THE EFFECT OF WATER DEFICIT ON PHOTOSYNTHETIC ELECTRON TRANSPORT AND NET CO$_2$ ASSIMILATION RATES IN FIELD-GROWN COTTON

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Introduction

Water availability is the primary limitation to crop productivity worldwide (Sharp et al., 2004) and water deficit is well-known to limit photosynthesis in upland cotton (*Gossypium hirsutum)* (Ennahli and Earl, 2005; Zhang et al., 2011)*.* Despite exhaustive literature describing drought stress effects on photosynthesis, the exact mechanism of photosynthetic inhibition is heavily debated (Flexas and Medrano, 2002; Loka et al., 2011).

For example, in some species, actual quantum yield and photosynthetic electron transport rate through photosystem II (ETR) are sensitive to drought stress conditions (Flexas et al., 1999; Flexas et al., 2002; Zhang et al., 2011). However, contrasting reports exist for *G. hirsutum*. For example, Pettigrew (2004) reported significant declines in photosynthetic electron transport rate (ETR), and actual quantum yield of photosystem II (Φ_{PSII}) even under water deficit conditions (Ψ_l = -2.36 MPa) producing no decline in net photosynthesis (P_N) for field-grown *G. hirsutum*. For greenhouse grown cotton, Ennahli and Earl (2005) reported substantial declines in P_N and ETR when Ψ_l declined from -1.6 to -2.0 MPa. More recently, some authors (Massacci et al., 2008; Zhang et al., 2011) have reported increased ETR under water deficit conditions for field-grown *G. hirsutum*. Additionally, Snider et al. (2013) recently reported either stable or increased midday ETR at times during the growing season coinciding with extreme water deficit conditions (Ψ_l = -3.1 MPa).

It is hypothesized that electron transport rate through photosystem II would not be limited even under a wide range of Ψ_l sufficient to significantly limit P_N. Consequently, the objective of the current study was to quantify the relationship between Ψ_l, P_N, and primary photochemistry under a wide range of leaf water status.

Materials and Methods

Plant Material and Study Sites
Experiments (one dryland and one irrigated) were conducted at one site near Tifton, Georgia and another site near Camilla, Georgia (a randomized arrangement of dryland and irrigated plots) in 2012. Seeds of two commercially-available cultivars [PHY499 WRF (PhytoGen, Dow AgroSciences) and DP 0912 B2RF (Delta and Pine Land, Monsanto Company)] were sown on May 2, 2012 (Tifton, GA) and three cultivars (PHY499 WRF, DP 0912 B2RF, and DP 1050 B2RF) were sown on May 5, 2012 (Camilla, GA) at a 0.91m inter-row spacing and at a rate of 11 seeds m^{-1} row. Plots for each cultivar (n = 4) were four rows wide, 12.2 m long, and had 3 m bare-soil alleys. Plots were arranged using a randomized complete block design at each location. All replicate plots at the Tifton site were well-watered, whereas at the Camilla study site, all cultivars were grown under both dryland and well-watered conditions to generate variation in leaf water supply at different times during the growing season.

Dryland plots are defined as those plots only receiving water via rainfall during the growing season, and well-watered plots received supplemental irrigation to meet weekly water requirements for cotton as defined using University of Georgia Cooperative Extension "Checkbook" recommendations.

Midday quantification of Ψ_l, P_N, ETR, and Φ_{PSII}

To evaluate the relationships between P_N, Φ_{PSII}, ETR, and Ψ_l in field-grown *G. hirsutum*, all measurements were conducted at midday (1200-1400 h), under saturating light intensity (PAR > 1200 µmol m^{-2} s^{-1}) using the fourth main-stem leaf below the apical meristem. This measurement time was chosen because ETR rates were maximal and stable during this time frame (data not shown), and this is one of the most stable time frames to measure leaf water potential during daylight hours (Grimes and Yamada, 1982). For each sample date and location, three readings were taken per plot for each parameter, and the average of those readings was used for subsequent statistical analysis. The resulting data set encompassed 76 replicate samples at two study sites in Georgia from July 9 to July 26, 2012.

Actual quantum yield of electron transport through photosystem II (Φ_{PSII}) was measured *in-situ* using the OS5p Modulated Fluorometer (Opti-Science, Tyngsboro, MA). Φ_{PSII} was calculated according to the equations given in Maxwell and Johnson (2000). Electron transport rate (ETR) through photosystem II was calculated for each leaf by multiplying ΦPSII × PAR (at the leaf surface) × 0.5 (excitation energy is divided between two photosystems) × 0.84 (a common leaf absorbance coefficient for C_3 plants) (Flexas et al., 1999). Single-leaf gas exchange (P_N quantification) was performed immediately following chlorophyll fluorescence measurements using an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE), where all leaves were measured under natural irradiance (PAR > 1200 µmol m^{-2} s^{-1}) and chamber CO_2 concentration of 380 p.p.m. For Ψ_l determinations, immediately following ETR and gas exchange measurements, leaves were excised from the same position on the plant as those that were used for the previous measurements. The leaf petiole was immediately sealed in a compression gasket with the cut surface of the petiole exposed. The leaf blade was sealed in a pressure chamber (Model 615; PMS Instruments, Albany, OR) and the chamber was pressurized using compressed nitrogen at a rate of 0.1MPa s^{-1} until water first appeared at the cut surface of the stem. The total elapsed time from when the leaf was cut from the plant to the initial pressurization of the chamber was 5-10 s. The relationship between midday Ψ_l and primary photochemistry was evaluated by plotting Ψ_l versus Φ_{PSII} and ETR.

Statistical Analysis

Prior to regression analysis, mean midday Ψ_l, P_N, ETR, and Φ_{PSII} values for each cultivar × sample date × location × irrigation treatment were determined. A total of 19 means for each parameter were generated, where each value is the average of four replicate plots. On the aforementioned data set, regression analyses to determine the relationship between Ψ_l, P_N, and primary photochemistry were performed using Sigma Plot 11 (Systat Software Inc., San Jose, CA).

Results and Discussion

The relationships between midday Ψ_l, P_N, Φ_{PSII}, and ETR are presented in Figure 1. Midday values for Ψ_l ranged from -1.0 to -2.9 MPa. There was a strong non-linear (quadratic; $r^2 = 0.755$) relationship between Ψ_l and midday P_N (Fig. 1A), where the maximum predicted value for P_N was 32.1 µmol m^{-2} s^{-1} at Ψ_l = -1.1 MPa and declined 57.9% to 13.5 µmol m^{-2} s^{-1} at Ψ_l = -2.9MPa. In contrast, there was not a significant relationship between Ψ_l and ETR (Fig. 1B; $r^2 = 0.075$), and there was not a significant relationship between Ψ_l and midday Φ_{PSII} (Fig 1C; $r^2 = 0.0002$).

In this study, the range of Ψ_l values was much broader than in previous studies with field-grown cotton (-1.0 to -2.36; Pettigrew, 2004; Zhang et al., 2011), and many of the Ψ_l values were well below those previously reported to cause significant declines in net photosynthesis (-1.9; Zhang et al., 2011) and yield (< -2.0; Grimes and Yamada, 1982), yet ETR remained stable. Our findings are not in agreement with those of Ennahli and Earl (2005), who reported declines in ETR at Ψ_l = -2.0 MPa. However, the aforementioned study was conducted under greenhouse conditions with potted plants. Because root growth can be restricted in such studies, drought stress undoubtedly occurs much more rapidly than under field conditions, limiting the acclimation response of the plant that is normally observed under field conditions (Kitao and Lei, 2007). Similar to the findings of the present study, previous authors have reported either stable or increased ETR for field grown *G. hirsutum* (Kitao and Lei, 2007; Massacci et al., 2008; Snider et al., 2013).

It has been reported that photorespiration rates typically increase under water-deficit conditions, allowing for maintenance of electron flow through photosystem II and possibly protecting against oxidative stress (Kitao and Lei, 2007). Because P_N was substantially reduced under water-deficit (Ψ_l = -2.9 MPa) without concomitant changes in ETR (Fig. 1), we find no evidence for reduced electron flow under water-deficit in field-grown cotton, as reported previously under mild drought stress (Pettigrew, 2004). Our findings support the hypothesis that electron flow through photosystem II is insensitive to water-deficit stress in field-grown cotton.

Acknowledgements

References

Earl, Hugh J. and Said Ennahli. Estimating photosynthetic electron transport via chlorophyll fluorometry without photosystem II light saturation. Photosynthesis Research 82:177-186, 2004.

Ennahli, Said and Hugh J Earl. Physiological limitations to photosynthetic carbon assimilation in cotton under water stress. Crop Science 45:2374-2382, 2005.

Flexas, J., J.M. Escalona, and H. Medrano. Water stress induces different levels of photosynthesis and electron transport rate regulation in grapevines. Plant, Cell & Environment 22:39-48, 1999.

Flexas, J. and H. Medrano. Drought-inhibition of photosynthesis in C_3 plants: stomatal and non-stomatal limitations revisited. Annals of Botany 89:183-189, 2002.

Flexas, Jaume, Josefina Bota, Jos M. Escalona, Bartolom Sampol, Hipolito Medrano. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Functional Plant Biology 29:461-471, 2002.

Grimes, D.W. and H. Yamada. Relation of cotton growth and yield to minimum leaf water potential. Crop Science 22:134-139, 1982.

Kitao, M. and T.T. Lei. Circumvention of over-excitation of PSII by maintaining electron transport rate in leaves of four cotton genotypes developed under long-term drought. Plant Biology 9:69-76, 2007.

Loka, Dimitra A., Derrick M. Oosterhuis, Glen L. Ritchie. Water-deficit stress in cotton. In: Oosterhuis, D.M. (ed). Stress Physiology in Cotton. Cordova, TN: The Cotton Foundation; p 37-72, 2011.

Massacci, A., S.M. Nabiev, L. Pietrosanti, S.K. Nematov, T.N. Chernikova, K. Thor, et al. Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum*) to the onset of drought stress under field conditions studied by gas exchange analysis and chlorophyll fluorescence imaging. Plant Physiology and Biochemistry 46:189-195, 2008.

Pettigrew, W.T. Physiological consequences of moisture deficit stress in cotton. Crop Science 44:1265-1272, 2004.

Sharp, Robert E., Valeriy Poroyko, Lindsey G. Hejlek, William G. Spollen, Gordon K. Springer, Hans J. Bohnert, et al. Journal of Experimental Botany 55:2343-2351, 2004.

Snider, John L., Derrick M. Oosterhuis, Guy D. Collins, Cristiane Pilon, Toby R. FitzSimons. Field-acclimated Gossypium hirsutum cultivars exhibit genotypic and seasonal differences in photosystem II thermostability. Journal of Plant Physiology 170:489-496, 2013.

Valentini, R., D. Epron, P. De Angelis, G. Matteucci, and E. Dreyer. In situ estimation of net CO2 assimilation, photosynthetic electron flow and photorespiration in Turkey oak (Q. cerris L.) leaves: diurnal cycles under different levels of water supply. Plant, Cell and Environment 18**:**631-640, 1995.

Zhang, Ya-Li, Yuan-Yuan Hu, Hong-Hail Luo, Wah-Soon Chow, and Wang-Feng Zhang. Two distinct strategies of cotton and soybean differing in leaf movement to perform photosynthesis under drought in the field. Functional Plant Biology 38:567-575, 2011.
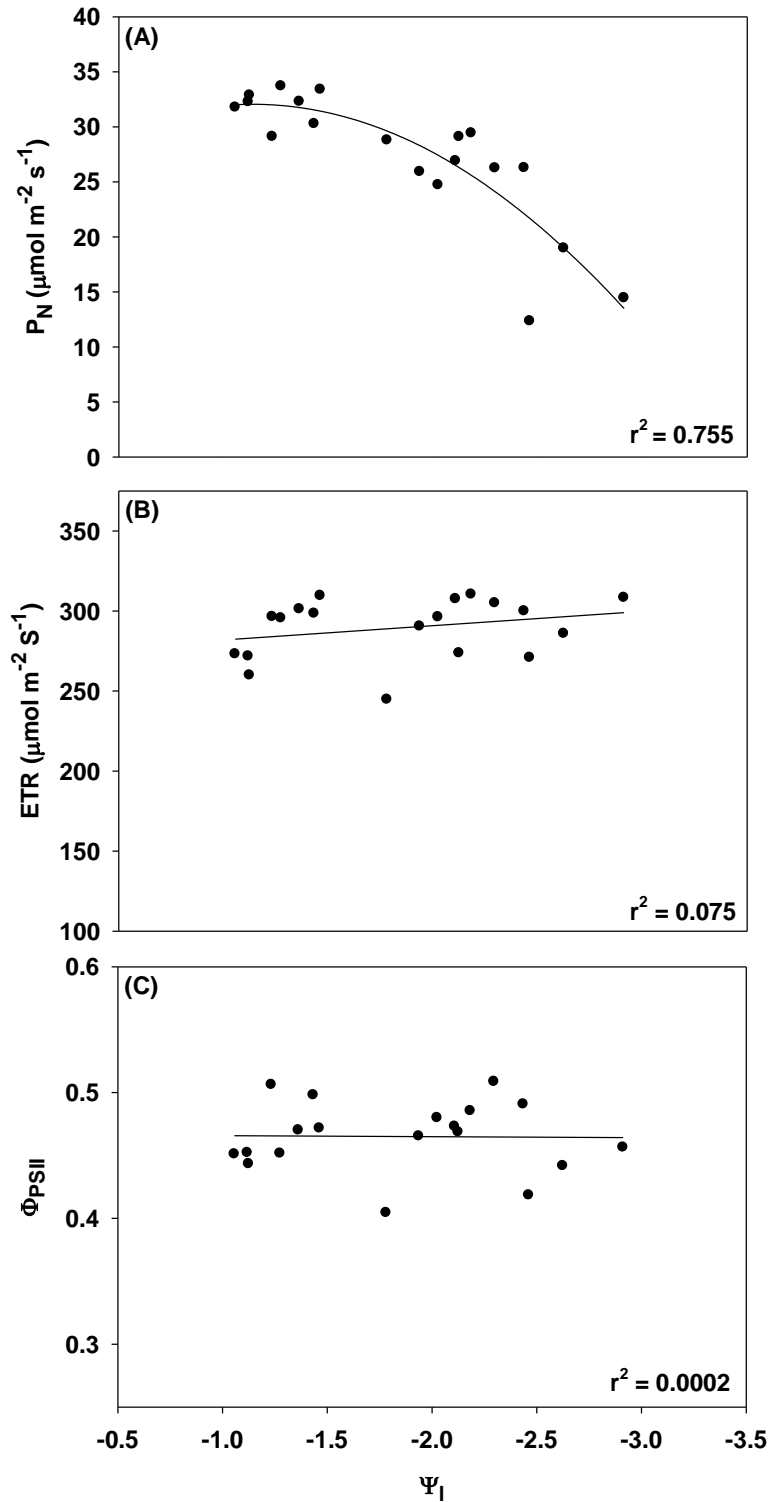
Figure 1. The relationship between midday (1200 to 1400 h) leaf water potential and net photosynthesis (P_N; A), electron transport through photosystem II (ETR; B) and actual quantum efficiency of photosystem II (Φ_{PSII}; C) Each data point represents an average of four replicate plots, where three measurements were taken in each replicate plot. The data presented in A-C were obtained from two study sites in Georgia on four sample dates from July 9 to July 26. All measurements were conducted on fourth-node, main-stem leaves.