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Original Research Article

Effects of the timing of water deficits on cotton water economy, growth, and yield

ABSTRACT

Aims: This study was conducted to evaluate the effects of water deficits applied at different phenological periods on whole-plant responses of cotton grown under rain-sheltered conditions.
Study design: A complete randomized design with four replications.
Place and Duration of Study: The study was conducted at the Drought Tolerance Laboratory at the Texas A&M AgriLife Research and Extension Center near Corpus Christi during the 2014 and 2015 cotton growing seasons.
Methodology: Individual plants of the commercial cultivar Phytogen 375 were grown in 13.5-L pots. Daily irrigation of individual pots was controlled with electronic timers and daily whole-plant transpiration was calculated lysimetrically from 10'-interval pot weights measured with electronic loadcells. Plant measurements included height, leaf area, total dry biomass and seed-cotton production.
Results: With only few exceptions, the water deficit treatments had significant impact on plant's production of biomass, leaf area, and seed-cotton, as well as whole-plant transpiration and transpiration per unit leaf area. The responses to these plant variables showed to be different between years as the environmental conditions were more stressful in 2014 than in 2015. Water deficits applied during FB-MB had the largest impact on plant growth, plant transpiration, and yield in both years; decreased dry biomass 32% in 2014 but had no effect in 2015, decreased seed-cotton 57% in 2014 and 23% in 2015, completely inhibited plant leaf area growth in both years, decreased cumulative whole-plant transpiration 46% in 2014 and 41% in 2015, decreased transpiration per unit leaf area 41% in 2014 and 37% in 2015.
Conclusion: Seed-cotton production per plant was most affected by water deficits during FB-MB stage (decreased 57% in 2014 and 23% in 2015), less affected during MB-OB stage (decreased 49% in 2014 and 0% in 2015), least affected during MH-FB stage (0% in 2014 and 17% in 2015).

Keywords: Drought; phenological stage; whole-plant responses; rain-sheltered conditions.

1. INTRODUCTION

The effect of drought on plants is complex involving interacting adaptive responses[1] . In general, the exposure of plants to soil water deficits results in the sequential inhibition of expansive growth, transpiration, and photosynthesis [2]. Under these conditions, plants conserve water by limiting leaf area growth and/or closing stomata [3], but ultimately adversely affecting plant performance and yield [4,5]. As with many other crop species, cotton plants must develop a vegetative framework of leaf area and fruiting positions big enough to sustain the development and growth of fruit and, hence, yield. Water deficits induced by low available soil water and/or high evaporative demand reduce the total number of fruiting positions in cotton as a result of a general reduction in shoot growth [6,7] and decreased fruit retention [8,9].

The effect of water stress on yield depends on its timing, intensity, and duration [10]. Studies have shown that cotton is sensitive to water stress during flowering and fruit development [11,12], but there has been no general agreement about which is the phenological stage most sensitive to water deficits. As cited by Loka et al. [13], studies have shown that early flowering is the most sensitive stage to water deficits [14], whereas other studies concluded that peak flowering is the most sensitive [15] or even at the end of flowering [16].

Most of the work on the effects of timing of water deficits on cotton has focused on yield under variable field growing conditions. Quantifying these effects under controlled environments, particularly in what

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relates to sheltering from rainfall, securing soil uniformity, and controlling irrigation water supply would allow for a greater accuracy in the assessment of the water economy, growth, and yield responses of cotton to timing of water deficits. This would lead to a better understanding of the complex responses of cotton to drought and help improve the management of cotton grown in dryland and deficit irrigated conditions.

In this paper we present data describing whole-plant responses of cotton grown in a rain-shelter under well-watered and water-deficient conditions, the latter applied at three phenological periods from match-head square stage to first open boll stage. The objectives of this study were to evaluate the effects of timed water deficits on plant biomass and seed-cotton production, plant leaf area, whole-plant transpiration, and transpiration per unit leaf area and to further our understanding of the ability of cotton plants to cope with water deficit.

2. MATERIAL AND METHODS

The study was conducted at the Drought Tolerance Laboratory at the Texas A&M AgriLife Research and Extension Center near Corpus Christi during the 2014 and 2015 cotton growing seasons. This facility consists of two joined greenhouse structures modified to operate as rain shelters and equipped with an automated irrigation system controlling the irrigation of individual pots and a computerized network of electronic loadcell-based lysimeters for high frequency measurement of individual pot weights from which to calculate daily whole-plant water transpiration.

Seeds of cultivar Phytogen 375 (PHY375), which is an early-medium maturity variety, were germinated at room temperature for planting. When the radicles reached a length of about 0.015 m, four germinated seeds were hand-planted in 13.5-L pots on April 2nd in 2014 and on April 8th in 2015. The planted pots were later thinned to one plant per pot when plants reached the 3rd true leaf stage. To minimize maximum soil water availability as a source of environmental variation affecting plant growth and plant water economy, all pots were equally filled with 11.4 L of dry fritted clay soil medium. This soil medium has a high volumetric water holding capacity of about 0.46 L L^{-1} [17]. Drained water holding capacity of pots was 4.1 L of which about 60% (2.46 L) was available to plants. Prior to planting, the soil in the pots was covered with finely perforated aluminum foil (60 uniformly distributed needle-size perforations) and thoroughly wetted. The aluminum foil was used with the double purpose of minimizing soil water loss due to evaporation and allowing uniform distribution of irrigation water across the soil surface. Two diagonal cuts were made in the aluminum foil to expose a central soil area for planting the seeds. Upon planting, all pots were subjected to a well-watered regime of daily nighttime excess irrigation to produce drainage with a modified Hoagland's nutrient solution [18]. Since the rates of plant transpiration during night are minimal, irrigation was applied during nighttime to prevent pot weight changes affecting the calculations of hourly daytime transpiration. The computerized system developed to convert whole-plant transpiration from changes in pot weight included an algorithm to remove nighttime weight data "noise" related to pot weight data collection during drainage of excess water that otherwise would affect hourly and daily whole-plant transpiration calculations. Irrigation was set at 1 minute per day at 0.8 L min^{-1} while plants were small, but increased to 2 minutes per day as plants increased in size until the start of water regime treatments.

The experimental design included a well-watered control treatment and three timed water deficit treatments applied at different phenological stages of development, namely from match head square (MH) to first bloom (FB), from FB to mid bloom (MB), and from MB to first open boll (OB). Daily irrigation varied throughout the test according treatments and plant development (Table 1). Length of daily irrigation times was maintained at 2 minutes from MH to FB, but increased to 3 minutes thereafter until the end of tests as plants increased in size. Irrigation flow rates were maintained at 0.8 L min^{-1} for well-watered treatments, but decreased to 0.25 L min^{-1} for the water deficit treatments. Upon termination of each water deficit treatment, irrigation was returned to the control's level. The tests were initiated on May 7th in 2014 and on May 14th in 2015 when plants reached the MH phenological stage and terminated on August 14th in 2014 and on July 20th in 2015. Unlike the protocol applied in 2014, irrigation of plants allocated to the deficit irrigation treatment during MH-FB was withheld for five days before the initiation of

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that treatment. The purpose of this protocol modification was to decrease stored soil water content to induce an early onset of water stress in the still small young plants and, therefore, be able to assess its impact on plant yield. As it will be shown in the results section, the onset of water stress during the MH-FB stage in 2014 occurred late likely as a result of the combined effects of an initial high soil water storage, young plants' small size, and lower atmospheric evaporative demand. The study was laid out as complete randomized design with four replications. Each replication of each treatment had three individually potted plants. Of these three plants, one was permanently assigned to a mini lysimeter. The other two plants were spares to be used as replacement if needed.

Table 1. Experimental water regime treatments designed to study the effects of one-time exposure to water deficits at different phenological stages; match-head square (MH) to first bloom (FB), FB to mid bloom (MB), MB to first open boll (OB).

	Phenological Stages			
Treatments	MH-FB	FB-MB	MB-OB	OB-Harvest
Well-Watered Control	1.6 L d^{-1}	2.4 L d^{-1}	2.4 L d^{-1}	2.4 L d^{-1}
Water Deficit MH-FB	0.5 L d^{-1}	2.4 L d^{-1}	2.4 L d^{-1}	2.4 L d^{-1}
Water Deficit FB-MB	1.6 L d^{-1}	0.75 L d^{-1}	2.4 L d^{-1}	2.4 L d^{-1}
Water Deficit MB-OB	1.6 L d^{-1}	2.4 L d^{-1}	0.75 L d^{-1}	2.4 L d^{-1}
Date range of stages 2014	May 8 - May 29	May 30 - Jun 19	Jun 20 - Jul 10	Jul 11 - Aug 14
Date range of stages 2015	May 14 - Jun 5	Jun 6 - Jun 23	Jun 24 - Jul 17	Jul 18 - Jul 20

Daily whole-plant transpiration (DWPT) was calculated as the 24-hr sum of hourly whole-plant transpiration. The hourly whole-plant transpiration was calculated as the pot weight differences between consecutives hours. It was assumed that changes in pot weight between consecutive hours was practically all due to transpiration and only minimally affected by changes in plant biomass. Soil evaporation was also assumed to be negligible, since the top surface of the pot was covered with reflective aluminum foil with needle-made tiny holes.

Plant height (PH) and plant leaf area (PLA) data were obtained at the start and end of each water regime treatments. At the end of the test, plants were harvested individually to measure their seed-cotton yield. In 2014, plant leaf area was calculated by applying a non-destructive method developed by Carbalho et al. [19] consisting in measuring the length of the central vein of main stem leaves and counting the number of leaves in the related branch. In 2015, plant leaf area was estimated using a linear regression of PLA on PH developed with 2014 data (PLA=0.5606*PH-0.0668; R2=0.89571).

Weather conditions during the studies, which are best summarized by the daily variation of reference potential evapotranspiration (RPET), were measured by an automated field weather station located approximately 100 m east of the Drought Tolerance Laboratory (Fig. 1). RPET was calculated at hourly steps using the Penman-Monteith equation and applying the reference standard method described by Pereira et al. [20]. Experimental data (sums, averages, standard deviations, and coefficients of variation) were summarized using Excel 2010 (Microsoft Corporation, Redmond, WA) and statistical analyses including ANOVA, mean separations, and contrasts were performed using SAS 9.2 (SAS Institute, Cary, NC).

3. RESULTS AND DISCUSSION

Weather conditions during the tests in 2014 and 2015 were different (Fig. 1). While the progression of RPET during the MH-FB stage was smoothly increasing with small daily variations in 2015, there were spikes of high evaporative demand early and about half-way during this development stage in 2014. A spike of high evaporative demand also occurred in 2014 at the start of the first bloom to mid bloom stage but then followed by smooth increase towards the end of this stage. Distinctly, the atmospheric

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evaporative demands during the first bloom to mid bloom and the mid bloom to first open boll stages were much lower in 2015 than in 2014, due primarily to cloudiness and high air humidity (data not shown).

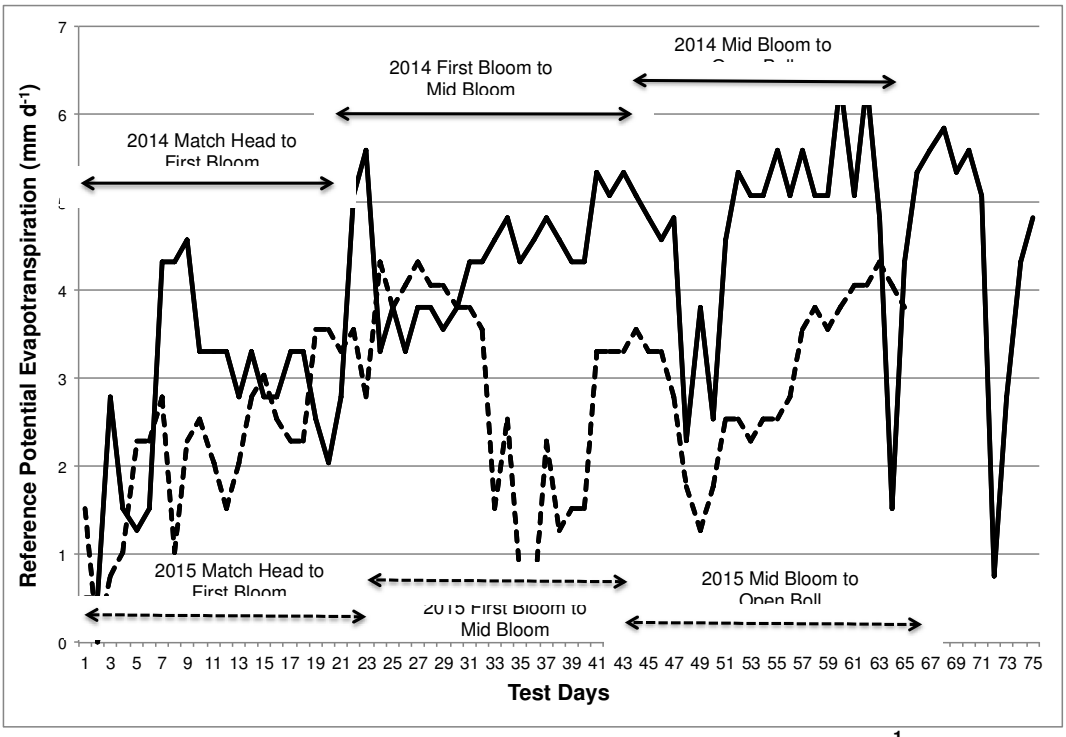

Fig. 1. Progression of reference potential evapotranspiration (mm d^{-1}) during the phenological treatment periods in 2014 and 2015.

Plant leaf area (PLA) at the start of tests (MH) was uniform among treatments in both years with the exception of the well-watered control in 2015, which was 21 to 29% lower than the water deficit treatments (Table 2). The well-watered control plants exhibited the largest PLA increase during the MH-FB phase of development; 4.4 fold in 2014 and 4.8 fold in 2015. Test plants not subjected to water deficits during the MH-FB stage exhibited also comparable PLA increases in both years ranging from 3.9 to 5.5 fold. PLA growth of well-watered controlled plants slowed down after the FB stage, exhibiting a modest increase from FB to MB of 36 and 33% in 2014 and 2015, respectively. Thereafter, PLA of the well-watered control plants did not exhibit growth from MB to OB. This slowing down and leveling of PLA resulted primarily from a decreasing production of new leaves as plants began to allocate more photosynthetic substrate to fruit growth than to vegetative growth; a long recognized cotton growth characteristic [21,22,23,24].

Water deficit applied during MH-FB stage inhibited PLA growth as compared to the well-watered control in 2014 and 2015, but this inhibition was more pronounced in 2015 than in 2014 (Table 2). This lower increase in PLA from MH to FB in 2015 as compared to 2014 was direct consequence of inducing an early onset of water stress in 2015 by depleting some of the water storage days before the MH stage.

Water deficit applied during FB-MB stage completely inhibited PLA growth in both years, as shown by the lack of significance between PLA values between FB and MB stages (Table 2). Water deficit applied during MB-OB decreased PLA in 2014 by eliciting leaf senescence; 30% decrease between MB and OB, and 17% less than the well-watered control at the OB stage. This water deficit treatment did not decrease PLA in 2015, which may be explained by the less harsh environment (low atmospheric evaporative demand) in 2015 compared to 2014 (Fig. 1). In 2015, there were no differences in PLA at the OB stage among treatments. The decrease of leaf area growth in cotton with limiting water supply is well documented [7,25,26,27,28,29,30,31]. After subjecting the test plants to water deficits during MH-FB and

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FB-MB and reinitiating full irrigation, test plants exhibited total recovery of PLA in both years (Table 2). PLA values in the MH-FB water deficit treatment were not different from the well-watered control at the MB stage and, likewise, PLA values in the FB-MB water deficit treatment were not different from the well-watered control at the OB stage.

Table 2. Plant leaf area (PLA) at four different phenological stages for the well watered control and three timed water deficit treatments in 2014 and 2015. PLA for 2015 was estimated using the linear regression of PLA on plant height PH data obtained in 2014 (PLA=0.5606*PH-0.0668; R^2=0.89571)*

	Plant leaf area (m^2)			
Year Treatment	**Match head square (MH)**	**First bloom (FB)**	**Mid bloom (MB)**	**First open boll (OB)**
2014				
Well Watered Control	0.111 a (c)	0.492 a (b)	0.668 a (a)	0.638 a (a)
Water Deficit MH-FB	0.096 a (c)	0.397 b (b)	0.754 a (a)	0.732 a (a)
Water Deficit FB-MB	0.100 a (c)	0.545 a (b)	0.512 b (b)	0.673 a (a)
Water Deficit MB-OB	0.095 a (c)	0.505 a (b)	0.758 a (a)	0.529 a (b)
2015				
Well Watered Control	0.108 c (c)	0.518 a (b)	0.687 a (a)	0.712 a (a)
Water Deficit MH-FB	0.152 a (d)	0.334 b (c)	0.610 b (b)	0.655 ab (a)
Water Deficit FB-MB	0.136 b (c)	0.548 a (b)	0.575 b (b)	0.640 b (a)
Water Deficit MB-OB	0.142 ab (c)	0.551 a (b)	0.714 a (a)	0.700 ab (a)

**Means with different letters are significantly different at the 5% level. First letters show significance of differences in PLA among treatments at a given phenological stage. Second letters inside parenthesis show significance of differences in PLA among phenological stages for each timed full irrigation treatment.*

Progressions of DWPT (daily whole-plant transpiration) during the span of the tests showed distinct patterns for each of the water regime treatments in both years (Figs. 2 and 3). Day-to-day variation of DWPT values throughout the tests resulted mostly from daily variations in weather conditions (Fig. 1), but variation trends over several days resulted from longer-term shifts in weather conditions and changes in PLA caused by leaf expansive growth, production of new leaves, and leaf senescence.

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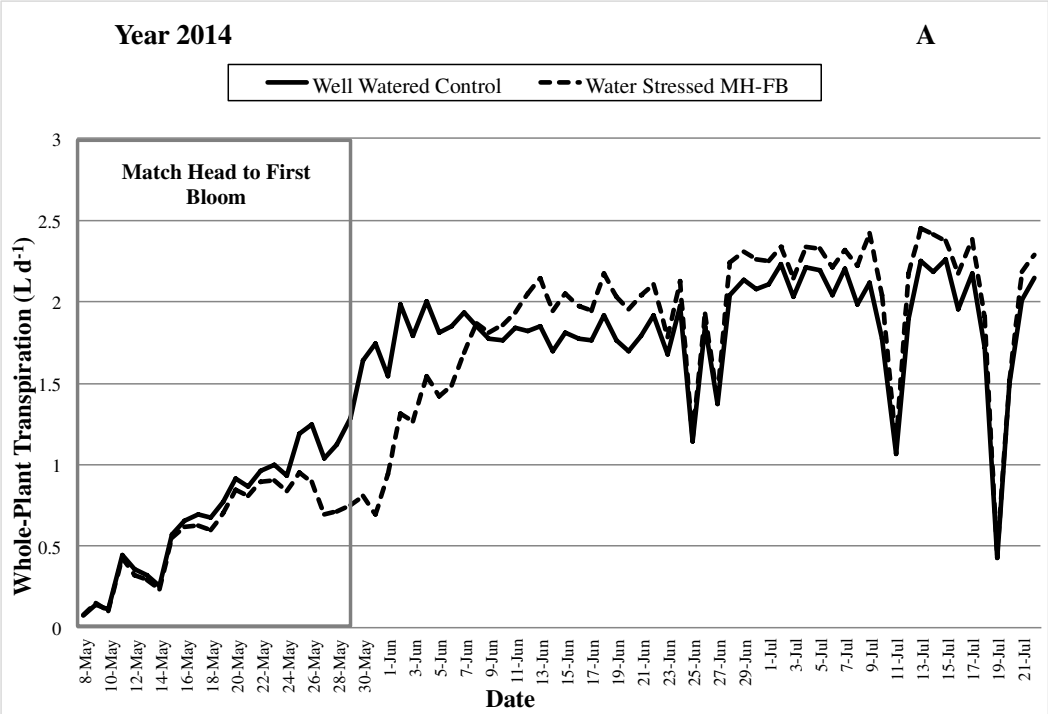

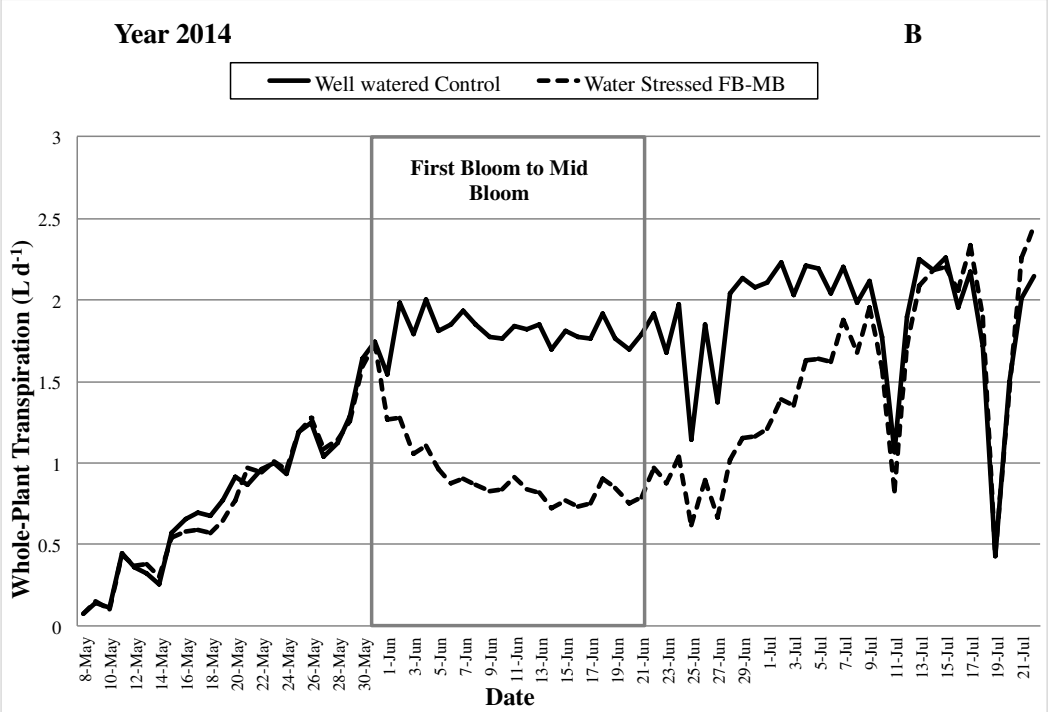

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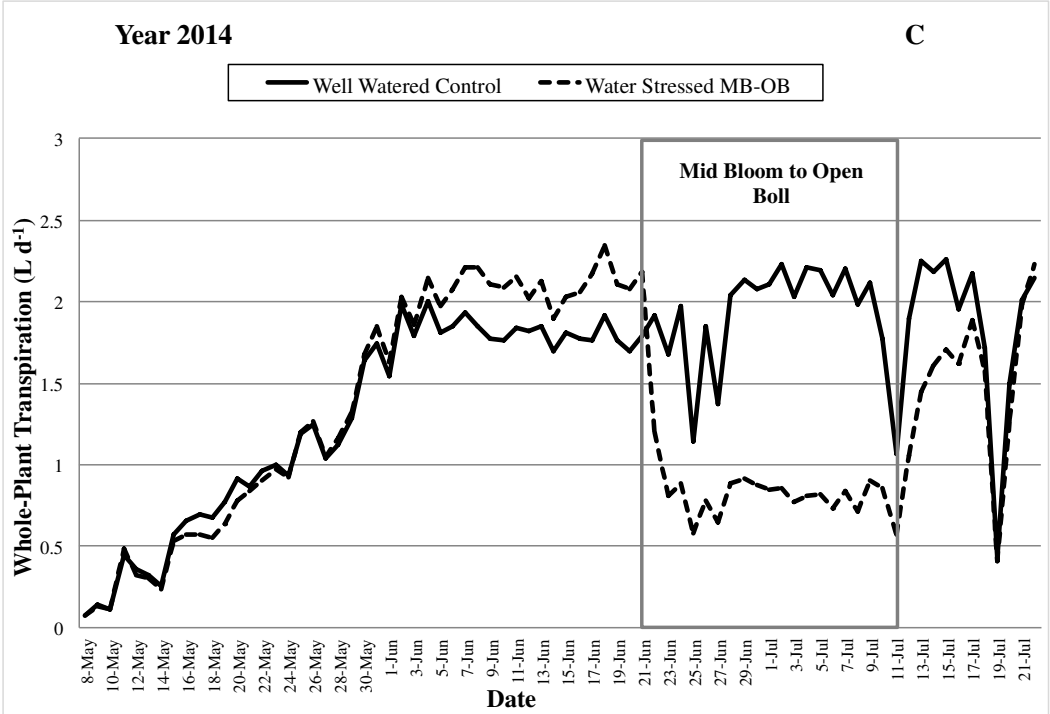
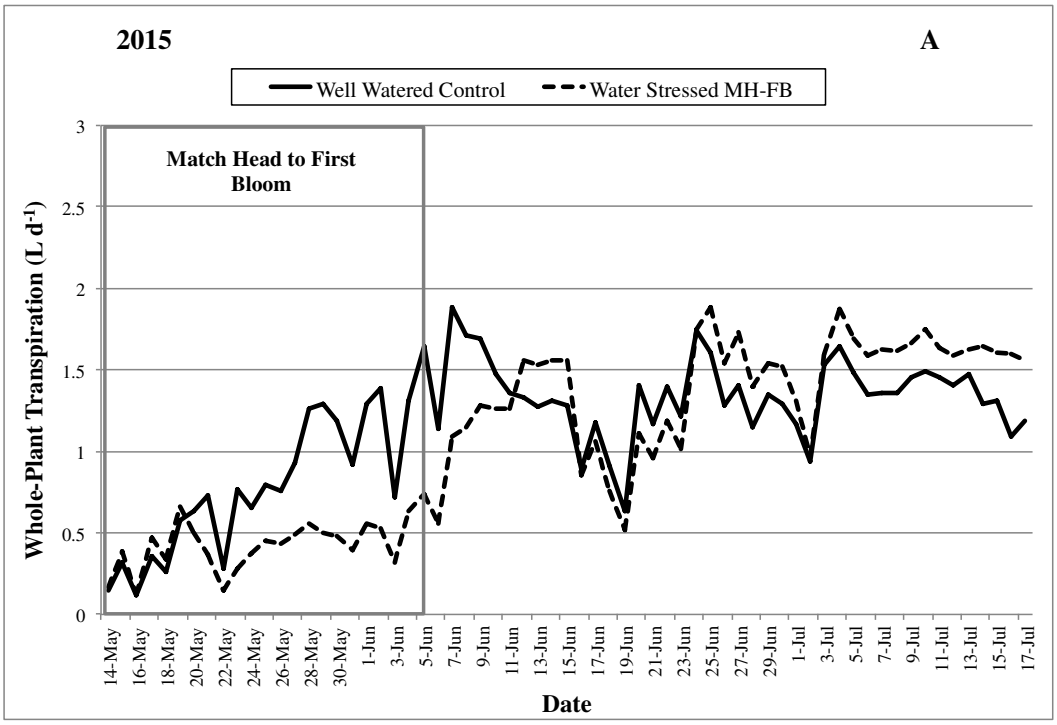


Fig. 2. Average whole-plant daily transpiration (L day^{-1}) data for the well-watered control and the three water deficit treatments during the 2014 season. (A) well watered control vs water deficit from match-head square to first bloom; (B) well watered control vs water deficit from first bloom to mid bloom; (C) well watered control vs water deficit from mid bloom to first open boll.



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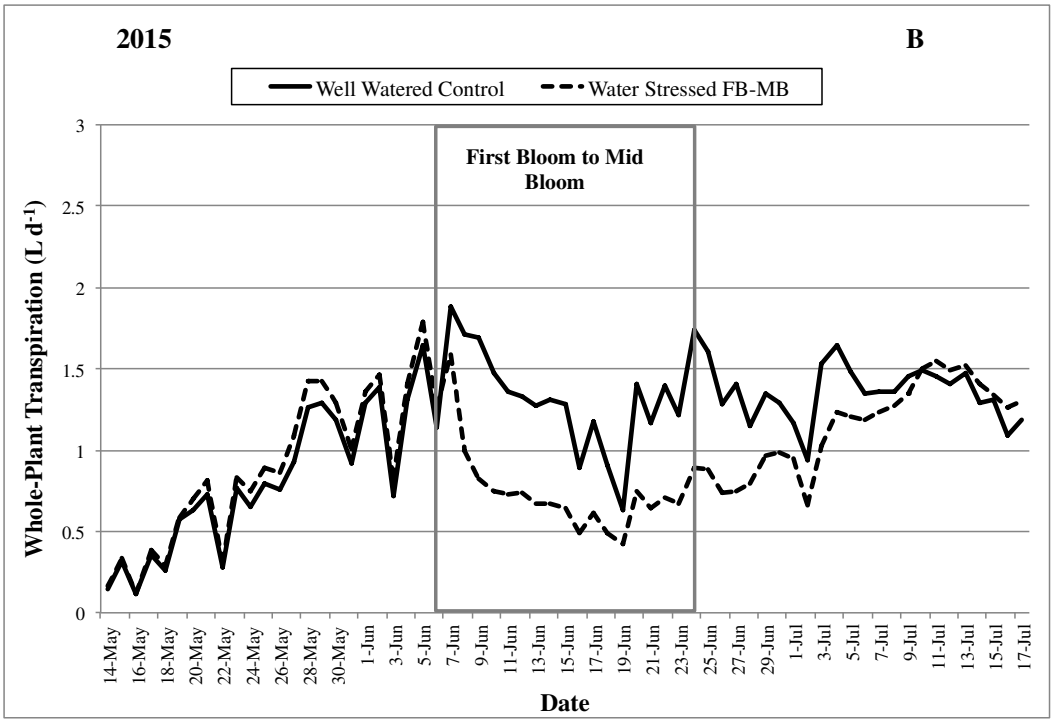

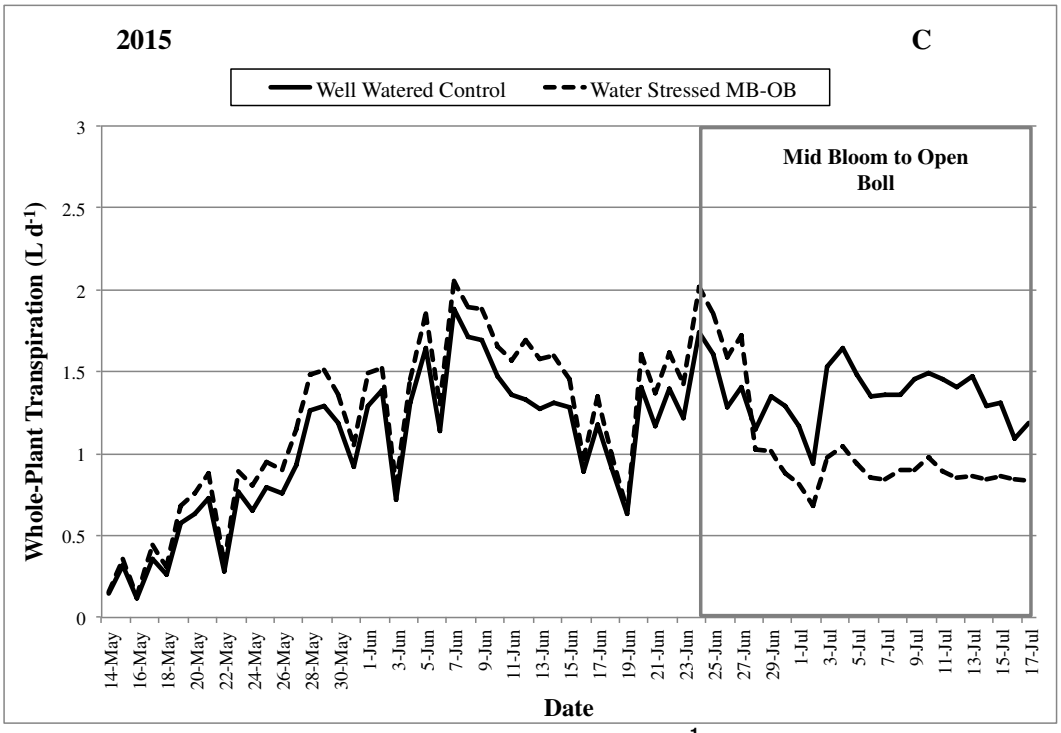

Fig. 3. Average whole-plant daily transpiration (L day^{-1}) data for the well watered control and the three water deficit treatments during the 2015 season. (A) well watered control vs water deficit from match-head square to first bloom; (B) well watered control vs water deficit from first bloom to mid bloom; (C) well watered control vs water deficit from mid bloom to first open boll.

DWPT in well watered control plants exhibited a marked increase during the MH-FB phase of development in both years (Figs 2 and 3). This increase in DWPT resulted from the combined effects of increasing leaf area per plant and increasing atmospheric evaporative demand (Table 2 and Fig. 1).

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DWPT values of well watered control plants at FB (June 3rd in 2014 and June 7th in 2015) were quite similar in both years; about 1.6 L d^{-1}. Thereafter in 2014, DWPT in the well watered control plants increased slightly to about 1.8 L d^{-1} during FB-MB, and to about 2.2 L d^{-1} during the MB-OB. This leveling of DWPT values resulted primarily from a slowed down production of new leaves as plants began to allocate more photosynthetic substrate to fruit growth than vegetative growth [21,22,23,24]. This slowed down production of new leaves occurred in 2014 and 2015, as shown in Table 2. In 2015, however, due to lower atmospheric evaporative demand, DWPT in the well watered control plants remained at about 1.5 L d^{-1} or below during the subsequent phases of development.

All three water deficit treatments showed marked declines in DWPT upon initiation of the water restrictions (Figs. 2 and 3). The decline exhibited during MH-FB was more pronounced in 2015 than in 2014 (Figs. 2A and 3A) due to the pre-conditioning protocol used to induce an onset of water stress earlier than the one in 2014. The small decline in DWPT observed in 2014 toward the end of the MH-FB growth period can be explained by a slower soil water depletion due to the combined effects of smaller plant size (Table 2) and low evaporative demand (Fig. 1). The large volumetric water holding capacity of the fritted clay soil medium also contributed to the slow onset of water stress. The declines in DWPT induced by the water deficit treatments during FB-MB and MB-OB were more pronounced in 2014 than in 2015 mainly due to a higher atmospheric evaporative demand in 2014 than in 2015 (Fig. 1).

The declines in DWPT upon subjecting the test plants to the water deficit treatments can be interpreted as primarily caused by decreasing leaf conductance to water loss by transpiration associated with stomatal closure. It has been long established and documented that stomatal closure is the main cause of the reduction in transpiration to water deficiency [2,32,33]. For the sake of simplification, the complex interconnected processes involved in the response of plants to water deficits can be described as follows. The decrease in soil water content due to watering restrictions leads to a decreased plant soil water uptake, decreased water transport to leaves, loss of leaves' turgor as water transport to leaves is insufficient to meet the transpiration demand, and stomata closure in response to loss of leaf turgor. More detailed descriptions of plant water stress dynamics in the form of simulation models have been published elsewhere [3,34,35,36,37,38]. Consequently, decreases in leaf conductance would lead to lower rates of transpiration per unit leaf area. All water deficit treatments resulted in lower values of daily transpiration per unit leaf area as shown in Table 3.

Upon cancelling the water restriction treatments and returning to full irrigation, test plants exhibited a gradual increase in DWPT in both years as shown in Figs. 2 and 3. It is noted, however, that no DWPT data is shown beyond the end of the MB-OB water deficit treatment for 2015. This gradual increase in DWPT upon returning to full irrigation can be attributed to a gradual increase of leaf conductance (as inferred from the gradual increase in transpiration per unit leaf area shown in Table 3) upon plants rehydrating and regaining leaf turgor [2], and also to a gradual increase in PLA as was shown in Table 2 for water deficit treatments MH-FB and FB-MB.

The distinct patterns of DWPT shown by the water regime treatments were reflected on the cumulative whole-plant transpiration values (CWPT) (Table 4). In 2014, there were no differences in CWPT among treatments when plants were subjected to water deficits during the MH-FB period. As interpreted above, this outcome resulted from the combined effects of low water loss by young small plants, low early season atmospheric evapotranspiration demand, and high soil water availability, which led to a late onset of water stress. In 2015, however, water deficit applied during the MH-FB period decreased CWPT 46%, but this response was consequence of a protocol change which allowed initiating this water deficit treatment at a lower soil water content to induce an early onset of water stress. Exposing plants to water deficits later in the season during the FB-MB and MB-OB periods decreased CWPT relative to the well-watered control in both years; during FB-MB, 46% in 2014 and 41% in 2015, and during MB-OB, 54% in 2014 and 24% in 2015.

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Table 3. Average daily transpiration per unit leaf area across phenological stages for the well watered control and the three timed water deficit treatments in 2014 and 2015. Phenological stages are: match-head square (MH) to first bloom (FB), FB to mid bloom (MB), and MB to first open boll (OB)*

	Daily Transpiration per Unit Leaf Area (L m^{-2})		
	Phenological Stages		
Year Treatment	**MHS-FB**	**FB-MB**	**MB-OB**
2014			
Well-Watered Control	2.376 a	3.122 a	2.913 a
Water Deficit MH-FB	2.402 a	2.925 a	2.771 a
Water Deficit FB-MB	2.160 a	1.844 b	2.068 b
Water Deficit MB-OB	2.272 a	3.276 a	1.391 c
2015			
Well-Watered Control	2.566 a	2.160 a	1.965 b
Water Deficit MH-FB	1.774 b	2.382 a	2.522 a
Water Deficit FB-MB	2.556 a	1.352 b	1.889 b
Water Deficit MB-OB	2.673 a	2.346 a	1.478 c

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

There were differences among experimental treatments in total plant dry biomass production, although these differences were not equal in 2014 and 2105 (Table 5). Water deficit applied during the MH-FB stage in 2014 did not affect plant dry biomass relative to the well watered control as a result of a late onset of water stress. However, this early water deficit treatment during MH-FB decreased 17% plant dry biomass in 2015, as a result of “forcing” an earlier the onset of water stress. Water deficit applied later during FB-MB and MB-OB stages decreased dry biomass per plant 32 and 19%, respectively, in 2014, but no differences relative to the well watered control were observed 2015. This lack of effect of later-applied water deficits in 2015 appear clearly related to a less stressful environment than in 2014 as shown by the lower atmospheric evapotranspiration demand in 2015 (Fig. 1). Studying plant responses to evaporative flux, Ritchie and Burnett [39] found that cotton crops greatly decreased growth and above ground dry biomass production when grown under droughty rain-fed conditions.

Likewise plant dry biomass responses to the water deficit treatments, seed-cotton yield per plant also exhibited differences among treatments, but these differences were not equal in 2014 and 2015 (Table 5). In 2014, there was no difference in seed-cotton yield per plant between the water deficit MH-FB treatment and the well watered control due to the late onset of water stress. In 2015, because the onset of water stress was “forced” to occur earlier by a pre-treatment partial depletion of soil water storage, seed-cotton yield of the water deficit MH-FB treatment was 17% lower than that of the well watered control. Seed-cotton yield per plant was decreased by the water deficit FB-MB treatment in both years, 57 and 23% in 2014 and 2015, respectively. The water deficit MB-OB treatment decreased seed-cotton yield per plant 49% in 2014, but it did not decrease with this late treatment in 2015. The lesser or nil effects of water deficit on seed-cotton yield per plant in 2015, when the treatments were applied during FB-MB and MB-OB, respectively, appear clearly related to a less stressful environment in 2015 than in 2014 as shown by the progressions of atmospheric evapotranspiration demand during the test periods in these two years (Fig. 1). Snowden et al. [40] studied the effects of the timing of episodic drought and found that events during early flowering and peak bloom caused significant reductions in yields, fruit retention, and fiber quality.

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Table 4. Cumulative whole-plant transpiration (CWPT) across phenological stages for the timed water deficit treatments in 2014 and 2015. Phenological periods are: match-head square (MH) to first bloom (FB), FB to mid bloom (MB), and MB to first open boll (OB)*

	Cumulative Whole-Plant Transpiration per Stage (L)		
Year	**Phenological Stages**		
Treatment	**MH-FB**	**FB-MB**	**MB-OB**
2014			
Well Watered Control	**15.6 a**	**39.6 b**	**39.9 a**
Water Deficit MH-FB	**13.0 a**	**36.9 b**	**43.1 a**
Water Deficit FB-MB	**15.3 a**	**21.4 c**	**25.9 b**
Water Deficit MB-OB	**14.9 a**	**44.8 a**	**18.5 c**
2015			
Well Watered Control	**18.3 b**	**23.2 b**	**32.8 b**
Water Deficit MH-FB	**9.92 c**	**20.3 c**	**38.3 a**
Water Deficit FB-MB	**20.1 ab**	**13.7 d**	**27.5 c**
Water Deficit MB-OB	**21.3 a**	**26.7 a**	**25.0 c**

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

Since CWPT integrates the effects of PLA and transpiration per unit leaf area over the phenological periods during which the water deficit treatments were applied, this variable was found useful for expressing the intensity of water deficits applied to the test plants. Linear regressions of dry biomass and seed-cotton production per plant on CWPT obtained by pairing 16 values (four water regime treatments x four replications) for both years 2014 and 2015 showed clear significant declining trends with decreasing values of CWPT from match-head square (MH) stage to first open boll (OB) stage (Fig. 4). With the exception of the coefficient of determination (R^2) of the regression of biomass on CWPT in 2015, which was significant at 5% probability level, all other coefficients of variation were significant at 1% level. The slopes of the linear regressions involving dry biomass and seed-cotton represent the average response of these two variables to changes in CWPT regardless of the timing of the water deficit treatments applied to the test plants. Total dry biomass per plant decreased 4.147 and 2.427 g per L of decline in CWPT in 2014 and 2015, respectively. Seed-cotton per plant decreased 2.752 and 1.692 g per L of decline in CWPT in 2014 and 2015, respectively. These declines in biomass and seed-cotton production per plant resulting from exposure to water deficits were respectively 41 and 39% lower in 2015 than in 2014, as a result of the lesser stressful environment (lower atmospheric transpiration demands) in 2015 than in 2014. Since total dry biomass values included seed-cotton mass, the slopes for biomass and seed-cotton clearly indicate that most of the declining responses in biomass with were related to decreases in seed-cotton production; the regression slopes for seed-cotton represented 66 and 70% of those for biomass in 2014 and 2015, respectively.

Table 5. Total dry biomass and seed-cotton per plant for the well watered control and the three timed water deficit treatments in 2014 and 2015*.

	2014		**2015**	
Treatment	**Biomass** (g plant^{-1})	**Seed-Cotton** (g plant^{-1})	**Biomass** (g plant^{-1})	**Seed-Cotton** (g plant^{-1})
Well Watered Control	418 a	152 a	310 a	103 a
Water Deficit MH-FB	416 a	163 a	258 b	85.3 b
Water Deficit FB-MB	286 c	65.9 b	293 a	78.9 b

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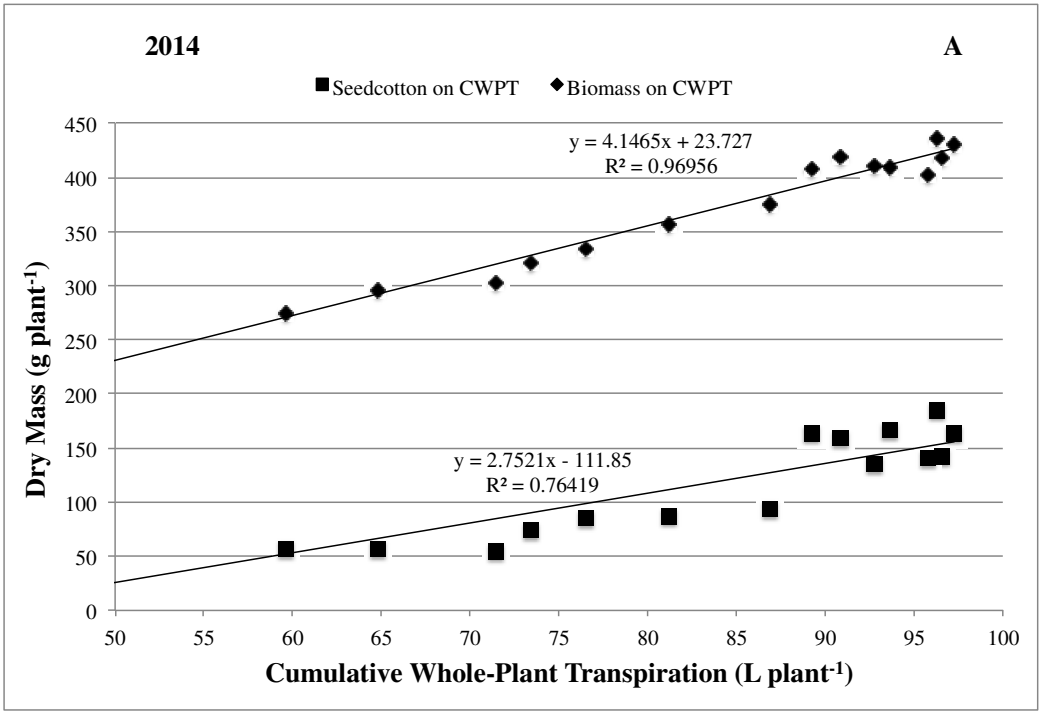
Water Deficit MB-OB	339 b	77.7 b	314 a	107 a

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



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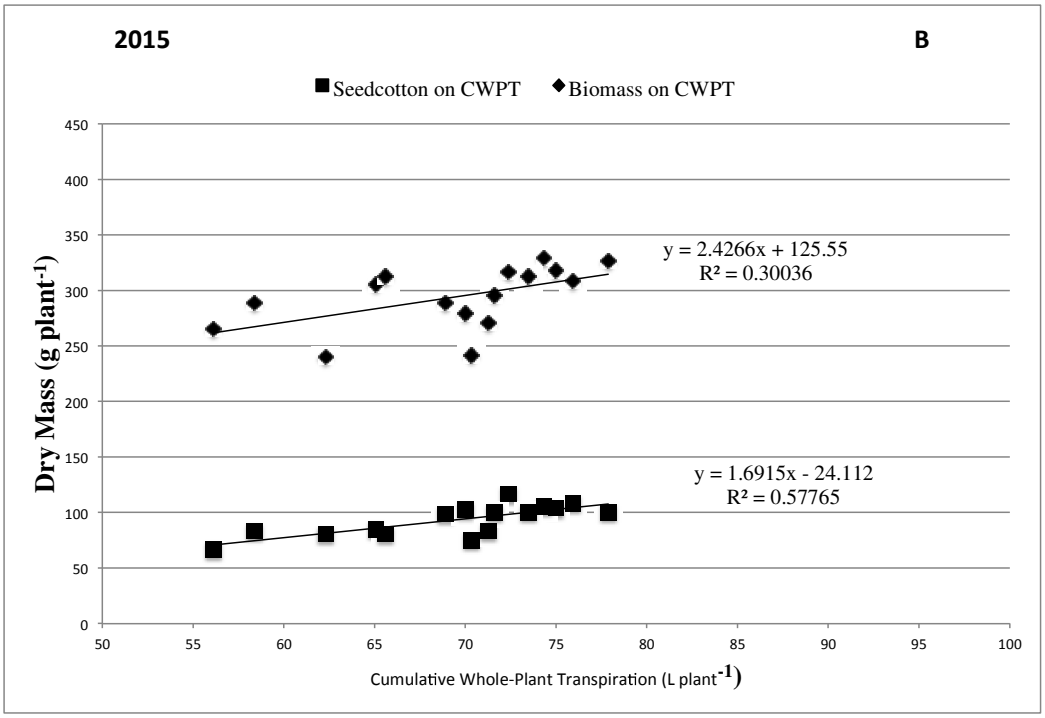

Fig. 4. Seed-cotton and total biomass per plant as a function of cumulative whole-plant transpiration from match-head square (MH) to first open boll (OB) during the 2014 (A) and 2015 (B) water deficit timing studies.

4. CONCLUSIONS

The two-year study conducted under rain-sheltered and controlled-irrigation conditions made possible to quantify significant cotton whole-plant responses to soil water deficits applied at three different phenological stages. The weather conditions during the two-year study (2014 and 2015), as assessed by the atmospheric evapotranspiration demand, were not too different during the MH-FB stage, but they were distinctly different during the phase of boll growth and development from first bloom to first open boll. The atmospheric evaporative demands were much lower in 2015 than in 2014. This incidental difference in environments amplified the range of conditions under which the responses to water deficits were quantified.

With only few exceptions, the water deficit treatments applied during different phenological stages had significant impact on plant's production of biomass, leaf area, and seed-cotton, as well as whole-plant transpiration and transpiration per unit leaf area. The responses to these plant variables showed to be different between years and this was attributed to environmental differences as assessed by the atmospheric evaporative demand, particularly from first bloom to first open boll. The water deficit treatment applied during MH-FB had only a small effect in 2014 (7% reduction in PLA growth) due to a late onset of water stress. But in 2015, this early water deficit treatment affected all these variables, as the onset of water stress was intentionally forced to occur at an earlier time in by decreasing the soil water content before the start of the treatment. This change in the experimental protocol was beneficial since the environmental conditions during MH-FB were not too different between years thus allowing to evaluate the effect of pre-bloom water stress on the plant variables studied. In 2015, water deficits during MH-FB decreased dry biomass and seed-cotton 17%, decreased PLA growth and CWPT 46%, and decreased average transpiration per unit leaf area 31%. Water deficits applied during FB-MB and MB-OB had generally a larger impact in 2014 than in 2015 as the environmental conditions during these development stages were more stressful in 2014 than in 2015. Water deficits applied during FB-MB decreased dry biomass 32% in 2014 but had no effect in 2015, decreased seed-cotton 57% in 2014 and

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23% in 2015, completely inhibited PLA growth in both years, decreased CWPT 46% in 2014 and 41% in 2015, decreased transpiration per unit leaf area 41% in 2014 and 37% in 2015. Water deficits applied during MB-OB did not affect dry biomass in neither year, decreased 30% PLA growth through increased leaf senescence in 2014 and but only completely inhibited it 2015, decreased seed-cotton 49% in 2014 but had no effect in 2015, decreased CWPT 54% in 2014 and 24% in 2015, decreased average transpiration per unit leaf area 52% in 2014 and 25% in 2015.

Seed-cotton production per plant was most affected by water deficits during FB-MB stage (decreased 57% in 2014 and 23% in 2015), less affected during MB-OB stage (decreased 49% in 2014 and 0% in 2015), least affected during MH-FB stage (0% in 2014 and 17% in 2015). The slope of the linear regression of seed-cotton on CWPT, which represents the overall impact of water deficits on plant seed-cotton production regardless of their timing, showed that seed-cotton per plant decreased 2.752 and 1.692 g per L of decline in CWPT in 2014 and 2015, respectively. This difference, which resulted from the less stressful environment in 2015, illustrates the effect of environmental conditions affecting the overall response of plant seed-cotton production to water deficits.

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