

MOLECULAR BIOLOGY AND PHYSIOLOGY

Stress Responses of Commercial Cotton Cultivars to Reduced Irrigation at Flowering and Maximization of Yields under Sub-Optimal Subsurface Drip Irrigation

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ABSTRACT

The gradual depletion of the Ogallala Aquifer in the Southern High Plains of Texas has resulted in reduced well capacities for cotton (*Gossypium* spp.) irrigation. This study investigated cotton cultivar responses to reduced irrigation from flowering to harvest (2013); and then evaluated season long water deficits and the impact of the timing and amount of irrigation (2014). In 2013, field-grown cotton irrigated with 5 mm per day irrigation showed relatively low stress levels as exemplified by efficiency of quantum yield values between 0.17 and 0.23. Reducing irrigation levels from 5 mm per day to 2.5 mm per day at flowering produced a range of stress levels of F_v/F_m from 0.28 to 0.54. In 2014, four of the commercial cultivars were grown in replicated plots receiving either 2.5 mm of irrigation per day or 17.5 mm of irrigation once a week. Cultivar differences in plant stress were detected for the daily and weekly irrigation strategies. Changes in plant size, boll production, and timing of cutout were observed. FM 2484B2F and PhytoGen 72 exhibited no yield differences between the irrigation regimes, while All-Tex Edge and PhytoGen 367 showed yield decreases (20% and 25%) under the 2.5 mm daily irrigation regime. Alteration of the timing for the limited irrigation can impact existing stress sensitivities by reducing stress levels and increasing yields.

Maximizing cotton (*Gossypium* spp.) yields under abiotic stress requires the synergistic effect of vegetative stress tolerance and reproductive stress tolerance. Significant diversity exists leading to a range of vegetative stress tolerances. Some of this diversity includes variation in root lengths (Bland and Dugas, 1989, Burke and Upchurch, 1995, Eissa,

et al., 1983, McMichael and Lascano, 2010, Schwab, et al., 2000), stomatal conductance (Ackerson and Hebert, 1981, McMichael and Lascano, 2010, Plaut and Federman, 1991, Singh, et al., 2013, Singh, et al., 2006), leaf area (Burke, et al., 1985, Dong, et al., 2006, Fernandez, et al., 1996b, Singh, et al., 2006), cuticle thickness (Bondada, et al., 1996, Oosterhuis, et al., 1991a, Oosterhuis, et al., 1991b, Shepherd and Wynne Griffiths, 2006), and accumulation of compatible solutes (Ackerson, 1981, Burke, 2007, Goldschmidt and Huber, 1992, Lopez, et al., 2008).

Many of the morphological and physiological responses of cotton to drought aid the plant in harvesting water from the soil and reducing water loss through the canopy. Burke and Upchurch (1995) described changes in rooting patterns associated with the warmer soil temperatures of non-irrigated cotton, increasing time within the optimum temperature range for growth and development. The observed root length densities of the irrigated plots peaked in the 10- to 20-cm zone at a value slightly greater than 2 (cm cm^{-3}) and declined with depth to low levels below 140 cm. The root length densities for the cotton in the non-irrigated plot showed a biphasic pattern. Root length density was 1.8 cm cm^{-3} in the 0- to 10-cm zone and declined to approximately 0.6 cm cm^{-3} between 30 and 60 cm, increased to approximately 1.0 cm cm^{-3} at 100 cm, and then declined with depth. The root length densities in the non-irrigated plot was four times that of the roots in the irrigated plots at 100 cm; and the soil temperatures of the non-irrigated plot at this depth were within the optimal temperature range for growth 10 times longer than in the irrigated plot. The observed cotton root responses are consistent with conclusions of Bland and Dugas (1989) that redistribution of dry matter invested in roots near the soil surface to deeper in the profile would probably increase total water extraction by the crop in fine textured soils. McMichael and Lascano (2010) investigated hydraulic lift in plants defined as the redistribution of water from wetter to drier soil through the plant roots in response to soil water potential gradients. Water is released from the roots into the dry soil when transpiration is low (night)

and reabsorbed by the plant when higher transpiration rates are resumed (daylight). They hypothesized based upon their findings that hydraulic lift was not of sufficient magnitude to meet total transpirational demands; however, there may be sufficient water transferred to maintain viable roots in the surface soil in anticipation of root water uptake for plant development from rain events before it is lost to water evaporation and/or runoff.

Above ground responses of cotton to water-deficit stresses include reductions in leaf area index (LAI), reduction in leaf size and thickness, alterations in thickness and chemical makeup of leaf cuticles, accumulation of compatible solutes (proline, glycinebetaine), reduction in plant height, and changes in solar tracking of leaves (Burke, et al., 1985, Ehleringer and Hammond, 1987, He, et al., 2007, Lv, et al., 2007, Oosterhuis, et al., 1991a, Oosterhuis, et al., 1991b, Zhang, et al., 2011). In a review article by Shepard and Wynne Griffiths (2006) the current knowledge relating to the effects of stress on cuticular waxes and the ways in which the wax provides protection against the deleterious effects of light, temperature, osmotic stress, physical damage, altitude, and pollution was discussed. They reported that development of leaf cuticles responds dynamically to environmental cues. Oosterhuis (1991a) reported that water deficit increased cotton leaf cuticle thickness by 33%, altered the epicuticular wax composition, and increased total wax content. Chloroform extracts of the leaf cuticle indicated that water-stressed leaves had increased concentrations of higher molecular weight waxes that increased the hydrophobicity of the leaf surface (Oosterhuis, et al., 1991a).

There are fewer reports of diversity in the levels of reproductive stress sensitivity compared with vegetative stress sensitivity. Loss of flowers and squares are commonly reported in response to water deficits and elevated temperatures associated with drought (Pettigrew, 2004, Ungar, et al., 1989, Wilson and Stapp, 1984). Pollen development and viability are also sensitive to elevated temperatures and dehydration associated with low humidity (Burke, et al., 2004, Kakani, et al., 2005). There are only a few reports of differential cultivar responses of pollen development under drought (Kakani, et al., 2005).

The goal of this study was to identify genetic diversity among commercial cotton cultivars in plant responses to suboptimal irrigation during peak boll load, and evaluate potential changes in irrigation

management for varieties that exhibit water deficit stress sensitivities. Vegetative stress responses were determined using the modified water-deficit stress bioassay for cotton described by Burke (2007) and Burke et al. (2010). The study evaluated commercial cotton varieties under 5 mm/day and 2.5 mm/day drip irrigation regimes during flowering and boll set. Selected lines were then evaluated using either 2.5 mm/day or a single 17.5 mm/week drip irrigation regime.

MATERIALS AND METHODS

Crop Management: 2013. The soil type is an Amarillo fine sandy loam, and the fields are located in Lubbock, TX. Four 15 m rows per plot of FM 2484B2F, Nitro44, NexGen 4111RF, PhytoGen 375, ST4946GLB2, DP 0912, PhytoGen 802, All-Tex_EpicRF, FM 8270GLB2, All-Tex Edge, NexGen 1511B2RF, DP 1212, PhytoGen 367, PhytoGen 499WRF, FM 2011GT, FM 9180, PhytoGen 72, and DP 1219B2RF cotton were planted in a North-South orientation per replication in a randomized complete block design using a John Deere 7300 MaxEmerge2 VacuMeter Planter. The field was part of an annual sorghum-cotton rotation. The plots were pre-plant irrigated by furrow irrigation and the plots subsequently irrigated with sub-surface drip. All plants received 5 mm/day from sub-surface drip in the furrows on 80-inch centers until initiation of flowering. Irrigation was then reduced to 2.5 mm/day on half of the plots (four replicates), while maintaining the 5-mm/day irrigation on the remaining plots (four replicates). The plots were sprayed with Ginstar (Bayer CropScience, Research Triangle Park, NC) and Prep (Bayer CropScience, Research Triangle Park, NC) according to manufacturer's instructions. The center two rows of each plot were harvested with a two-row John Deere 484 plot stripper that allowed collection of rows independently. Seed cotton weights for the two rows per plot were measured for four replicates per line per treatment.

Crop Management: 2014. Eight 20 m rows of All-Tex Edge, FM 2484B2F, PhytoGen 72 and PhytoGen 367 were planted per replication in a complete block design in a North-South orientation using a John Deere 7300 MaxEmerge2 VacuMeter Planter. The soil type was an Amarillo fine sandy loam, and the fields are located in Lubbock, TX. The plots were pre-plant irrigated by furrow irrigation and the plots subsequently irrigated with sub-surface drip. Plants received either 2.5 mm/day on half of the plots, and

17.5 mm irrigation once a week on the remaining plots. The plots were sprayed with Ginstar (Bayer CropScience, Research Triangle Park, NC) and Prep (Bayer CropScience, Research Triangle Park, NC) according to manufacturer's instructions on 3 October 2014, and the plots were harvested on 30 October 2014. Four-1 m sections were hand harvested from each replicate and evaluated for yield. The hand-harvested cotton was ginned on a bench-top saw gin and the percent turnout determined. Fiber characteristics were determined at the United States Department of Agriculture (USDA) Cotton Classing Office, Lubbock, TX.

Stress Test Bioassay. A 1-cm² leaf punch was harvested from the fifth mainstem leaf from the top of the plant. This was repeated on five separate plants. The punches were transferred to a well in a Costar[®] 3524 24-well cell culture cluster (Corning Inc., Corning, NY) that had been 1/2 filled with water. The lid was returned to the cell culture plate immediately following addition of the leaf punches. This process was repeated until samples from all treatments had been harvested. In the lab, the punches were placed on moistened Model 583 Gel Dryer Filter Paper (Bio-Rad Laboratories, Hercules, CA) in a Pyrex baking dish. The leaf punches and filter paper were covered with Glad[®] ClingWrap [CO₂ permeable] (The Glad Products Company, Oakland, CA) and pressed flat with a speedball roller for Microseal film (MJ Research, Inc., Waltham, MA) to remove air bubbles and ensure good contact between the tissue and filter paper. Initial chlorophyll fluorescence yield of quantum efficiency (Fv/Fm) levels were determined using an Opti-Science OS1-FL Modulated Fluorometer and then samples were placed in the dark in a VWR Model 2005 incubator (Sheldon Manufacturing, Inc., Cornelius, OR) set to 39°C. The samples were heat treated for 30 min at 39°C, and then removed from the incubator and placed on the bench top at 25°C for 30-min. The decline in fluorescence yield (Fv/Fm) over time was used as a relative measure of the stress level of the plant. Stressed plants exhibit a slow decline and non-stressed plants exhibit a more rapid decline.

Measurement of Relative Root Length. In an effort to evaluate relative rooting patterns in the upper 0.5-meter of soil, the root systems of three plants per plot were harvested using "The Uprooter" (Grants Pass, OR). The root systems were photographed on a 2-inch by 2-inch grid and root lengths determined by tracing the roots in Photoshop and comparing the total pixel number to the number of pixels in a 2-inch line.

Meteorological Measurements. The USDA - PSWC Meteorological Tower is located immediately adjacent to the experimental plots. Five-minute measurements of temperature (C) were collected and hourly averages calculated. Average daily air temperatures were calculated and compared with the stress values obtained from the bioassay.

Experimental Design and Statistical Analysis. The field experiment in 2013 used a randomized complete block design with three replications. The 2014 experiment used a complete block design with two replications and four rows per cultivar per replication. Statistical significance among genotypes was analyzed using PROC GLIMMIX (SAS version 9.4, SAS) for the different values of response cultivars and interactions between irrigation regimes, using block and block by entry as random effect in the model with adjust=simulate (data not presented) for mean separation. Also, data were analyzed and presented herein for convenience of visualization and/or graphics, with a student's *t* test through the statistical applications of Excel software. Graphs were created using Kaleida-Graph Version 4.1.3 (Synergy Software, Reading, PA, http://www.synergy.com/wordpress_650164087/kaleidagraph/). Analyses provided similar outcomes for the most significance differences presented in this study.

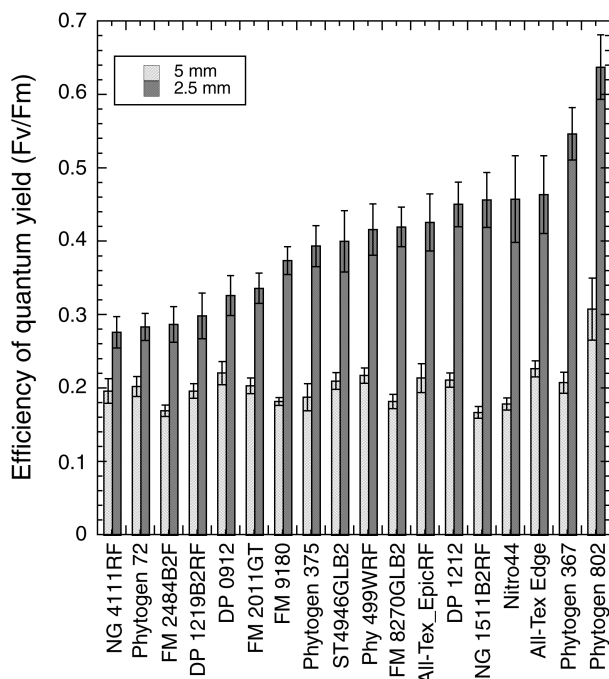
RESULTS

This study investigated the usefulness of a vegetative stress bioassay (Burke, 2007, Burke, et al., 2010) in identifying genetic diversity among commercial cotton cultivars in plant responses to suboptimal irrigation during peak boll load, and evaluated potential changes in irrigation management for varieties that exhibit water deficit stress sensitivities.

The 2013 vegetative stress responses of eighteen commercial cultivars to the initiation of irrigation regimes of either 5 mm per day or 2.5 mm per day of subsurface drip at flowering are shown in Figure 1. With the exception of Phytogen 802, all of the commercial cultivars exhibited low levels of water stress as exemplified by the 0.17 to 0.23 efficiency of quantum yield values observed under the 5 mm per day irrigation regime. The least stressed cultivars were NexGen 1511B2RF and FM2482B2F with a value of 0.17; and the most stressed cultivars were All-Tex Edge and Phytogen 802 with values of 0.23 and 0.31, respectively. When irrigation was reduced to 2.5 mm per day at flowering, increased stress levels were ob-

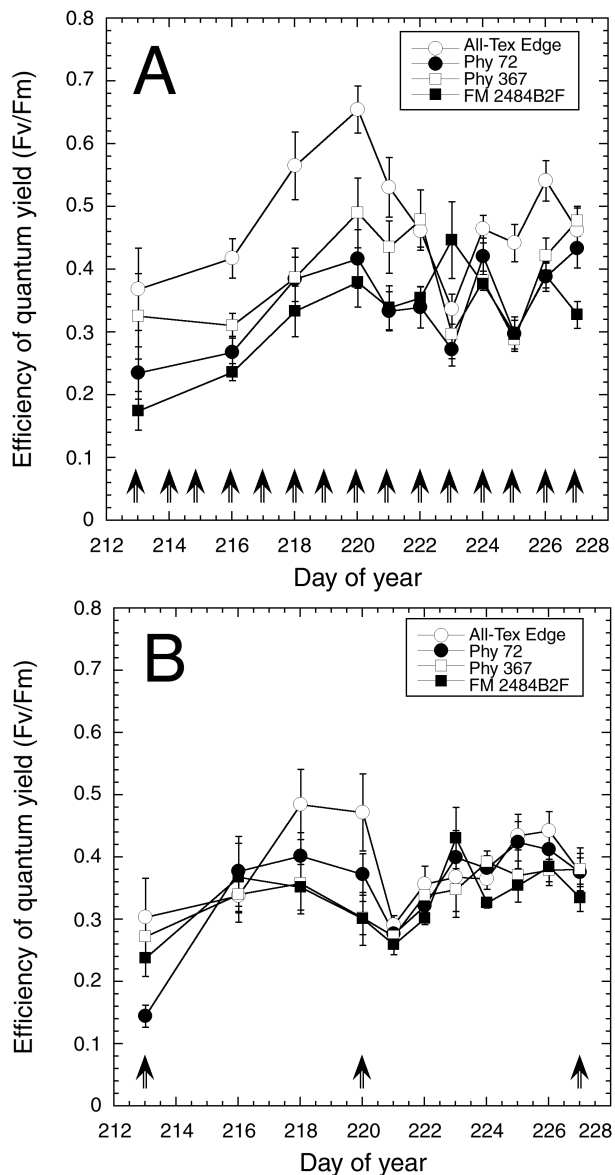
served. The efficiency of quantum yield values ranged from 0.28 to 0.54. NexGen 4111RF and Phytogen 72 had the lowest stress levels with values of 0.277 and 0.284, while Phytogen 367 and Phytogen 802 were the most stressed with values of 0.547 and 0.638.

Figure 1. Graph of the vegetative stress responses, measured using the efficiency of quantum yield, of eighteen commercial cultivars to the initiation of irrigation regimes of either 5 mm per day (dotted bar) or 2.5 mm per day (solid bar) of subsurface drip at flowering. Error bars indicate standard errors.



Two lines from the 2013 study that exhibited low stress levels under the 2.5 mm per day treatment (FM2482B2F and Phytogen 72) and two lines with elevated stress levels (All-Tex Edge and Phytogen 367) were evaluated in 2014 for their growth and development under an irrigation regime of either 2.5 mm per day or 17.5 mm once per week. Figure 2A is a graph of changes in stress levels under the 2.5 mm daily irrigation regime, and Figure 2B is a graph of changes in stress levels under the 17.5 mm weekly irrigation during the two-week period of peak bloom. The All-Tex Edge exhibited the highest stress levels under the 2.5 mm irrigation regime, followed by Phytogen 367, Phytogen 72 and FM 2484B2F. All-Tex Edge exhibited slightly higher stress levels than the other cultivars under the 17.5 mm per week treatment, but on the whole, efficiency of quantum yield values were reduced compared with the 2.5 mm per day treatment.

Figure 2. Graph of changes in stress levels, measured using the efficiency of quantum yield, under the 2.5 mm daily irrigation regime (Figure 2A), and changes in stress levels under the 17.5 mm once a week irrigation (Figure 2B) during the two-week period of peak bloom. Arrows indicate irrigation events and error bars indicate standard errors.



We observed that the efficiency of quantum yield values for All-Tex Edge dropped from a high of 0.65 on day of year 220 to a low of 0.33 on day of year 223 (Fig. 2A). This decrease in stress level occurred under a constant low level of irrigation and without any rain events during this period. We investigated a range of possible scenarios that would result in the observed pattern and discovered that the abiotic stress level provided by the stress bioassay tracked the average daily air temperature. Figure 3A is a graph of the All-Tex Edge cultivar under 2.5 mm per day irriga-

tion and the average daily air temperatures. A clear pattern is observed between these two parameters. Similarly, Figure 3B shows the All-Tex Edge stress levels under the 17.5 mm per week irrigation treatment compared with the average daily air temperatures. The lower stress level under the 17.5 mm irrigation once a week is clearly lower than that of the plants under the 2.5 mm per day treatment. Plotting the efficiency of quantum yield values against the average daily air temperatures shows a significant relationship between these parameters (Fig. 4). The 2.5 mm of irrigation per day produced a R² value of 0.58025 and the 17.5 mm of irrigation produced a R² value of 0.43095.

Figure 3. Figure 3A is a graph of the All-Tex Edge under 2.5 mm per day irrigation and the average daily air temperatures. Figure 3B is a graph of the All-Tex Edge under 17.5 mm once a week irrigation and the average daily air temperatures. Arrows indicate irrigation events. Error bars indicate standard errors.

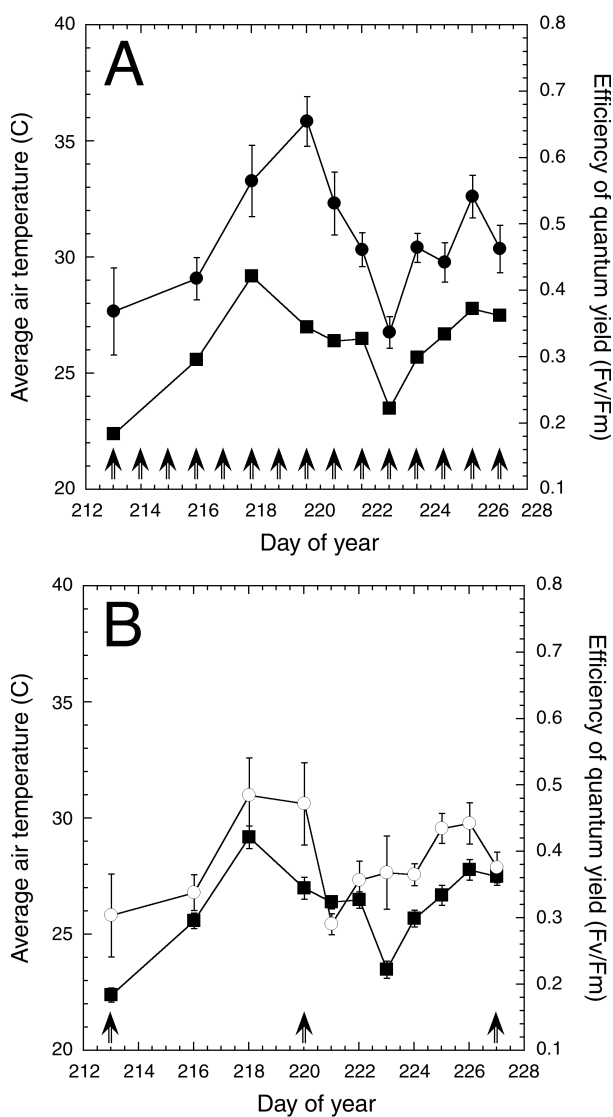
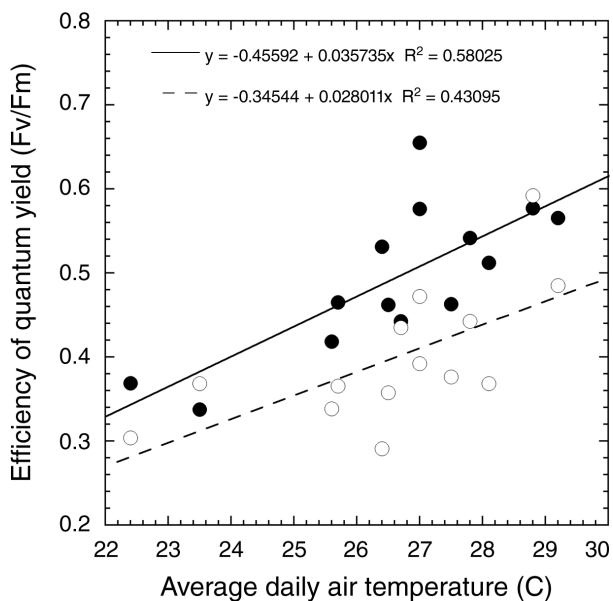


Figure 4. Graph of the efficiency of quantum yield values for All-Tex Edge 2.5 mm daily irrigation (closed circles) and the 17.5 mm once a week irrigation (open circles) plotted against the average daily air temperatures. Error bars indicate standard errors.



In light of the day-to-day variability in average daily temperature we chose to compare the mean efficiency of quantum yield value for the measurements obtained between 1 August 2014 and 15 August 2014. Figure 5 graphically shows the mean efficiency of quantum yield values for the 0.25 mm per day (double line) and 17.5 mm per week (solid line) for the four cultivars evaluated. Similar mean values were obtained among the four cultivars irrigated with 17.5 mm once a week. The values ranged from a high of 0.38 (All-Tex Edge) to a low of 0.33 (Phytogen 72). The mean values were obtained among the four cultivars irrigated with 2.5 mm once a day had a range from 0.48 (All-Tex Edge) to a low of 0.33 (Phytogen 72). The Phytogen 367 had a mean value of 0.39 under the 2.5 mm per day treatment.

Plant height was reduced in all cultivars under the 2.5 mm per day treatment compared with the 17.5 mm per week treatment (Fig. 6). FM 2484B2F, All-Tex Edge, and Phytogen 367 exhibited similar height reductions of 19-21%, while Phytogen 72 only exhibited an 8% reduction in plant height. The relative root lengths of the FM 2484B2F and All-Tex Edge cultivars showed no significant changes in length under the two irrigation treatments (Fig. 7). However, the Phytogen 367 cultivar had greater root lengths under the 17.5 mm per week irrigation treatment compared with the 2.5 mm per day treat-

ment. Phytogen 72 had greater root lengths under the 2.5 mm per day irrigation treatment compared with the 17.5 mm per week treatment.

Yield responses of the cultivars to the differential irrigation regimes is shown in Figure 8. Under the 17.5 mm irrigation treatment Phytogen 367 exhibited the highest yield of 1808 kg/ha, followed by the All-Tex Edge, Phytogen 72 and FM 2484B2F cultivars at 1630, 1544, and 1528

kg/ha, respectively. Under the 2.5 mm irrigation treatment FM 2484B2F exhibited the highest yield of 1549 kg/ha, followed by the Phytogen 72, Phytogen 367, and All-Tex Edge cultivars at 1377, 1350, and 1297 kg/ha, respectively. The All-Tex Edge and Phytogen 367 cultivars had significant reductions in yield under the 2.5 mm per day treatment, and no significant differences were observed for FM 2484B2F and Phytogen 72.

Figure 5. Graph of the comparison of the mean efficiency of quantum yield values for the measurements obtained between day of year 213 and 227. The mean efficiency of quantum yield values for the 0.25 mm per day are represented by the red line and the 17.5 mm per week by the blue line for the four cultivars. Error bars indicate standard errors.

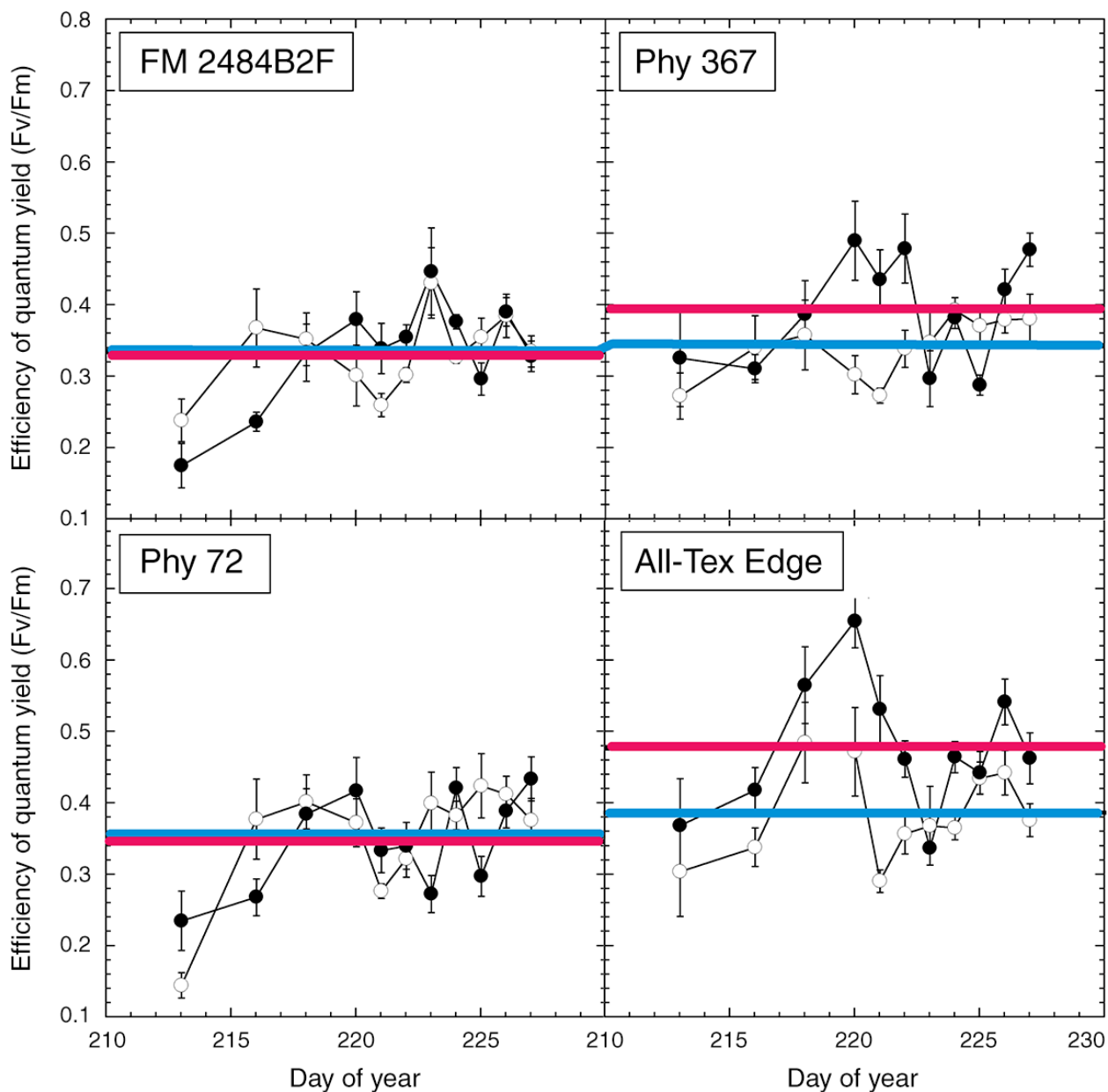


Figure 6. Graph of plant heights at the end of the season grown under the 2.5 mm per day irrigation treatment (black bar) compared with the 17.5 mm per week treatment (grey bar). Error bars indicate standard errors. A single asterisk indicates significance <0.05, and NS stands for not significant.

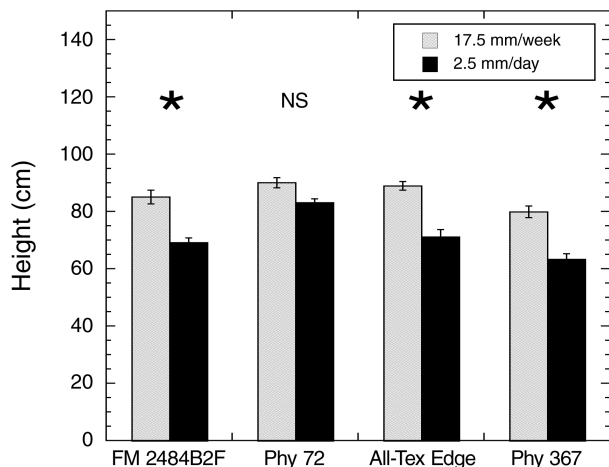
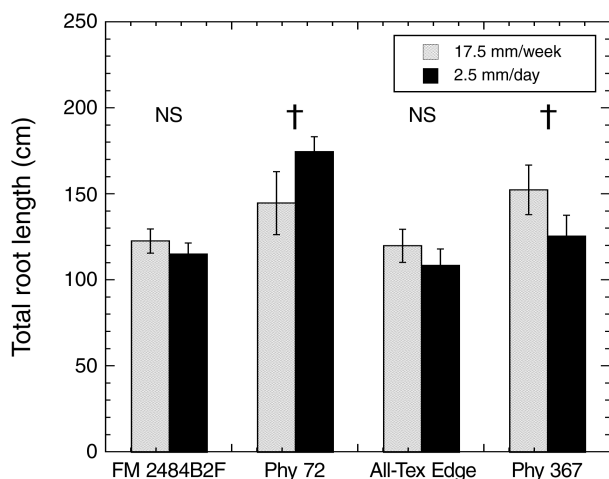


Figure 7. Graph of the relative root lengths under the 2.5 mm per day irrigation treatment (black bar) compared with the 17.5 mm per week treatment (grey bar). Error bars indicate standard errors. The symbol † indicates significance <0.10. NS stands for not significant.



Fiber lengths of the cultivars under the differential irrigation regimes are shown in Figure 9. No significant changes in length were observed for FM 2484B2F (2.85 and 2.94 cm) or Phytogen 72 (2.84 and 3.00 cm) respectively. A reduction in fiber lengths was observed for All-Tex Edge (2.76 and 2.90 cm) and Phytogen 367 (2.73 and 2.99 cm) respectively. Micronaire measurements showed increased micronaire values in the FM 2484B2F (4.22 and 4.5) and Phytogen 367 (4.52 and 4.85) cultivars respectively. No significant changes in micronaire were observed in the Phytogen 72 (4.55 and 4.59) and All-Tex Edge (4.81 and 4.84) cultivars respectively as shown in Figure 10.

Figure 8. Graph of the calculated kg/ha under the 2.5 mm per day irrigation treatment (black bar) compared with the 17.5 mm per week treatment (grey bar). Error bars indicate standard errors. A single asterisk indicates significance <0.05, and NS stands for not significant.

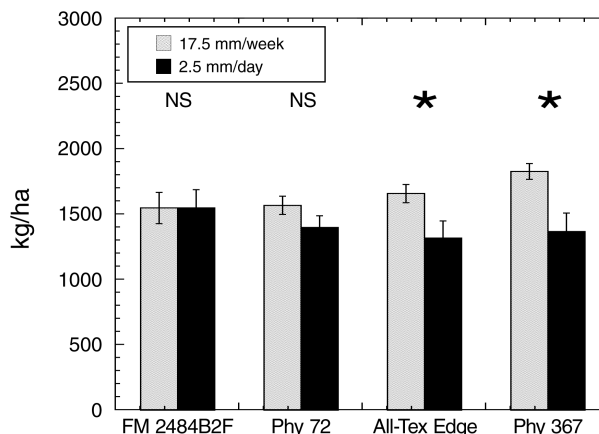


Figure 9. Graph of the cotton fiber lengths under the 2.5 mm per day irrigation treatment (black bar) compared with the 17.5 mm per week treatment (grey bar). Error bars indicate standard errors. The symbol † indicates significance <0.10 and a single asterisk indicates significance <0.05. NS stand for not significant.

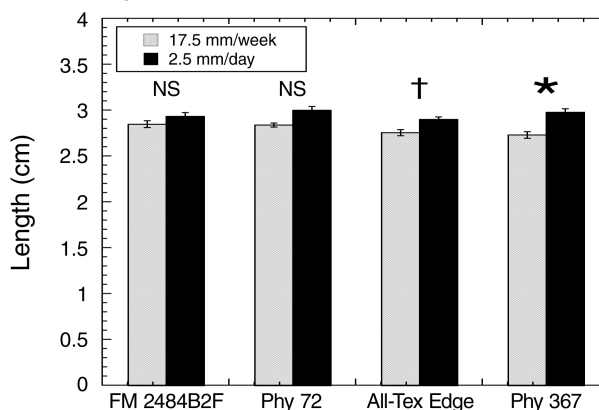
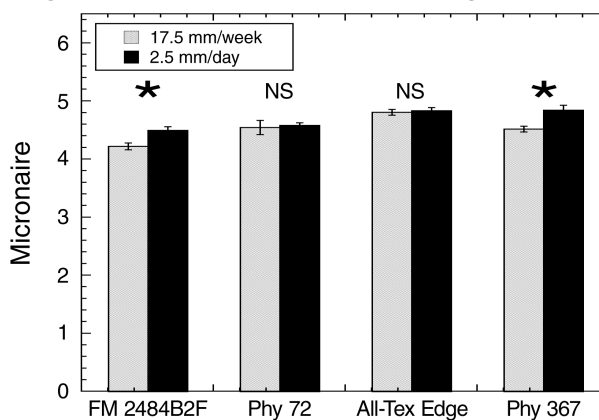


Figure 10. Graph of the micronaire values under the 2.5 mm per day irrigation treatment (black bar) compared with the 17.5 mm per week treatment (grey bar). Error bars indicate standard errors. A single asterisk indicates significance <0.05, and NS stands for not significant.



DISCUSSION

Over the past three decades, cotton (*Gossypium hirsutum* L.) production on the High Plains of Texas has been transitioning from full to reduced irrigation because of declines in the Ogallala Aquifer (Musick and Lamm, 1990, Wheeler-Cook, et al., 2008), the cost of pumping (Baumhardt, et al., 2009), and the reduced price of cotton on world markets (Starbird, 1985). Identifying cotton cultivars better suited for supplemental irrigation and rain fed agricultural practices is essential to maintain producer profitability.

There have been numerous studies of the impact of reduced irrigation on cotton performance, physiology, and fiber quality (Attia, et al., 2015, Basal, et al., 2005, Baumhardt, et al., 2009, Bondada, et al., 1996, Burke, 2007, Burke, et al., 1985, Burke and Upchurch, 1995, Carmi, et al., 1993, Chastain, et al., 2016, Da Costa and Cothren, 2011, Dagdelen, et al., 2009, DeTar, 2008, Dumka, et al., 2004, Enciso, et al., 2003, Feng, et al., 2014, Fernandez, et al., 1992, Fernandez, et al., 1996a, Fernandez, et al., 1996b, Gerik, et al., 1996, Hozain, et al., 2012, Ko and Piccinni, 2009, Leidi, et al., 1999, Levi, et al., 2009, Liu, et al., 2008, Mahan, et al., 2012, Meek and Oosterhuis, 2000, Oosterhuis, et al., 1991a, Pace, et al., 1999, Payton, et al., 2011, Pettigrew, 2004, Pettigrew, 2004, Pilon, et al., 2016, Radin, et al., 1992, Rahman, et al., 2008, Showler, 2002, Snider, 2015, Snider, et al., 2013, Snowden, et al., 2013, Snowden, et al., 2013, Tsonev, et al., 2011, Ullah, et al., 2008, Ünlü, et al., 2011, Voloudakis, et al., 2002, Wilson, et al., 1987). The present study was unique in that it evaluated the water stress sensitivity of commercial cultivars using a water-stress bioassay to determine the characteristics of contemporary germplasm; and then evaluated the timing of sub-optimal irrigation on the yield of two water-deficit stress tolerant cultivars and two water-deficit stress sensitive cultivars identified by the stress bioassay.

The initial evaluation of water stress responses of commercial cotton cultivars was performed from flower initiation to plant termination. This scenario was chosen to mimic much of High Plains agriculture where well capacities are sufficient to start the crop, but decline as the season progresses. With the exception of Phytogen 802, all of the cultivars studied showed relatively low stress levels as exemplified by efficiency of quantum yield values between 0.17 and 0.23 (Fig. 1) under the 5 mm per day irrigation regime. Possible reasons for the elevated efficiency

of quantum yield values in Phytogen 802 compared with the other commercial lines is that it is a Pima (*G. barbadense*) cultivar and the other cultivars are upland (*G. hirsutum*) cottons. Comparative studies reported by Reddy et al. (1992, 1992b) evaluated vegetative and reproductive responses of a *G. hirsutum* cultivar (DES 119) and a *G. barbadense* cultivar (Pima S-6). The vegetative data showed that maximum stem elongation rates in *G. hirsutum* peaked under the 30C/22C day/night cycle, while the *G. barbadense* maximum stem elongation rate occurred under the 35C/27C day/night cycle. Lubbock has cooler night temperatures than those regions where the Pima cotton was originally bred and is routinely grown. We have reported previously that cool nights restrict carbohydrate mobilization from upland cotton leaves thereby leaving un-mobilized photosynthate in the leaves and reducing photosynthesis in the leaves the next day (Warner and Burke, 1993, Warner, et al., 1995). Similar increases were observed in the efficiency of quantum yield from upland cotton experiencing low night temperatures in the field (Burke, unpublished data) during the development of the stress bioassay (Burke, 2007). Although genetic differences were observed within the efficiency of quantum yield values under the high-water regime, all of the cultivars performed well. Reducing irrigation levels from 5 mm per day to 2.5 mm per day at flowering produced a range of stress levels (efficiency of quantum yield) from 0.28 to 0.54 (Fig. 1). Increases in the water stress levels were seen in all cultivars. The percent increase in stress level ranged from a low of 50% for DP 0912 (0.22 to 0.33) to a high of 265% for Phytogen 367 (0.20 to 0.53). These data suggest differential stress sensitivities among the modern cultivars studied. Average combined seed cotton yields of the three lines (NexGen 4111RF, FM 2484B2F, and Phytogen 72) showing low levels of stress under the 2.5 mm per day irrigation were 25% lower than the 5 mm per day treatment plants. Average combined seed cotton yields of the three lines (All-Tex Edge, Phytogen 367, and Nitro44) showing high levels of stress under the 2.5 mm per day irrigation were 43% less than the 5 mm per day plants. These findings suggested that some of the modern commercial cotton cultivars do not respond well to irrigation reductions at flowering.

Next, we evaluated whether the commercial lines showing high levels of water-deficit stress sensitivity when water was reduced at flowering would express season long stress responses. Additionally,

we investigated whether the cultivars would respond similarly if they experienced daily irrigation events or a single weekly irrigation event. The efficiency of quantum yield values obtained in the 2.5 mm per day irrigation treatment were similar to those obtained in 2013 (Fig. 2A). The FM 2484B2F and Phytogen 72 cultivars exhibited lower efficiency of quantum yield values than the Phytogen 367 and the All-Tex Edge cultivars under the 2.5 mm per day irrigation treatment. The average quantum yield values were 0.33 for FM 2484B2F and Phytogen 72, while Phytogen 367 and All-Tex Edge had values of 0.39 and 0.48 for the same period. Watering once a week with 17.5 mm of irrigation made no difference to the stress levels of the FM 2484B2F or Phytogen 72 cultivars; however, Phytogen 367 and All-Tex Edge showed reduced stress levels with the weekly irrigation event (Figs. 2 and 5). Interestingly, the Phytogen 367 and All-Tex Edge quantum yields were similar to those of FM 2484B2F or Phytogen 72 under the single weekly irrigation regime (Fig. 2 and 5). Enciso et al. (2003) evaluated deficit irrigation frequencies on a commercial farm in St. Lawrence, Texas and found no significant differences between irrigation frequency treatments in lint yield, micronaire, fiber length, fiber strength, uniformity, or gross returns for Deltapine NuCoTN 33 B or Deltapine 458BR. They concluded that with no major advantage in increasing irrigation frequency using SDI under deficit conditions, these results might have an impact on the agronomic practices of the region where water is very limited. They stated that low frequency irrigation might allow farmers to have more flexibility in managing their irrigation systems and avoid the additional expense of automating a microirrigation system. Our results for FM 2484B2F and Phytogen 72 were similar to those of Enciso et al. (2003) in that no yield advantage was observed between the daily and weekly-irrigated regimes. The All-Tex Edge and the Phytogen 367 did show yield reductions under the daily irrigation further supporting the recommendation for weekly instead of daily drip irrigation schemes.

The pattern of the stress accumulation in All-Tex Edge was surprising in that the efficiency of quantum yield values showed a rise and fall pattern between 1 August 2014 and 11 August 2014 (Figs. 2 and 3). A gradual increase in the stress level was expected as plants increased in size and no additional water except for the 2.5 mm per day irrigation were received by the plant. An evaluation of the environmental components known to contribute to plant water

losses revealed a significant correlation between the efficiency of quantum yield values and the average daily air temperature measured at 2 m above the ground (Figs. 3 and 4). The plots receiving 2.5 mm of irrigation per day were more tightly coupled to the environmental changes as exemplified by the higher R^2 values compared with the plots watered only once a week (Fig. 4). The All-Tex Edge plots receiving 2.5 mm of irrigation per day exhibited an R^2 value of 0.58025, while the All-Tex Edge plots receiving 17.5 mm once a week exhibited an R^2 value of 0.43095. This pattern was true of all four cultivars evaluated in this study (data not shown). One possible reason for the lower R^2 values under the weekly irrigation is that sufficient water was available the first few days after the irrigation event to meet evaporative demands and keeping the plants less stressed than those plants receiving the daily small irrigation. It is reassuring that the stress bioassay tracked the average daily air temperatures, further suggesting that the stress bioassay truly measures the plant's water stress level.

Morphological data collected for the cultivars to the differential irrigation treatments showed that all of the cultivars were reduced in plant height when watered daily with 2.5 mm of irrigation compared with the 17.5 mm of irrigation once per week (Fig. 6). Phytogen 72 showed the least reduction in height suggesting that this cultivar could avoid or respond to the water stress better than the other cultivars during vegetative growth and development. One possible explanation might be differences in rooting patterns among the cultivars (Fig. 7). Phytogen 72 showed greater root development under 2.5 mm daily irrigation compared to the other cultivars. This may explain, in part, the maintenance of plant height in Phytogen 72 under the 2.5 mm irrigation regime. Fiber length was reduced in both All-Tex Edge and Phytogen 367 under the 2.5 mm daily irrigation, but no significant reductions in fiber length was observed in FM 2484B2F and Phytogen 72 (Fig. 9). Water status of the plant during the elongation period influences fiber length (Davidonis, et al., 2004, Ramey, 1986, Snowden, et al., 2013). Snowden et al (2013) showed significant reductions in fiber lengths when stress occurred in DP 0912, DP0935, FM 9170 and FM 9180 at early flowering, peak bloom, and peak bloom to termination. It is interesting that All-Tex Edge and Phytogen 367 exhibiting the high water stress levels as measured by our bioassay, were the two cultivars showing reduced yields and fiber lengths (Figs. 8 and 9).

Cultivar differences in micronaire were observed with FM 2484B2F and Phytogen 367 showing significant increases in micronaire under the 2.5 mm per day irrigation treatment. When you look at absolute micronaire levels the FM 2484B2F values were well within the desired range for cotton. The acceptable micronaire range is 3.5 to 4.9 and any fiber outside that range is subject to a price penalty (Davidonis, et al., 2004). The only cultivars exhibiting micronaire values of individual replicates above 4.9 were All-Tex Edge under either irrigation regime, and Phytogen 367 under the 2.5 mm irrigation regime. All other cultivars fell within the accepted range.

In summary, this study identified cultivar differences in water stress responses when irrigation was decreased from 5 mm per day to 2.5 mm per day at flowering; and the same cultivar differences were seen when these cultivars received 2.5 mm/day and 17.5 mm/week drip irrigation regimes. In addition, a linkage between observed stress levels provided by the chlorophyll fluorescence bioassay and average daily air temperature was identified. This linkage between daily air temperature and observed stress Fv/Fm levels during the cotton reproductive window may provide new insights into breeding cotton germplasm for drought tolerance. Moreover, the water-deficit stress bioassay used in this study can provide useful information on cultivar differences in water stress sensitivities to cotton breeders and producers. Yield differences and fiber quality component changes reflected the stress levels experienced by the cultivars.

Finally, weekly versus daily irrigation lessened existing stress sensitivities and increase yields.

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