiment 1 and the slope of NTR decrease after the FTSW threshold from the soil drying experiment ($R^2 = 0.55$, $p = 0.03$) (Fig. 13a). The slope of NTR decrease after the FTSW threshold is the rate at which NTR decreases after the initial decrease in NTR in response to soil drying. A more negative NTR slope indicates a higher rate of NTR reduction after FTSW threshold. A plant which decreases NTR earlier in the soil drying cycle can conserve soil moisture for longer use throughout the soil drying process (Shekoofa et al., 2013; Shekoofa and Sinclair, 2018; Devi and Reddy 2018). The soil water conservation, a consequence of limited K_{shoot}, would allow for a more gradual reduction in NTR through the soil drying process. The gradual reduction in NTR maximizes water use efficiency, allowing a plant to maintain physiological functions for a longer period under soil drying. This results in a less rapid decline in TR as soil drying progresses (Devi et al., 2009). On the contrary, a cultivar with no limitation of K_{shoot}

under high evaporative stress will have maximum NTR, and rapid depletion of soil water leading to a lower FTSW threshold and a steeper transpiration decline (Jørgensen et al., 2010).

To allow for a more gradual NTR reduction, NTR must be limited earlier in the soil drying cycle. In support of this, a negative correlation was found between the NTR slope and FTSW threshold among cotton cultivars in the soil drying experiment (R^2 = 0.84, p = 0.001) (Fig. 13b), indicating a more negative NTR slope is associated with a lower FTSW threshold. Thus, the later in the soil drying cycle a plant begins to decrease NTR, the steeper the slope of NTR decrease will be.

Shoot hydraulic conductance (K_{shoot}) leaf characteristics

In Experiments 1 and 2, differences were observed among cultivars for total leaf area (cm^2) and dry leaf weight (g) (Table 9). In Experiment 1, a positive correlation was found between K_{shoot} and total leaf area (R^2 = 0.56, p = 0.01) (Fig. 14), indicating that under high evaporative demand, cultivars with a larger leaf area will likely exhibit a higher K_{shoot}.

Since leaves are intricately connected to a plant's vascular system and represent between 30 to 90% of the hydraulic resistance of the whole plant (Brodribb and Holbrook, 2006), as more leaf area is amassed, a greater amount of water must be supplied to the leaves to avoid desiccation under high evaporative stress (Brodribb, 2009). Therefore, from a drought tolerance perspective it would be advantageous for a cultivar to have limited leaf area. When high evaporative demand occurs, the plant will

then not be required to transport as much water to support leaf functions and will conserve soil water (George-Jaeggli et al., 2017).

Conclusion

This study provides information to researchers and breeders on the K_{shoot} response of a variety of cotton cultivars under high evaporative demand. These experiments highlight the importance of K_{shoot} in influencing the limitation of TR under high temperature and evaporative demand. Correlations of K_{shoot} under high evaporative demand with a variety of drought tolerance parameters such as g_s, WUE_k, FTSW threshold, VPD breakpoint, and leaf area indicate that K_{shoot} is associated with drought tolerance in plants. Differences in K_{shoot} response to high evaporative demand among cotton cultivars reveal that genotypic differences can lead to diverse hydraulic conductance within plant shoots. These findings suggest that those cultivars with low sensitivity in K_{shoot} have restricted shoot water flow which can be used as an effective measurement to screen cultivars for drought tolerance traits under high evaporative demand and soil drying environments. Further experimentation is needed to investigate root hydraulic conductance.

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Appendix

Table 7. Shoot hydraulic conductance (K_{shoot}) (kg $H_2O \cdot s^{-1} \cdot MPa^{-1}$), Standard Error, and R^2 response of cotton cultivars in Experiment 1: (38ºC, vapor pressure deficit (VPD) > 3 kPa) and Experiment 2: (32ºC, VPD = 1-1.5 kPa) in controlled environments Cultivars followed by the same letter within each experiment are not significantly different based on the 95% confidence interval.

		Experiment 1	
Cultivar	**K_{shoot}**	**Standard Error**	**R^2**
ST 6182 GLT	6.80×10^{-9} a	3.08×10^{-11}	0.99
PHY 330 W3FE	6.23×10^{-9} ab	6.40×10^{-11}	0.99
PHY 490 W3FE	5.62×10^{-9} ab	3.65×10^{-11}	0.99
PHY 340 W3FE	5.43×10^{-9} ab	6.10×10^{-11}	0.99
ST 4949 GLT	3.97×10^{-9} ab	1.04×10^{-11}	0.99
Arkot 9704	3.55×10^{-9} b	7.41×10^{-12}	0.99
Arkot 0705	3.37×10^{-9} b	3.79×10^{-11}	0.99
DP 393	3.18×10^{-9} b	7.10×10^{-12}	0.99
UA 107	3.16×10^{-9} b	7.77×10^{-12}	0.99
DP 1612 B2XF	3.10×10^{-9} b	1.47×10^{-11}	0.99
		Experiment 2	
Cultivar	**K_{shoot}**	**Standard Error**	**R^2**
PHY 490 W3FE	1.97×10^{-9} ns	7.78×10^{-11}	0.97
DP 393	1.87×10^{-9}	3.70×10^{-12}	0.9
ST 6182 GLT	1.83×10^{-9}	5.99×10^{-11}	0.98
Arkot 0705	1.58×10^{-9}	1.76×10^{-12}	0.99
PHY 340 W3FE	1.53×10^{-9}	2.20×10^{-11}	0.99
Arkot 9704	1.53×10^{-9}	1.74×10^{-11}	0.99
UA 107	1.37×10^{-9}	3.21×10^{-11}	0.99
DP 1612 B2XF	1.28×10^{-9}	9.69×10^{-12}	0.99
PHY 330 W3FE	1.25×10^{-9}	4.75×10^{-11}	0.99
ST 4949 GLT	1.23×10^{-9}	8.04×10^{-12}	0.99

ns= Not significant ($p<0.05$)

Table 8. Stomatal conductance ratios (g_s, $mmol \cdot m^{-2} \cdot s^{-1}$) for cultivars from Experiment (Exp.) 1 (38ºC, vapor pressure deficit (VPD) > 3 kPa) and Exp. 2: (32ºC, VPD = 1-1.5 kPa) in controlled environments. Cultivars followed by the same letter within each experiment are not significantly different based on the 95% confidence interval.

	Stomatal Conductance (g_s)		
Cultivar	**Exp. 1 (High VPD)**	**Exp. 2 (Low VPD)**	**Exp. 1 – Exp. 2**
DP 1612 B2XF	0.29 a	0.45 a	-0.16
DP 393	0.22 ab	0.48 a	-0.26
UA 107	0.19 abc	0.51 a	-0.32
ST 6182 GLT	0.17 abcd	0.23 b	-0.06
PHY 330 W3FE	0.15 bcd	0.42 a	-0.27
PHY 490 W3FE	0.13 bcd	0.23 b	-0.10
Arkot 9704	0.12 bcd	0.57 a	-0.45
ST 4949 GLT	0.10 bcd	0.53 a	-0.43
Arkot 0705	0.07 cd	0.54 a	-0.47
PHY 340 W3FE	0.06 d	0.21 b	-0.15

Table 9. Leaf area (cm^2) and dry leaf weight (g) of cotton cultivars from Experiment 1: (38ºC, vapor pressure deficit (VPD) > 3 kPa) and Experiment 2: (32ºC, VPD = 1-1.5 kPa) in controlled environments. Cultivars followed by the same letter within each measurement are not significantly different based on the 95% confidence interval.

	Experiment 1	
Cultivar	**Leaf Area (cm^2)**	**Dry Leaf Weight (g)**
ST 6182 GLT	556.3 a	4.25 a
PHY 340 W3FE	565.8 a	4.22 ab
ST 4949 GLT	499.2 ab	3.49 c
PHY 330 W3FE	491.4 ab	3.89 abc
PHY 490 W3FE	470.8 ab	3.94 abc
Arkot 9704	496.0 ab	3.57 bc
Arkot 0705	440.2 b	3.37 c
DP 1612 B2XF	435.4 b	3.77 abc
DP 393	421.3 b	3.56 bc
UA 107	412.8 b	3.49 c
	Experiment 2	
Cultivar	**Leaf Area (cm^2)**	**Dry Leaf Weight (g)**
PHY 490 W3FE	304.6 a	2.67 a
ST 6182 GLT	277.0 a	2.57 a
PHY 340 W3FE	275.5 a	2.47 a
Arkot 9704	145.5 b	1.17 b
Arkot 0705	141.9 b	0.95 b
ST 4949 GLT	139.1 b	0.92 b
UA 107	133.1 b	0.92 b
DP 393	125.6 b	0.82 b
DP 1612 B2XF	113.7 b	0.87 b
PHY 330 W3FE	104.7 b	0.70 b

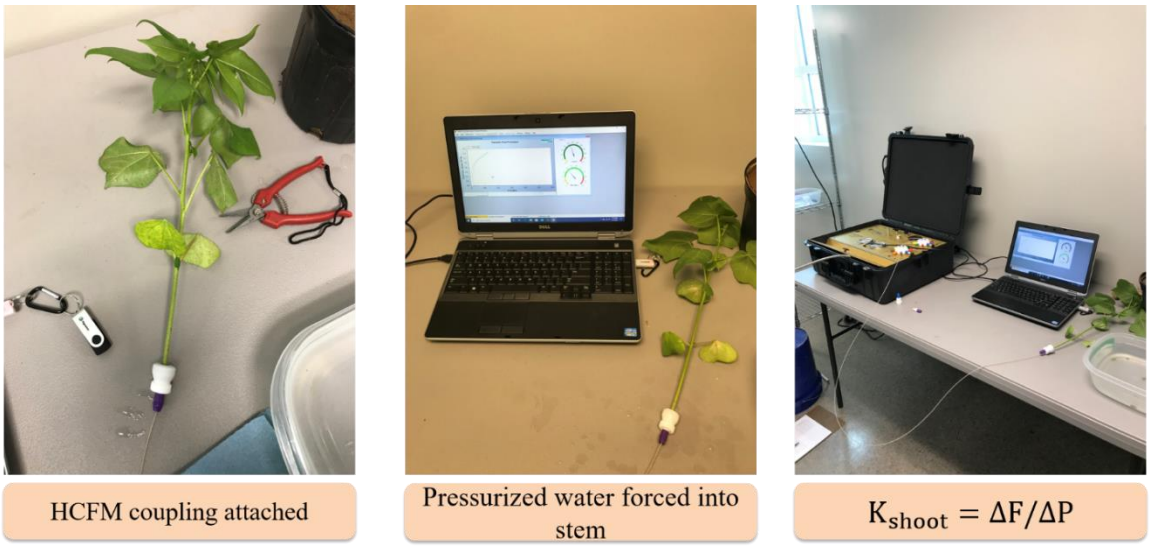


Figure 9. Process of measuring shoot hydraulic conductance [K_{shoot} = change in water flow (F) / change in pressure (P) (kPa)] using a hydraulic conductance flow meter (HCFM).

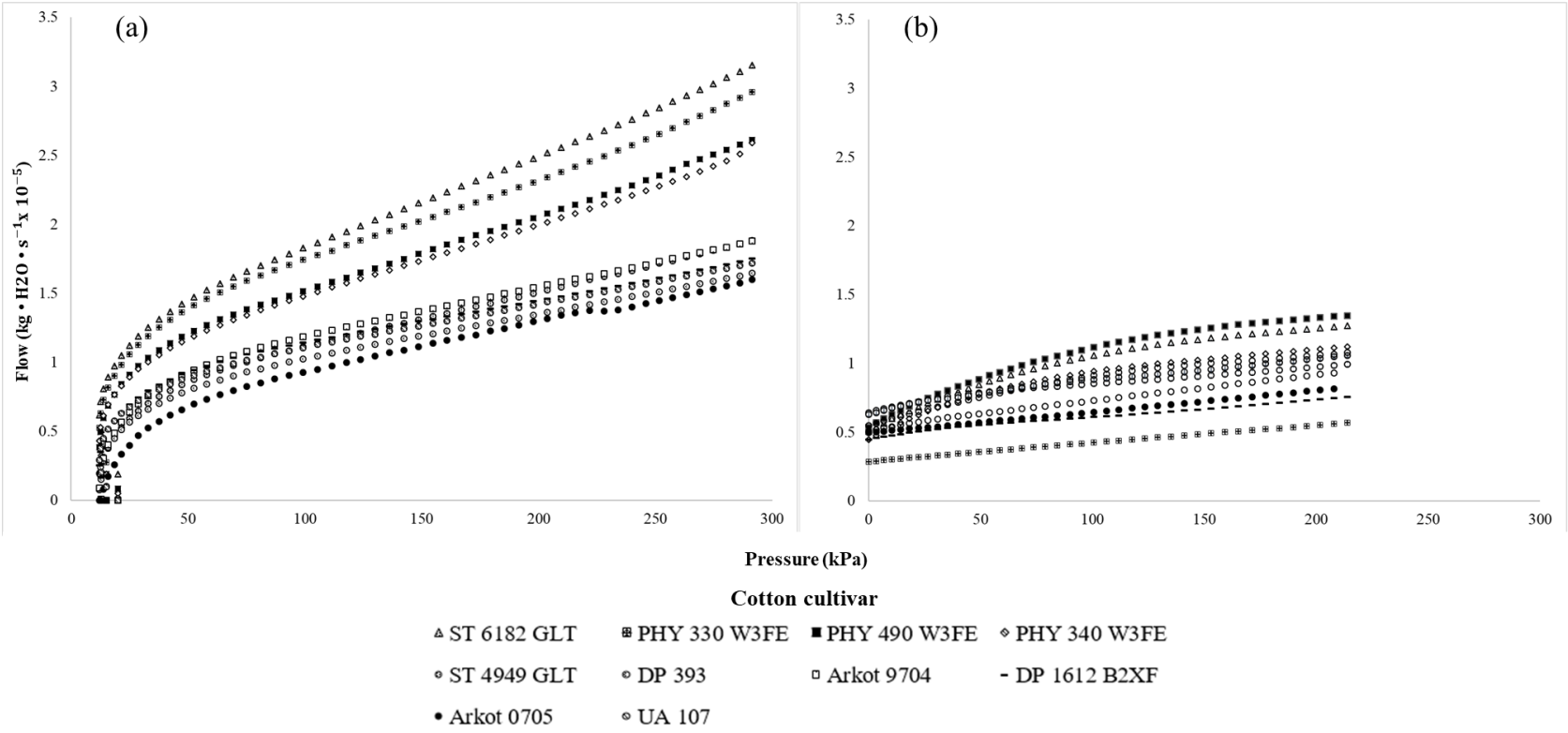


Figure 10. Average water flow of each cotton cultivar over the total average increase in pressure from each measurement in (a) Experiment 1: [38ºC, vapor pressure deficit (VPD) > 3 kPa] and (b) Experiment 2: (32ºC, VPD = 1-1.5 kPa) in controlled environments.

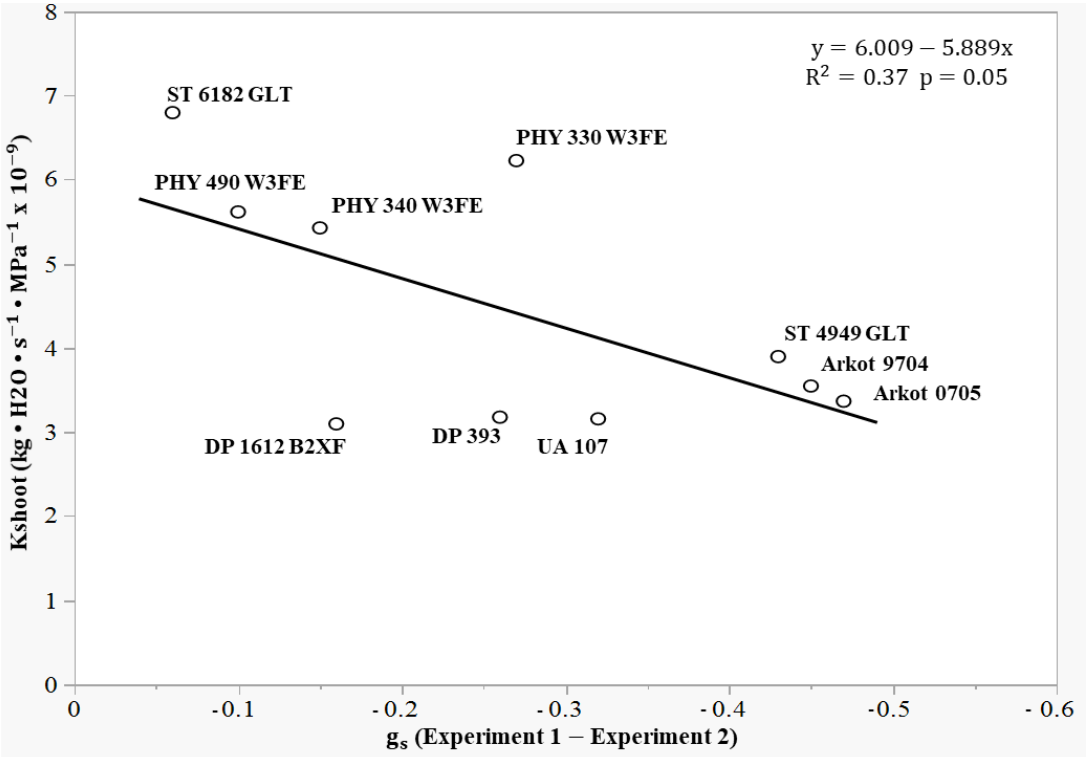


Figure 11. Correlation between cotton cultivar's average shoot hydraulic conductance (K_{shoot}) under a high evaporative demand [38ºC, vapor pressure deficit (VPD) > 3 kPa] and difference in stomatal conductance (g_s) between a low evaporative demand (32ºC, VPD = 1-1.5 kPa) and a high evaporative demand environment in controlled environments. $R^2 = 0.37$, $p = 0.05$

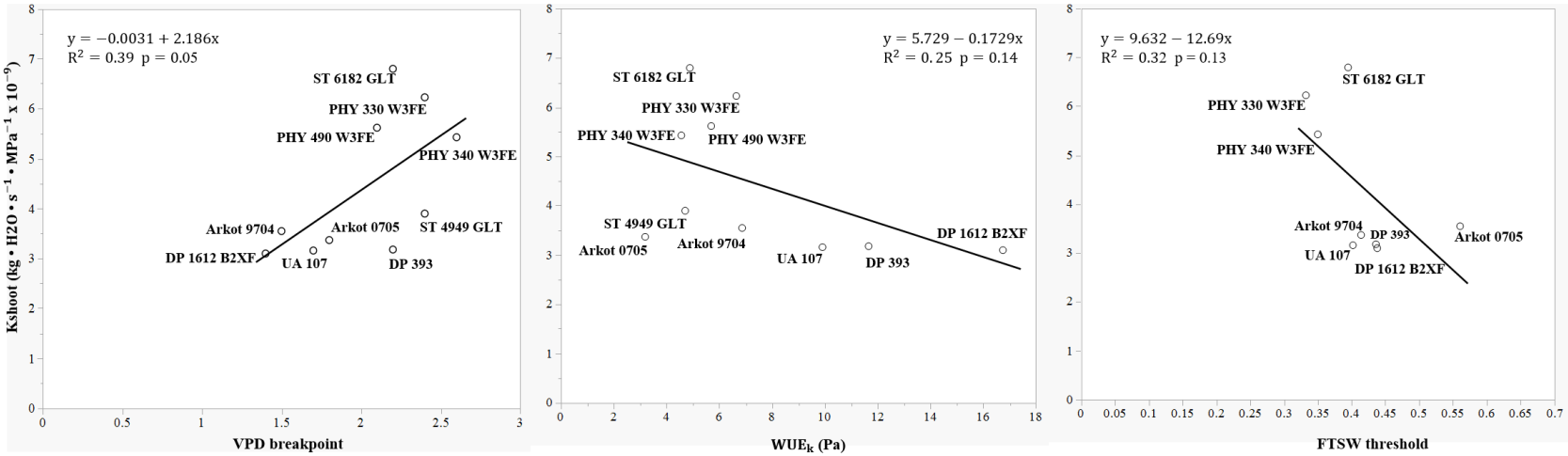


Figure 12. Graphs of values of vapor pressure deficit (VPD) breakpoint (a), water use efficiency coefficient (WUE_k, Pa) (b), and fraction of transpirable soil water (FTSW) threshold under progressive soil drying (c) versus shoot hydraulic conductance (K_{shoot}) under a high evaporative demand (38ºC, VPD > 3 kPa) among cotton cultivars in a controlled environment. $R^2 = 0.39$, $p = 0.05$ (a), $R^2 = 0.25$, $p = 0.14$ (b), $R^2 = 0.32$, $p = 0.13$ (c).

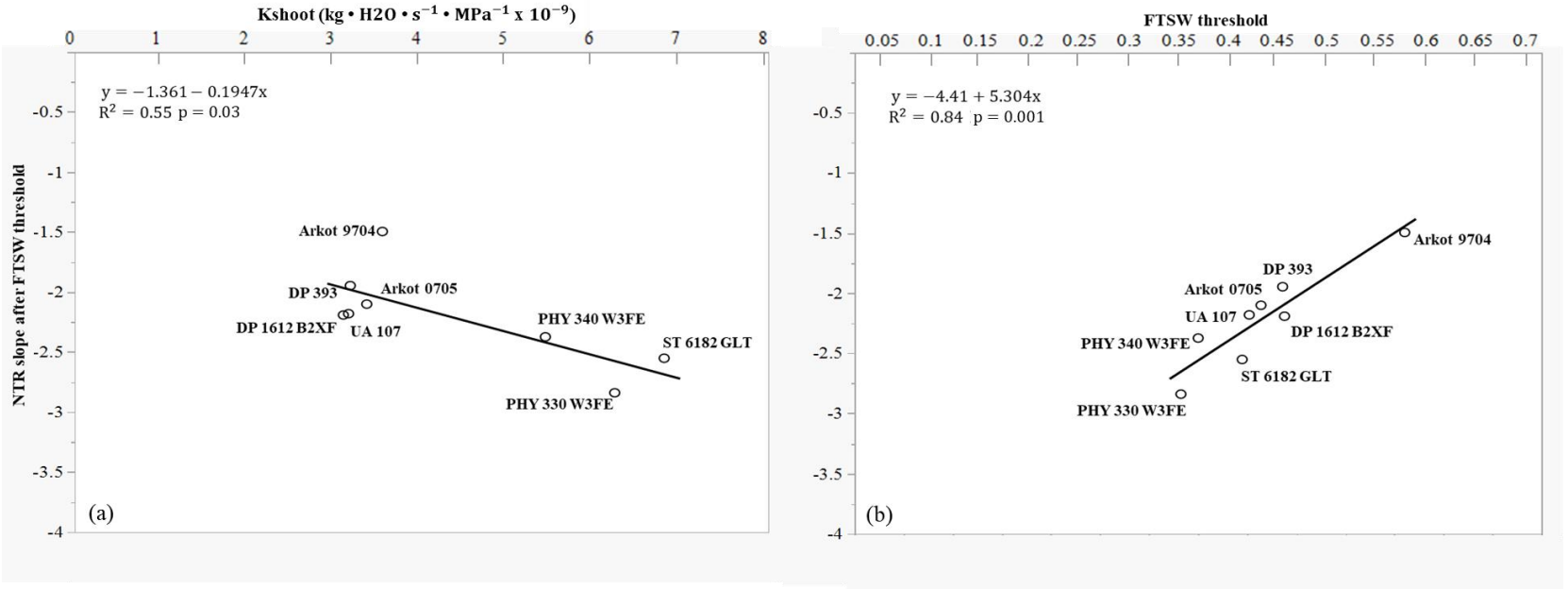


Figure 13. Graphs of values of shoot hydraulic conductance (K_{shoot}) under a high evaporative demand [38ºC, vapor pressure deficit (VPD) > 3 kPa] in a controlled environment (a) and the fraction of transpirable soil water (FTSW) threshold under progressive soil drying (b) versus the normalized transpiration rate (NTR) slope after FTSW threshold. $R^2 = 0.55$, $p = 0.03$ (a). $R^2 = 0.84$, $p = 0.001$ (b).

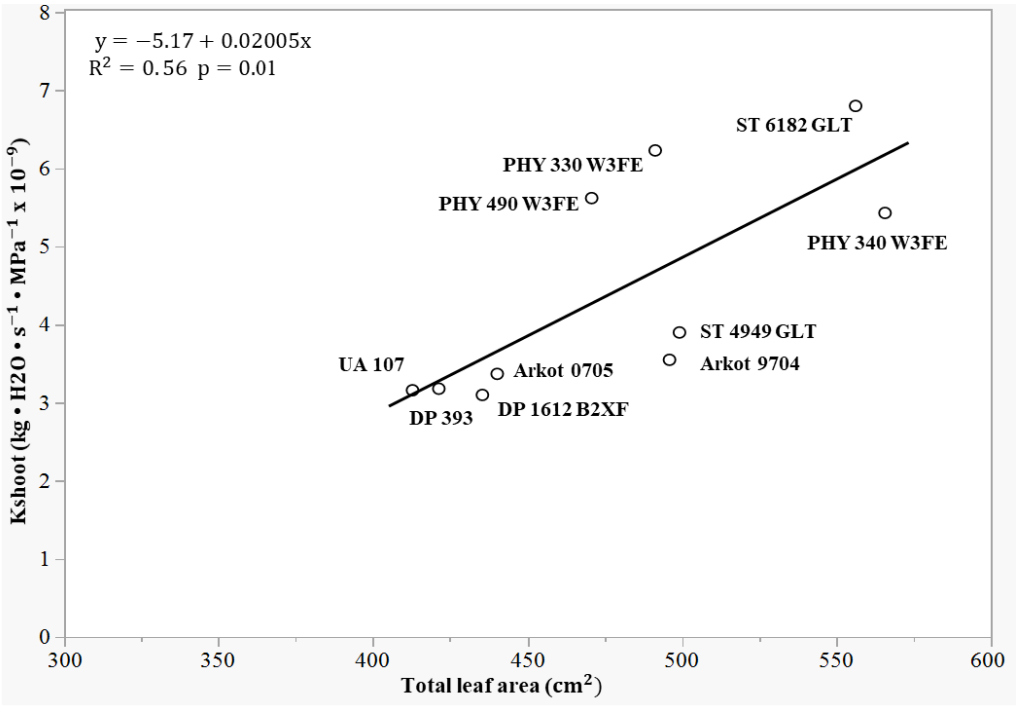


Figure 14. Correlation between cotton cultivar's average shoot hydraulic conductance (K_{shoot}) under a high evaporative demand [38ºC, vapor pressure deficit (VPD) > 3 kPa] and total leaf area (cm^2) in a controlled environment. $R^2 = 0.56$, $p = 0.01$.

CHAPTER IV
COTTON PHYSIOLOGICAL RESPONSES TO VARIOUS RATES OF MEPIQUAT CHLORIDE UNDER RAINFED AND IRRIGATED CONDITIONS

Abstract

Plant growth regulators (PGRs) are often used in cotton production to regulate vegetative growth, increase the efficiency of foliar insecticide and defoliant applications and to increase harvest efficiency. Mepiquat chloride (MC) is the most common PGR used in cotton production. Many environmental and plant physiological factors such as temperature, soil water status, plant maturity, and cultivar can affect the efficacy of MC. Furthermore, irrigation increases plant height and slows maturity, while the addition of growth-suppressing PGRs decreases height and increases maturity. Therefore, a study was conducted during the 2021 growing season to investigate the physiological effects of various rates of MC [passive, moderate and aggressive; 4.2, 9.1, and 14.0 g active ingredient (a.i.) $\cdot ha^{-1}$, respectively] on four varying cotton cultivars including PHY 400 W3FE, DP 1646 B2XF, UA 107 and Arkot 9704 under irrigated and rainfed conditions. Overall, increased rate of MC significantly reduced plant height and specific leaf area (SLA) under both rainfed and irrigated water regimes. Cultivars varied in plant height, stomatal conductance (g_s), and yield response to irrigation and MC treatments. All cotton cultivars growing under irrigated treatment had significantly higher plants height than rainfed, regardless of MC treatments. Results indicate a need for improved MC management strategies under irrigation, given the significant negative correlation found between plant height and machine harvested lint yield.

Introduction

Cotton (*Gossypium hirsutum* L.) plants are managed as annual row crops despite having an inherent perennial growth habit (Ritchie et al., 2007). Subsequently, one major challenge is balancing vegetative and reproductive growth (Cook & Kennedy, 2000; Gwathmey & Clement, 2010) by the use of plant growth regulators (PGR). Failure to regulate growth can result in excessive vegetative growth and subsequent fruit shed, difficulty in machine harvest, boll rot, increased insect and disease pressure, decreased lint quality, and decreased yield (Nichols et al., 2003). The most commonly used PGR is mepiquat chloride (MC) which inhibits gibberellin biosynthesis and reduces cellular elongation (Rademacher, 2000). The key consequence of a MC application is a reduction in plant height and number of main-stem nodes (Reddy et al., 1990). Reduced plant height in cotton can increase the efficiency of foliar insecticide and defoliant applications as well as increase harvest efficiency (Gianfagna, 1995).

The appropriate timing and amount of a MC application is essential when it comes to cotton management. However, making such decisions can be difficult because many environmental factors need to be considered including temperature (Kerby, 1985; Reddy et al., 1990; Rosolem et al., 2012), soil water content (Fernandez et al., 1991; Reddy et al., 1992; Cook and Kennedy, 2000), growth stage (Zhao et al., 2017; Tung et al., 2018), and cultivar (Jost et al., 2006). Thus, growers often struggle to make proper MC applications during a growing season. Mismanagement of MC can negatively impact lint yield due to excessive vegetative growth inhibiting harvest efficiency (Oosterhuis et al., 1998).

Cotton cultivars can vary in their response to PGR application rates and timings (York, 1983; Jost et al., 2006). In general, cultivars with high vegetative growth potential require aggressive MC management and cultivars that are more sensitive to MC applications typically require passive management (Jost et al., 2006). With the use of irrigation for cotton production increasing in the Mid-South of the USA (Vories and Evett, 2014), questions have been raised about how MC management will differ under irrigation, especially with new cotton cultivars with more aggressive growth patterns. Under irrigated conditions, growth potential of cotton is increased and MC application strategies may need to be altered, especially because both irrigation and MC application have associated application costs. Therefore, the objective of this study is to determine how modern cotton cultivars with high vegetative growth potential respond to varying MC application rates under irrigated and rainfed conditions.

Materials and Methods

Field setup

A field experiment evaluating the influence of various rates of MC and irrigation on four cotton cultivar's physiological parameters and yield was conducted in 2021 at the West Tennessee Research and Education Center (WTREC) in Jackson, TN. Four cotton cultivars: Phytogen (PHY) 400 W3FE (Phytogen Cottonseed, Corteva Agroscience, Indianapolis, IN); Deltapine (DP) 1646 B2XF (Deltapine Cottonseed, Bayer CropScience, St. Louis, MO), UA 107 (Bourland & Jones, 2018) and Arkot 9704 (Bourland & Jones, 2009) were chosen for this experiment based on differences in

vegetative growth potential. DP 1646 B2XF and UA 107 are known to have high vegetative growth potential (UTIA, 2018; Bourland & Jones, 2018), while PHY 400 W3FE and Arkot 9704 have low vegetative growth potential (Bourland & Jones, 2009; Agrilife, 2020).

The experiment was arranged in a randomized complete block design with split-split plot arrangement in four replications. The experiment was blocked on irrigation regime: (1) irrigated- plants were watered with a lateral variable-rate irrigation system (Valley, Valley, NE) when a soil matric potential value of -75 to -80 (kPa) was detected by a ZL6-Meter logger (METER Group Inc., Pullman, WA) using soil matric potential sensors at depths 15 and 70 cm, and (2) rainfed- plants only received rainfall with no water supplement.

Each irrigation block was split into three main plots to allow for passive, moderate, and aggressive application rates of MC (Pix Plus, BASF, Research Triangle Park, NC). Each main plot was split into four sub-plots to contain the four cotton cultivars. Within each irrigation block, main plots and sub plots were replicated four times. Irrigation blocks were not replicated. A total of 96 experimental sub plots were evaluated in this experiment.

On May 18th, 2021, cotton seeds were direct seeded at a depth of 2.5 cm in a Lexington silt loam (fine-silty, mixed, active, thermic Ultic Hapludalf). Each subplot was planted with one cultivar in 4, 9.1 m long rows with 96 cm row spacing. The two center rows of each subplot served as experimental rows, while the outer two rows of each subplot served as border rows. All measurements were conducted on the two center rows

of each subplot. The research field was soil-sampled prior to planting, and fertilizer and lime were applied according to the current University of Tennessee Soil and Plant Pest Center recommendations (UTIA, 2021a). The cotton crop was managed for insects and weeds according to University of Tennessee recommendations for pesticide application (UTIA, 2021b).

Mepiquat chloride (MC) and defoliant application

Forty-three days after planting (DAP), a standard management application of MC (4.1 g a.i.·L^{-1}) was applied to the entire field at a rate of 1.2 g a.i.·ha^{-1}. The experimental MC treatments were applied on two separate dates, 57 DAP and 79 DAP. Treatments received: passive (0.0, 2.4), moderate (2.4, 4.9), and aggressive (4.9, 14.0) g a.i.·ha^{-1} for each application, at 57 and 59 DAP, respectively (Table 10). The MC applications were made using a MudMaster Multipurpose Sprayer (Bowman, Newport, AR). Travel speed, tank pressure, and spray volume were 6.5 km·h^{-1}, 317 kPa, and 140 L·h^{-1}, respectively.

At 149 DAP, defoliants including thidiazuron (Daze, Winfeld Solutions LLC, St. Paul, MN), tribufos (Folex, Amvac Chemical Corporation, Newport Beach, CA), cyclanilide, and ethephon (Finish, Bayer Crop Science, St. Louis, MO) were applied at a rate of 87.5, 210.1, 78.8, and 1,260.0 g a.i.·ha^{-1}, respectively, to all research plots. To expedite boll opening, at 157 and 167 DAP, ethephon (Finish, Bayer Crop Science, St. Louis, MO) was sprayed again onto the irrigated plots at a rate of 945.8 and 1,260.0 g a.i.·ha^{-1}, respectively. All defoliation applications were made using a LEE Spider spray tractor (Leeagra Inc., Lubbock, TX). Travel speed, tank pressure, and spray volume were 6.5 km·h^{-1}, 275 kPa, and 140 L·h^{-1}, respectively.

Irrigation application

Water was applied to the irrigated block at 70, 98, and 103 DAP. At 70 DAP, 25 mm of water was applied and for the rest of irrigation events only 13 mm of water were applied. At 2 DAP, all 96 plots received 13 mm of irrigation to help with seed germination due to dry weather before and after planting. Other than the initial irrigation at 2 DAP, the water treatment blocks received a total of 724.5 [irrigated (rainfall + irrigation)] and 673.5 mm [rainfed (only rainfall)] during the growing season (May-October) (Fig. 15).

Plant physiological measurements

Plant height and leaf number measurements were taken in each subplot 51, 69, 84, 90, 133, and 140 DAP. Before initiating the plant height and leaf number measurements, the base of five plants from the middle of the center two rows in each plot were flagged. These five plants were monitored throughout the season and their plant height and leaf number were measured. A meter stick was used during the season for collecting plant height data.

Specific leaf area (SLA) measurements were taken in each subplot under the rainfed water treatment 64, 81, 90, 97, 118, and 133 DAP. Subplots under the irrigated water treatment had leaves sampled 64 and 134 DAP, only. The SLA measurements were taken as described by Wilson et al. (1999). The youngest, but fully expanded leaves from the upper canopy of a plant were sampled in the center of each subplot on sunny days between 1100 and 1400 h.

Wilt scoring was measured in each subplot under the rainfed water treatment 74, 81, 84, 86, 91, 118, and 132 DAP. Scores were taken on sunny days between 1100 and 1400 h with the following scoring scale: 0-5, 0 = no wilting, 1 = a few leaves wilted, 2 = half of leaves wilted, 3 = most leaves wilted, 4 = plant severely wilted, 5 = plant dead.

Stomatal conductance (g_s, mol $H_2O \cdot m^{-2} \cdot s^{-1}$) and photosynthesis values (P, μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) were measured in each subplot under each water treatment using a portable photosynthesis machine (LiCor 6400-XT, Li-Cor Inc., Lincoln, NE) on sunny days between 1100 and 1400 h. Measurements were taken on one separated fully expanded leaf of an individual plant from the center of each subplot. A 6-cm^2 of the leaf was enclosed in the instrument chamber for 60 s to allow for equilibration before a measurement was taken. The section of leaf within the LiCor chamber was exposed to 2000 μmol PAR$\cdot m^{-2} \cdot s^{-1}$ using a 6400-01 light source at 36°C. The CO_2 concentration was maintained at 400 μmol $CO_2 \cdot mol^{-1}$ air with a flow rate was 500 μmol$\cdot s^{-1}$. These settings were meant to replicate the ambient environmental conditions the plant would experience during a typical midday. Licor 6400-XT measurements were taken in the rainfed water treatment 64, 81, 97, and 109 DAP. The measurements in the irrigated water treatment were conducted at 66 and 134 DAP.

In mid-November, 181 DAP, cotton was harvested from each subplot by a two-row picker outfitted with an in-basket, catch-and-weigh system. Each subplot was subsequently harvested, weighed, and ginned. Samples were ginned at the University of Tennessee Cotton MicroGin located at the WTREC in Jackson, TN.

Statistical analysis

Physiological parameters were analyzed with a mixed model ANOVA and mean separation conducted with Tukey's HSD ($p = 0.05$) (JMP, version Pro 15, SAS Institute Inc., Cary, NC). Correlations were computed using bivariate and linear analysis in JMP.

Results and Discussion

Plant height and leaf number

When the MC rate increased from passive to aggressive plants were shorter regardless of irrigation treatment. (Fig. 16). Plants were taller in irrigated than rainfed plots under all three MC treatments (Fig. 16).

Cultivars varied significantly in their growth rate over time, particularly the percent increase in height from 69 DAP (2 weeks after first MC application) to 140 DAP (10 weeks after first MC application) (Table 11). Under rainfed conditions, the height of PHY 400 W3FE increased 29% when treated with a passive MC rate and only 5% when treated with aggressive MC rates. Comparatively, the height of DP 1646 B2XF increased 32.9 and 17.5% when treated with a passive and aggressive rate of MC, respectively (Table 11).

Under irrigated conditions, this trend in height increase over time was even more noticeable among cultivars (Table 11). PHY 400 W3FE height increased 41.8 and 22.3% with passive and aggressive MC rates, respectively. The height of DP 1646 B2XF cultivar increased 60.5 and 54.1% with passive and aggressive MC rates, respectively (Table 11).

A reduction in plant height increase is an indication of MC efficacy in balancing vegetative and reproductive growth. The avoidance of excessive vegetative growth is critical in maintaining cotton yield (Oosterhuis, 1990). Excessive vegetative growth can cause shading of the lower plant canopy which can lead to the abscission of early, lower canopy fruit (Oosterhuis, 2001). Therefore, more sunlight reaching the lower canopy in shorter plant leads to decreased boll rot, higher fruit retention, and increased yield. In support of this hypothesis, a negative correlation was found when the final plant height (140 DAP) was plotted against the lint yield ($kg \cdot ha^{-1}$) among three of the four cultivars (DP 1646 B2XF, PHY 400 W3FE, and UA107) (R^2 = 0.42, 0.28, 0.15; p-value 0.0006, 0.01, 0.06, respectively) (Fig. 17). This indicates that a reduction in plant height is associated with an increase in lint yield.

In their study, Zhao et al. (2019) showed that applying MC resulted in a lower and more compact plant canopy, which is attributed to the effective control of MC in plant vegetative growth. A reduced plant height and more compact plant canopy could allow for greater penetration of end-of-season defoliant applications. Proper defoliation of a cotton crop is critical for improved harvest efficiency and fiber quality (Faircloth et al., 2004). Therefore, the observed increase in yield with a decrease in plant height could be a result of shorter plants having increased efficiency of end-of-season defoliant applications.

Through regulation of vegetative growth, MC can manipulate cotton growth to increase fruit retention (Nuti et al., 2006). This effect was observed when individual cotton plants (PHY 400 W3FE and DP 1646 W3FE) were selected from passive and

aggressive MC treatments under the irrigated water treatment and photographed (Fig. 18). A mixed model analysis of variance (ANOVA) showed a significant interaction between irrigation regimes, MC rates, and cultivars for plants height 69 DAP, when plants were sampled for Fig. 18 (Table 12). This interaction indicates that treatments had effect on plant height, which may have led to the observed difference in boll retention among plants (Fig. 18). At 120 DAP plants treated with aggressive MC had higher boll retention compared to passive MC. Across both MC treatments, PHY 400 W3FE had higher boll retention than DP 1646 W3FE (Fig. 18).

Specific leaf area (SLA)

An increase in MC led to lower SLA among all cultivars under both water treatments (Table 13). Overall, plants under the rainfed water treatment had lower SLA (113.3 $cm^2 \cdot g^{-1}$) than those under irrigation (117.3 $cm^2 \cdot g^{-1}$).

A reduced SLA is beneficial to plants grown in water limited (i.e., rainfed) conditions, causing cell wall thickening which could help increase the ability of a leaf to accommodate variations in leaf water potential during successive drying cycles (Marron et al., 2003). Reddy et al. (1996) reported that leaf area growth rate, total node number, and plant height decrease linearly with MC concentrations increasing from 0 to 30 µg g^{-1}. Reddy et al. (1990) demonstrated MC rate effect on container-grown cotton and found the reduced SLA response to MC was temperature dependent. Plants exposed to MC under cooler temperatures (20/15°C day/night) had a more reduced SLA than plants exposed to MC under higher temperature (35/25°C). Rosolem et al. (2012) indicated that at high temperatures, the rate of MC to be applied must be disproportionately increased,

because either plant growth is impaired by high temperature lessening the effect of MC, or degradation of MC within the plant is too rapid.

Overall plants growing in the irrigated water treatment had a higher SLA rate when compared to rainfed in our study. This effect is likely due to the higher rate of plant growth observed under irrigation. The results show that responses from differing MC application rates are highly variable and closely related to the environment in which the crop is grown. Therefore, cotton grown under irrigation may require a higher rate or more frequent application of MC to achieve its desired effects.

Wilting score

Under the rainfed water treatment throughout the entire growing season, PHY 400 W3FE and DP 1646 B2XF consistently had the lowest and highest wilt scores, respectively (Fig. 19). The observed difference in wilt scores between these two cultivars could be why the efficacy of MC application varied. The first application of MC was 57 DAP, when no wilting was observed. However, the second application of MC was 79 DAP, when early wilting was observed among plants under the rainfed water treatment. Wilt score was measured two and five days after the second MC application (81 and 84 DAP). In both measurements, DP 1646 B2XF had a higher wilt score than all other cultivars. This higher instance of wilting observed in DP 1646 B2XF could lead to lower leaf water potentials (O'Toole and Cruz, 1980), which in turn could decrease the amount of MC translocated throughout the plant (Munns and Pearson, 1974; Ackerson and Herbert, 1980; Nuti et al., 2006). This reduction in MC translocation could have decreased the efficacy of MC, increasing vegetative growth rate that was recorded for this

cultivar. Meanwhile, PHY 400 W3FE had limited to no wilting observed throughout the growing season, indicating this cultivar had higher leaf water potential, thus improved MC translocation, and a greater response to MC witnessed through decreased vegetative growth rates.

Stomatal conductance (g_s)

Figure 21 illustrates the average of g_s measurements taken throughout the growing season for plants under each water treatment. Cultivar DP 1646 B2XF had higher g_s under the rainfed water treatment than all other cultivars (Fig. 20). There were no g_s differences among cultivars under the irrigated water treatment (Fig. 20). High g_s rate under rainfed conditions can cause limited soil water availability (Shekoofa et al., 2020), and likely contributes to the high wilt score observed throughout the growing season (Fletcher et al., 2007; Sheldon et al., 2021; Purdom et al., 2021). Therefore, when the second application of MC was applied, MC efficacy was lessened for DP1646 B2XF due to wilting. When a rainfall event of 15 mm occurred 10 days after the second MC application, DP 1646 B2XF growth rate rapidly changed and a limited impact of MC application was observed.

Yield

Overall under rainfed conditions, all cotton cultivars yielded higher than those under irrigated conditions (Fig. 21). This is likely due to a greater efficacy of defoliant under the rainfed water treatment. Near the end of season (i.e. 140 DAP), plants under the rainfed water treatment were shorter than those under irrigated conditions (Table 14).

Landivar et al. (1997) reported the effect of MC in reducing seed abortion and yield was more evident under dryland than under irrigated conditions. The lower lint yield observed among all MC treatments under irrigation indicates that even the aggressive MC treatment was insufficient at controlling vegetative growth under irrigated conditions.

Increased MC rates beyond passive rate led to improved yield under rainfed and irrigated conditions for most cotton cultivars (Fig. 21). This trend was observed for almost all cultivars except Arkot 9704. Cultivar PHY 400 W3FE had a 34% increase in lint yield when MC rate was changed from passive to aggressive under the irrigated water treatment. A similar trend was observed for PHY 400 W3FE with an 18% increase in yield under the rainfed water treatment.

Correlations in plant height and yield varied by cultivar (Fig. 17). Smaller plants due to MC application rates led to fewer leaves, and fewer leaves could lead to a higher turnout percentage (ratio of the ginned lint mass to the initial mass of seed cotton) and final yield. Controlling plant height and leaf number in modern cultivars, such as DP 1646 B2XF with an aggressive growth, is critical for maintaining yield. Of the two cultivars with strong correlations between plant height and yield (PHY 400 W3FE and DP 1646 B2XF) the cultivar with greater response to MC, PHY 400 W3FE, had significantly higher yield under both water treatments (Fig. 21).

Irrigated and rainfed cotton plots received their first harvest aid application at the same time (149 DAP). Excessive vegetative growth under irrigation prevented penetration of the defoliant into the canopy and led to the need for an additional application. Furthermore, lodging was observed in some Arkot 9704 plots leading to

decreased defoliant efficacy and reduced yield. Results are in agreement with those of Nelson et al. (1990), who reported that several factors including the vegetative condition of the irrigated cotton plants may have reduced the effectiveness of the defoliation treatments.

Conclusion

Overall, irrigation, MC rate, and cultivar impacted cotton plant height which led to variability in lint yield. Plants grown under irrigation mostly escaped the MC or had limited response to application. This led to a reduction in fruit retention and created leaf defoliation difficulties which negatively impacted yield. Because both irrigation and MC application have associated application costs, the benefits of these field management options might be improved by further research in irrigated cotton. An adjustment of either early MC application or shortened application interval is needed to address application amount and timing issues under irrigation conditions.

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Appendix

Table 10. Amount of mepiquat chloride (MC) applied on days after planting (DAP) for each MC treatment (passive, moderate, and aggressive).

Amount of applied MC (g a.i.·ha^{-1})				
	DAP			
MC Treatment	**43**	**57**	**79**	**Total**
Passive	1.8	0.0	2.4	4.2
Moderate	1.8	2.4	4.9	9.1
Aggressive	1.8	4.9	7.3	14.0

Table 11. Plant growth rate over time the increase in height (%) from 2 weeks after first mepiquat chloride (MC) application, 69 days after planting (DAP) to 10 weeks after first MC application (140 DAP) among cultivars under irrigated and rainfed conditions for passive (4.2 g a.i·ha^{-1}) and aggressive (14.0 g a.i.·ha^{-1}) MC treatments.

Plant Height (cm)						
Irrigated						
	2 weeks		10 weeks		The increase in height (%)	
Cultivar	**Passive**	**Aggressive**	**Passive**	**Aggressive**	**Passive**	**Aggressive**
PHY 400 W3FE	89.5	70.4	127.0	86.2	41.8	22.3
Arkot 9704	91.3	77.4	144.1	98.2	57.8	26.7
UA 107	89.9	67.1	135.5	97.4	50.7	45.1
DP 1646 B2XF	94.7	72.0	152.1	111.0	60.5	54.1
Rainfed						
PHY 400 W3FE	88.7	72.9	114.4	76.6	29.0	5.1
Arkot 9704	96.0	72.5	125.0	79.8	30.1	9.9
UA 107	86.9	72.5	123.6	79.6	42.2	9.8
DP 1646 B2XF	95.1	80.0	126.5	94.0	32.9	17.5

Table 12. Mixed model analysis of variance (ANOVA) of plant height (cm) taken 69 days after planting (DAP).

Source	DF	Sum of Squares	F Ratio	Prob > F
Irrigation	1	532.27	7.4759	0.0065
MC	2	30560.342	214.6156	<.0001
Cultivar	3	3877.465	18.1535	<.0001
Cultivar × Irrigation	3	9.18	0.043	0.98
Cultivar × MC	6	424.703	0.9942	0.42
Irrigation × MC	2	244.381	1.7162	0.18
Cultivar × Irrigation × MC	6	896.907	2.0996	0.05

Table 13. Specific leaf area (SLA) of all cotton cultivars under rainfed and irrigated water treatments among each mepiquat chloride (MC) treatment. SLA followed by the same letter under each water treatment are not significantly different (alpha = 0.05).

	SLA ($cm^2 \cdot g^{-1}$)	
	Water treatment	
MC treatment	**Rainfed**	**Irrigated**
Passive	118.4 a	124.19 a
Moderate	113.02 ab	115.52 ab
Aggressive	108.63 b	112.21 b

Table 14. Average plant height (cm) among meiquat chloride (MC) treatments under rainfed and irrigated water treatments near the end of season, 140 days after planting (DAP). P-value measured at alpha = 0.05

	Plant height (cm) 140 DAP			
	Water treatment			
MC treatment	**Rainfed**	**Irrigated**	**Height difference**	**p-value**
Passive	122.4	141.0	18.6	<.0001
Moderate	92.7	106.8	14.1	<.0001
Aggressive	82.5	98.2	15.7	<.0001

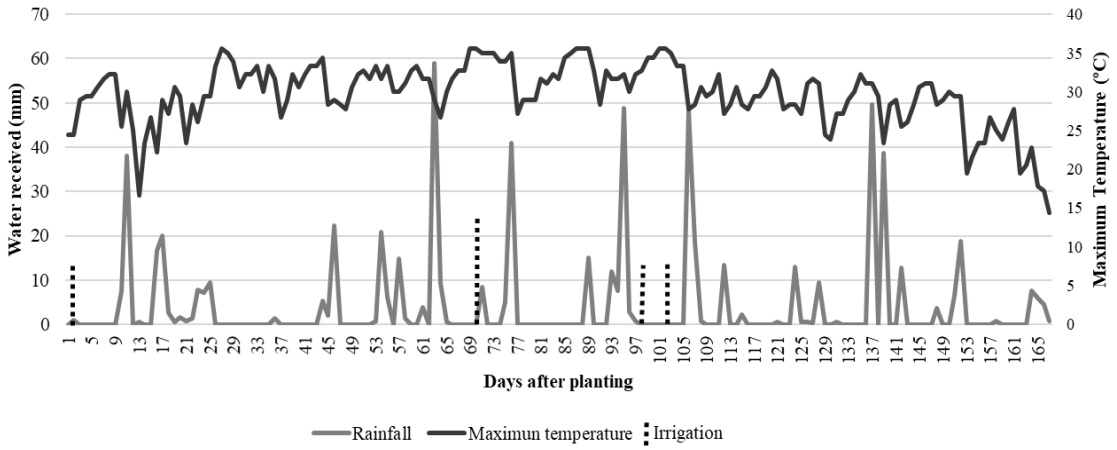


Figure 15. Rainfall, irrigation (mm), and maximum daily temperature (ºC) during the 2021 growing season at the West Tennessee Research and Education Center in Jackson, TN.

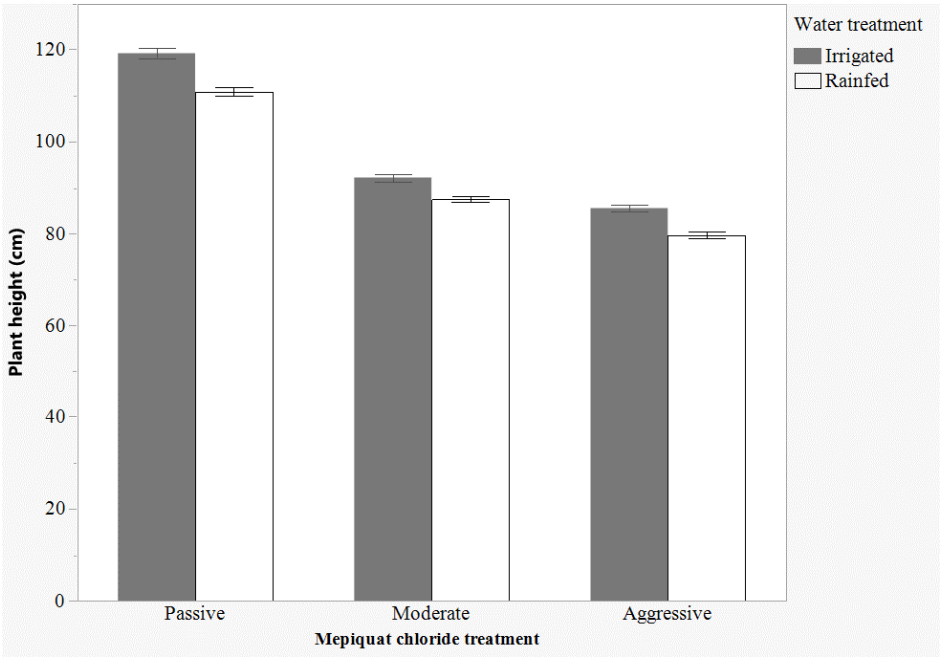


Figure 16. Average plant height (cm) among all cotton cultivars under each mepiquat chloride (MC) treatment [passive (4.2 g a.i.·ha^{-1}), moderate (9.1 g a.i.·ha^{-1}), and aggressive (14.0 g a.i.·ha^{-1})] under irrigated and rainfed conditions. Error bars represent the standard error of the mean.

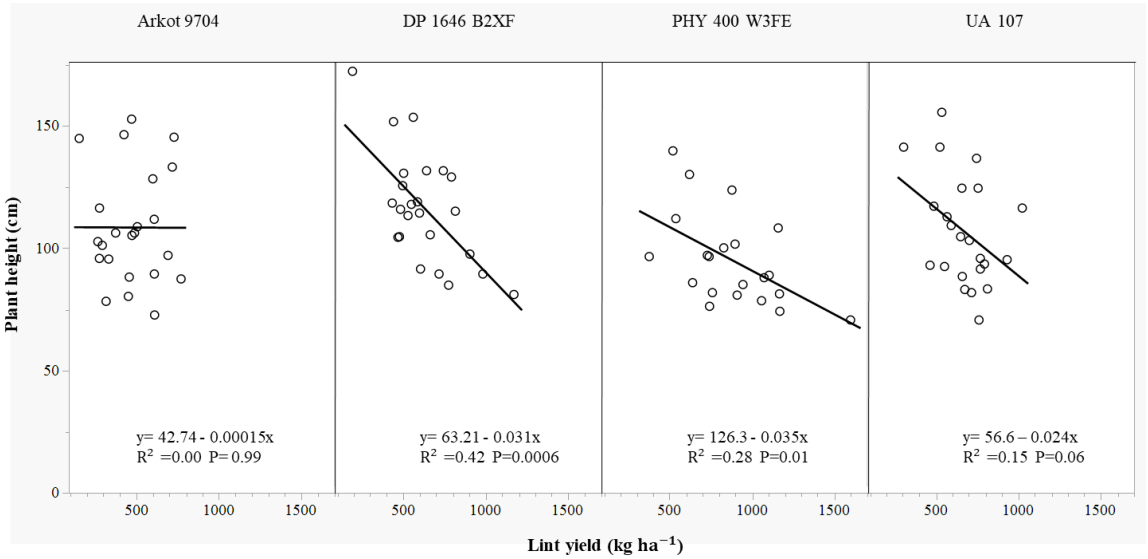


Figure 17. Correlations between plant height (cm) and lint yield ($kg \cdot ha^{-1}$) among cotton cultivars under ranfed and irrigated water treatment. Plant height was measured near the end of the season 140 days after planting. P-value was measured at alpha = 0.05.



Figure 18. Plant height (cm) response to passive (4.2 g a.i.·ha^{-1}) and aggressive (14.0 g a.i.·ha^{-1}) mepiquat chloride (MC) treatments between cotton cultivars PHY 400 W3FE (left) and DP 1646 B2XF (right) during growth [120 days after planting (DAP)] (upper) and before harvest (185 DAP) (lower). A 152 cm measuring stick is included as a reference for scale. Plants were grown under the irrigated water treatment.

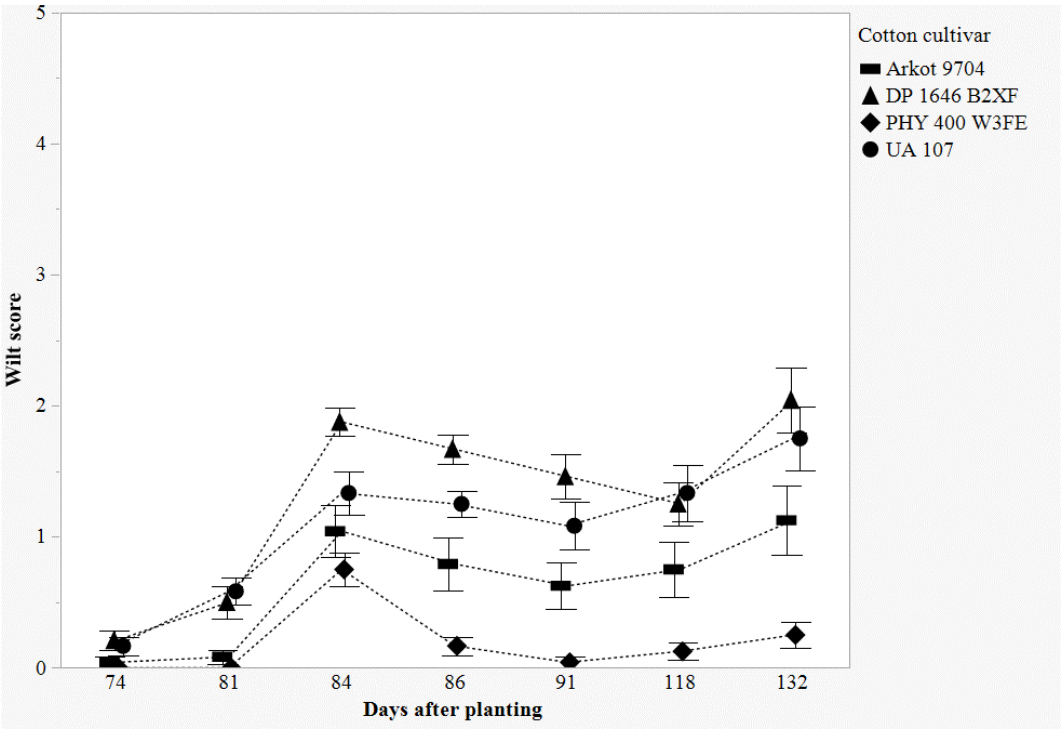


Figure 19. Wilt scores of cotton cultivars under the rainfed water treatment throughout the growing season. 0-5, 0 = no wilting, 1 = a few leaves wilted, 2 = half of leaves wilted, 3 = most leaves wilted, 4 = plant severely wilted, 5 = plant dead. Error bars represent the standard error of the mean.

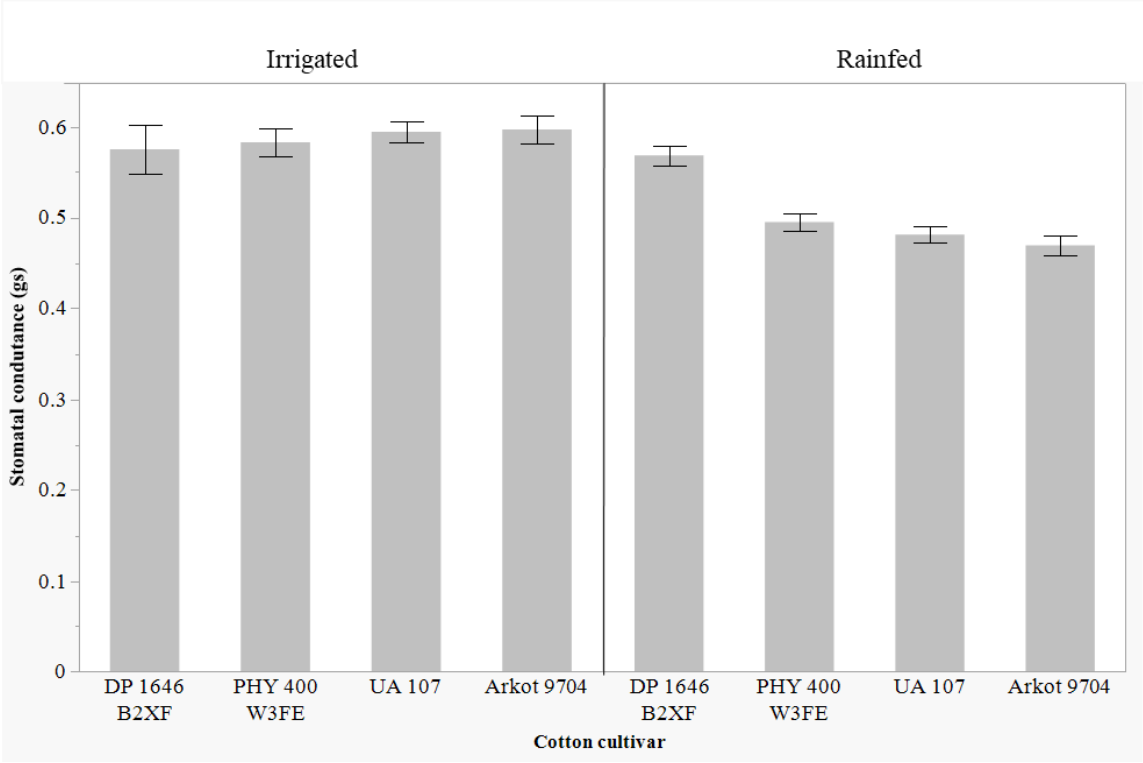


Figure 20. Cultivar's average stomatal conductance (g_s, mol $H_2O \cdot m^{-2} \cdot s^{-1}$) through the 2021 growing season under the irrigated and rainfed water treatments. Error bars represent the standard error of the mean.

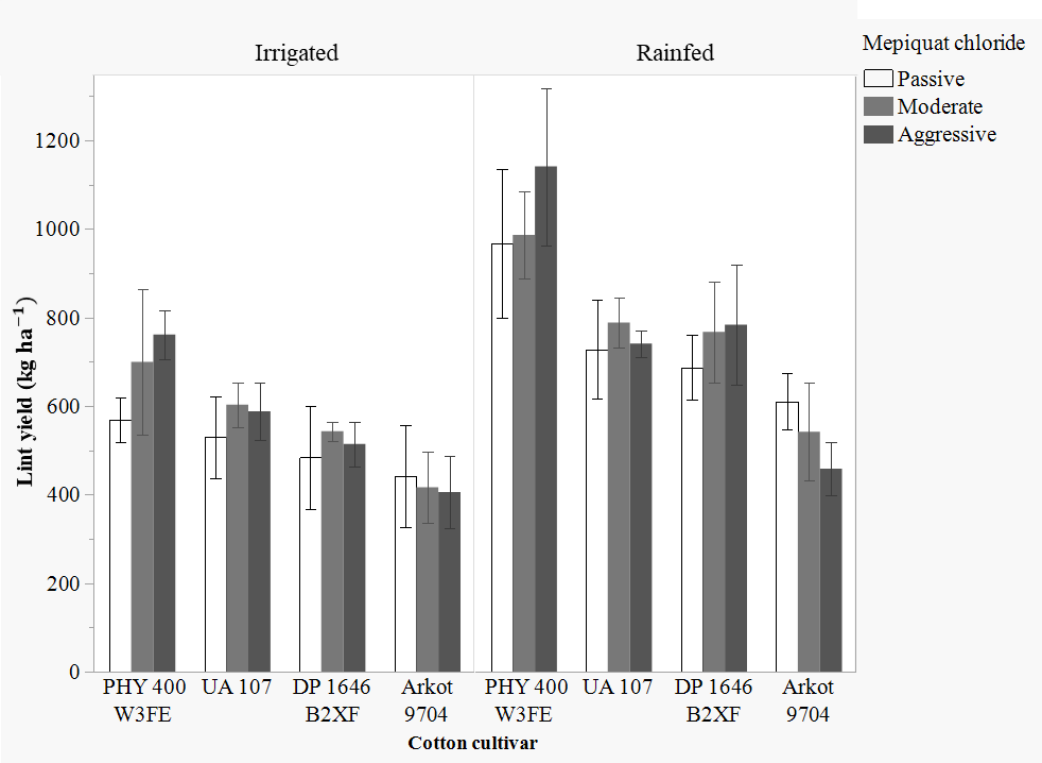


Figure 21. Cotton cultivar's lint yield (kg·ha^{-1}) under water (irrigated and rainfed) and mepiquat chloride (MC) [passive (4.26 g a.i.·ha^{-1}), moderate (9.16 g a.i.·ha^{-1}), and aggressive (14.06 g a.i.·ha^{-1})] treatments. Error bars represent the standard error of the mean.

CHAPTER V
CONCLUSION

Discussion and Conclusion

In Tennessee, only 12% of cotton is grown under irrigation (UTIA, 2021), leaving the majority of cotton acreage dependent on rainfall to supply all required water for a growing season. Furthermore, cotton is grown in regions with high temperature and evaporative demand rates. As a result, water shortages during the growing season are common and have a negative influence on yield (Devi and Reddy, 2018). Cultivar selection can be beneficial in minimizing the detrimental impacts of water-deficit stress due to variances in water use. Therefore, the discovery of drought tolerance traits among cotton cultivars is needed for yield protection under dryland environments.

In response to the unpredictability of rainfall, an increasing number of growers in the Mid-South region of the United States have installed irrigation systems for use in cotton production (Vories and Evett, 2014). Cotton grown under irrigation has a higher capacity for vegetative development, which could necessitate changes in plant growth regulator (PGR) management (Reddy et al., 1992).

This project sought to identify the limited transpiration (TR_{lim}) trait among a variety of newly released cotton cultivars under progressive soil drying, high atmospheric vapor pressure deficit (VPD), and extreme water-deficit stress in a greenhouse, growth chamber, and field settings. An investigation into shoot hydraulic conductance (K_{shoot}) was completed to assess if hydraulic movement within plant shoots was associated with the TR_{lim} trait. Furthermore, evaluation into the physiological effects of various rates of PGR on modern, aggressive growth cultivars under irrigated and rainfed environments was completed to determine if changes to management strategies were warranted.

In the greenhouse, growth chamber, and field studies discussed in Chapter 2, four cultivars, PHY 400 W3FE, PX 3C06 W3FE, PHY 500 W3FE, and PX 3D32 W3FE, were evaluated for their transpiration (TR) response to a variety of water-deficit stresses. The most significant finding from this study was that PHY 400 W3FE, the only cultivar to demonstrate TR_{lim} trait under both controlled environments, had significantly higher yield under extreme water-deficit stress in the field than cultivars that did not express TR_{lim} trait, in particular PHY 500 W3FE. The results indicate that the stability of early stomatal closure and TR_{lim} from controlled environments translate to the field. Results highlight the potential for future cultivar screening of drought tolerance traits through similar methods. Furthermore, the field results indicate that TR_{lim} trait can have a positive influence on yield under water-deficit stress (Sinclair et al., 2014; Shekoofa et al., 2020), considering that PHY 400 W3FE, a cultivar with observed TR_{lim} trait, yielded 37% higher under the extreme water-deficit stress condition than PHY 500 W3FE, a cultivar without expression of TR_{lim}.

In Chapter 3, K_{shoot} measurements among cultivars under high temperature and evaporative demand (38ºC and >3 kPa, VPD) showed that hydraulic flow of water through a plants shoot is critical in influencing the limitation of TR. Correlations between several drought tolerance parameters and K_{shoot} indicate that K_{shoot} is associated with drought tolerance traits (i.e., TR_{lim} trait and early stomatal closure) in plants. These connections and significant differences observed among cultivar's K_{shoot} response to high evaporative demand suggest that K_{shoot} can be used as an effective measurement to screen cultivars for drought tolerance traits. Results also indicate that genotypic variation among

cultivars can influence the limitation of shoot water flow under high evaporative demand. Therefore, the inclusion of K_{shoot} measurements into drought tolerance assessment among new and existing cultivars is justified. Further research is needed to investigate root hydraulic conductance given the correlations found between K_{shoot} and soil-water saving traits during progressive soil drying.

In Chapter 4, observations and measurements gathered in the field suggest that current PGR management strategies under irrigation need to be adjusted, especially with modern, aggressive growth cultivars such as Deltapine (DP) 1646 B2XF. Plants grown under irrigation were less responsive PGR applications. This led to a higher rate of plant growth and significantly taller plants at the end of the season among all tested cultivars. The increased plant height under irrigation led to a reduction in fruit retention and created difficulties in the end of season defoliation which led to a significant reduction in yield. Therefore, adjustment is needed under irrigation to either apply PGR earlier in the season or shorten the interval of application rates to avoid excessively tall plants. Given the application costs of PGR and irrigation, the benefits of these field management options could be improved by further research on irrigated cotton.

Collectively, the results of these studies provide valuable information to cotton researchers, breeders, and growers about the physiological differences cotton cultivars demonstrate under water-deficit stress environments. Differences in TR_{lim} trait through stomatal closure in response to water-deficit stress can lead to significant impacts on crop production, as was demonstrated in Chapters 2, 3, and 4. From a plant breeding perspective, information about genotypic differences in TR_{lim} trait has the potential to be

incorporated into breeding programs to develop cultivars with greater water-use efficiency for water-deficit stress environments. From a research perspective, additional studies should continue to investigate the physiological mechanisms responsible for TR_{lim} trait under varying field management strategies such as PGR application rates and irrigation regimes and work to discover additional cultivars among a variety of breeding lines that demonstrate the TR_{lim} trait. From a grower's perspective, information on cultivars that demonstrate water-saving potential under rainfed environments can be used to make better cultivar selection decisions. This can lead to improved drought tolerance, greater yields, and increased sustainability of cotton production as the climate changes.

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VITA

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