



Response of upland cotton (*Gossypium hirsutum* L.) leaf chlorophyll content to high heat and low-soil water in the Arizona low desert

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Abstract

The sustainable production of upland cotton, an economically important fiber crop, is threatened by changing environmental factors including high temperatures and low-soil water content. Both high heat and low-soil water can reduce net photosynthesis resulting in low fiber yields or poor fiber quality. Leaf chlorophyll content has a direct relationship with photosynthetic rate. Understanding how high heat and low-soil water affect chlorophyll content can identify opportunities for breeding improvement that will lead to sustainable fiber yields. A two-year field trial located in Maricopa Arizona measured leaf chlorophyll content, available soil water, ambient air temperatures, and cotton growth measurements collected by a high-clearance tractor equipped with proximal sensors. The results showed that low-soil water significantly increased leaf chlorophyll content, while high temperatures significantly reduced content. Structured equation modeling revealed that cotton may divert available resources to leaf area and chlorophyll content for the production of photosynthates during periods of high temperatures.

Keywords: abiotic stress; leaf chlorophyll content; proximal sensing; upland cotton.

Introduction

Upland cotton (*Gossypium hirsutum* L.) is an important natural fiber resource and supports a multi-billion-dollar industry worldwide. The United States (US) is the third largest producer of cotton, behind India and China. The US is the leading global exporter of cotton with approximately 70% going to foreign markets. As the global population continues to rise, demand for high-quality fiber has increased to meet the global textile manufacturing needs

(Smith and Coyle 1997). In response, US cotton breeders have focused on increasing cotton fiber yields and fiber quality (Bridge *et al.* 1971, Campbell *et al.* 2011). However, like many crops, global climate uncertainty threatens the future of sustainable cotton production. A study using nine predicted global climate parameters and the *DSSAT CSM-CROPGRO-Cotton* model predicted cotton fiber yields could be reduced by as much as 78% for the Arizona low desert (Ayankojo *et al.* 2020). The model showed that the primary loss of yield was due to increased air

Highlights

- High heat and low-soil water have opposing effects on cotton leaf chlorophyll content
- Structural equation modeling informs source/sink relationships in yield components
- Irrigation management strategies can mitigate leaf chlorophyll degradation

Received 15 November 2021

Accepted 2 March 2022

Published online 13 April 2022

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CWSI – crop water stress index; DOY – day of year; H – height; L1 – level one; L2 – level two; LAI – leaf area index; NDVI – normalized difference vegetation index; PD – planting dates; RTK – real time kinematic; SEM – structural equation modeling; Ta – ambient temperature; Tc – canopy temperature; TRT – treatment; UTM – universal transverse mercator.

Acknowledgments: We would like to thank Matthew McGuire, Paloma Fajardo, Dusti Baker, Scott Machtley, Sharette Rockholt, Kathy Johnson, Suzette Maneely, Pernell Tomasi, Sara Harders, Matthew Hagler, and Melissa Stefanek for assistance with data collections and field management; and Mike Roybal and Bill Lockett for assistance with proximal sensing data handling and management. This work was supported by a Cotton Incorporated Research Grant 13-738 and the United States Department of Agriculture-Agricultural Research Service 2020-2100-013-00D.

Conflict of interest: The authors declare that they have no conflict of interest.

temperatures leading to low boll retention and reduced boll size.

Optimum day/night temperatures for cotton growth, reproductive development, and photosynthesis are 30/20°C, which is routinely exceeded in the southern US (Reddy *et al.* 1991, Brown 2008). The effects of high heat on cotton include reduced net photosynthesis (Law and Crafts-Brandner 1999, Hejník *et al.* 2015) and leaf chlorophyll (Chl) content (Reddy *et al.* 2004, Snider *et al.* 2010), increased minimum (F_0) chlorophyll fluorescence (van der Westhuizen *et al.* 2020), and inhibition of Rubisco activase (Law and Crafts-Brandner 1999, Salvucci and Crafts-Brandner 2004) under controlled conditions. High temperatures also cause pollen sterility (Marshall *et al.* 1974), flower and boll abscission (Reddy *et al.* 1992, Brown 2008), and reduced biomass and leaf area (Reddy *et al.* 1991, Bange and Milroy 2004). To avoid heat stress, cotton plants will maintain stomatal conductance and transpiration rates for evaporative cooling under well-watered conditions (Radin *et al.* 1994, Carmo-Silva *et al.* 2012). While this is an effective heat-avoidance mechanism, evaporative cooling can have adverse effects by depleting soil water in the root zone, leading to severe water deficit without supplemental irrigation (Carmo-Silva *et al.* 2012). Like heat stress, low-soil water has been shown to reduce net photosynthesis which limits cotton growth (Carmo-Silva *et al.* 2012). To improve sustainable cotton production for the future, breeding strategies need to focus on adaptation mechanisms for high temperatures and low-soil water (Araus *et al.* 2002, Sun *et al.* 2009).

Pettigrew and Turley (1998) suggested breeders focus on photosynthesis and its principal components, such as gas exchange and Chl fluorescence. However, the relationship between photosynthetic components and yield in cotton is not well understood, making it difficult to rely on these measurements in a breeding program (Singh *et al.* 2007, Turley and Pettigrew 2011). The relationship is further complicated with high heat and low-soil water conditions, as plant adaptation and recovery mechanisms, preserving net photosynthesis, may not lead to increased yield or fiber quality (Singh *et al.* 2007). To provide a comprehensive evaluation of photosynthetic components and cotton growth, Turley and Pettigrew (2011) focused on Chl-deficient mutants grown in the field. Chl is the primary light-harvesting pigment located in the chloroplast and is responsible for driving photosynthesis for the production of photosynthates. Chl content has a direct relationship with the photosynthetic rate (Anjum *et al.* 2011). Reduced Chl content will limit the energy available for photosynthesis which will reduce plant growth and development. If plants produce too much Chl, more light energy will be absorbed than can effectively be used for photosynthate production and may become harmful. Turley and Pettigrew (2011) found that reduced Chl content was consistently and significantly associated with reduced height, carbon exchange ratio, and minimum Chl fluorescence (F_0), however, they did not examine the effects on fiber yield, nor did they examine the Chl mutants under adverse environmental conditions. There is a need for breeding efforts to optimize cotton leaf Chl content

and maintain photosynthate production and yield under adverse environmental conditions.

The goal of this research was to understand the effects of high heat and low-soil water on leaf chlorophyll content and plant growth in upland cotton (*Gossypium hirsutum* L.). Understanding these relationships will help identify where breeding improvements can be most beneficial to increase photosynthate production that leads to sustainable fiber yields. The objectives were to (1) evaluate the effects of high heat and low-soil water on leaf chlorophyll content; (2) evaluate the relationships between leaf chlorophyll content and cotton growth parameters including height, the normalized difference vegetative index, and the leaf area index; and (3) evaluate the relationships between leaf chlorophyll content and cotton fiber yield and seed.

Materials and methods

Field experimental design: As described by Thorp *et al.* (2018) upland cotton field trials were conducted in 2016 and 2017 at the University of Arizona, Maricopa Agricultural Center (33.068°N, -111.971°W, 360 m above sea level), in Maricopa, Arizona. Eight upland cotton entries representing region-adapted cultivars (DP 1044B2RF, DP 12R244R2, and DP 1549B2XF), germplasm from the national breeders testing network (Ark0712-9, Arkot 9704, and PD 07040), and common check varieties (FM 958 and Siokra L23) were grown under four irrigation treatments (TRT) with two planting dates (PD). The irrigation treatments were 60, 80, 100, and 120% of the recommendation from an irrigation scheduling tool (Thorp *et al.* 2017). The first planting date treatments were sown on 26 April 2016 [day of the year (DOY) 117] and 19 April 2017 (DOY 109), and the second planting date treatments were sown on 18 May 2016 (DOY 139) and 10 May 2017 (DOY 130). Irrigation treatments started at first square (early June) and continued to 90% open boll (early September). The experimental design was a randomized complete block design with planting date as the block and the irrigation treatments nested within the blocks. The cotton entries were replicated three times for each treatment, with a total of 96 plots per block. In 2016, the plots were 3.0 × 4.6 m, with a 1.5-m alley between adjacent plots, and a density of ~ 8.6 plant m⁻². In 2017, the plots were reduced to 2.0 × 4.6 m, with a 1.5-m alley between adjacent plots, and a density of ~ 8.6 plant m⁻². Nitrogen was managed and applied as described by Thorp *et al.* (2020). In 2016, three applications of liquid ammonium nitrate (UAN 32-0-0) were uniformly applied in three split applications on 3 June (DOY 155), 16 June (DOY 168), and 8 July 2016 (DOY 190) totaling 111 kg(N) ha⁻¹. In 2017, the applications were applied in four split applications on 17 May (DOY 137), 8 June (DOY 159), 21 June (DOY 172), and 5 July 2017 (DOY 186) totaling 148 kg(N) ha⁻¹. Plots were harvested by a two-row cotton picker on 1 November 2016 (DOY 306) and 8 November 2017 (DOY 312). The seed cotton mass, plot area, and lint percent turnout were used to calculate cotton fiber yield [kg ha⁻¹]. Before mechanical harvest, 25-boll samples

were hand-picked from each plot and ginned with a tabletop 10-saw gin (*Compass Systems*, Barberton, OH, USA) to determine lint percent turnout, boll size, seed per boll, and the seed index.

Field measurements: The environmental conditions for both field seasons were recorded by the *Arizona Meteorological Network* (AZMET) weather station located 1.2 km away from the field. The weather station measured hourly humidity, temperature, and solar radiation which were used to calculate Level 1 and Level 2 heat stress conditions. A Level 1 heat stress condition was determined when the average daily canopy temperature was between 28 and 30°C and a Level 2 heat stress condition was determined when the average daily canopy temperature exceeded 30°C ([Brown 2008](#)). Soil water content was measured bi-weekly using a neutron moisture meter (*model 503, Campbell Pacific Nuclear*, Martinez, CA, USA) and steel access tubes as described by [Thorp et al. \(2020\)](#). The total available water [%] was calculated to a depth of 120 cm using the drained upper (0.16–0.22 cm³ cm⁻³) and lower (0.08–0.11 cm³ cm⁻³) soil water limits ([Thorp et al. 2018](#)) and the soil water content readings from the neutron moisture meter.

Leaf chlorophyll extractions: Four leaf discs from the uppermost fully expanded leaves were collected from each plot between 09:00 and 12:00 h into 96-well deep well microplates (*VWR International*, Radnor, PA, USA) and kept on ice in the field until they could be stored at –80°C in the laboratory. Chl was extracted by adding 1 mL

of cold (4°C) 100% methanol (*Fisher Scientific*, Waltham, MA, USA) to each well of the plates. Samples were covered, inverted several times, then placed in an orbital shaker (*Stovall Life Sciences*, Greensboro, NC, USA) on the max setting (~ 1.5 rotations per second). The samples were kept at 4°C in the dark for 48 h with shaking. After 48 h, 200 µL of the extracted Chl in methanol from each sample was transferred to a clear, 96-well flat-bottom microplate (*Fisher Scientific*, Waltham, MA, USA). Sample fluorescence was measured using a *Synergy HT* (*BioTek Instruments*, Winooski, VT, USA) plate reader with 665 and 652 nm wavelengths. A correction factor for each wavelength was developed by *BioTek* for the 96-well microplate. Chl concentration [µg mL⁻¹] from each sample was calculated following [Porra et al. \(1989\)](#) then divided by the total leaf area per sample (1.2 cm²) to get µg cm⁻². Collections were performed between June and August in 2016, and between June and September in 2017 ([Fig. 1](#)).

Cotton growth measurements: A retrofitted *Avenger Pro high-clearance* tractor (*LeeAgra Inc.*, Lubbock, TX, USA) was used for the acquisition of canopy temperature, plant height, and the normalized difference vegetative index (NDVI) using proximal sensors. The front boom was modified using 0.04 × 0.04 m extruded aluminum T-slot tubing, framing members, and hardware (*Rexroth Bosch Group*, Charlotte, NC, USA) to accommodate the proximal sensors as described by [Thompson et al. \(2020\)](#). The proximal sensors included *Pepperl+Fuchs UC2000* (*Pepperl+Fuchs Group*, Twinsburg, OH, USA) ultrasonic transducers to measure canopy height, *Crop*

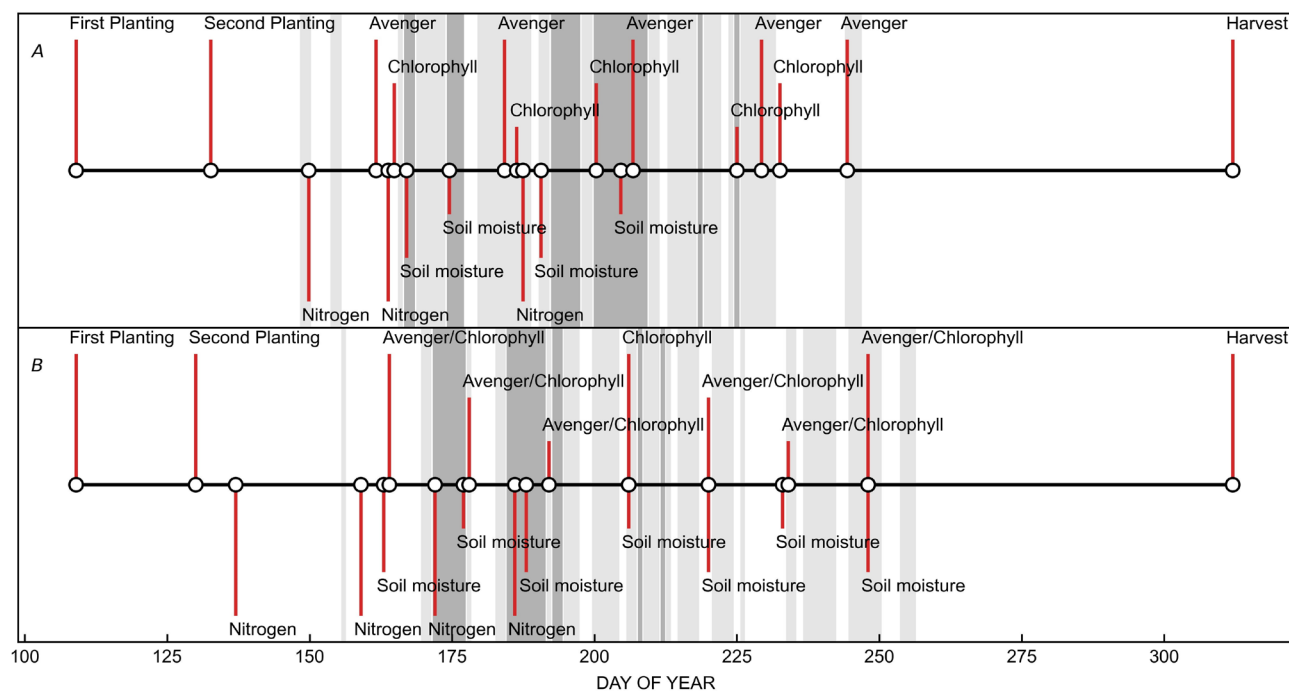


Fig. 1. The timeline of activities and field collections for 2016 (A) and 2017 (B) by day of the year. The light grey vertical lines represent the Level 1 heat stress days and the dark grey represents the Level 2 heat stress days as calculated using data from the Arizona Meteorological (AZMET) weather station located 1.2 km away from the field.

Circle ACS-470 (Holland Scientific, Lincoln, NE, USA) active spectral reflectance sensors to measure NDVI, and Apogee SI-131 (Apogee Instruments, Logan, UT, USA) infrared thermometers to measure canopy temperature. Measurements from each sensor were georeferenced by simultaneously recording the geographic position from a Trimble R6 real-time kinematic (RTK) GPS receiver (Trimble Inc., Sunnyvale, CA, USA) and heading from an inertial measurement unit sensor (VN-100, VectorNav Technologies, LLC, Dallas, TX, USA). All sensor information was recorded on a PXIe-1085 system (National Instruments, Austin, TX, USA) and assigned a timestamp. The latitude and longitude coordinates from the RTK-GPS receiver position were projected to the Universal Transverse Mercator (UTM) coordinate system (units of meters); vehicle heading and sensor configuration information were used to compute the UTM coordinates of each sensor at each timestamp (Wang *et al.* 2016). Collections by the Avenger tractor were made between June and August in 2016 and between June and September in 2017 (Fig. 1).

Proximal sensor data processing: Previous calibration studies under controlled temperature conditions revealed the Crop Circle ACS-470 sensors were sensitive to the high heat ($> 40^{\circ}\text{C}$) typical in the Arizona low desert which caused the reflectance data to drift (result not shown). To correct for the temperature-induced drift, a linear interpolation method was used to adjust the field reflectance data based on reflectance measurements over a white reference panel taken before (warm-up period) and after (cool-down period) the field outing. The panel was made of plywood $0.6 \times 3.05 \times 0.02$ m (l \times w \times h) and painted with a flat ultra-white titanium dioxide, which reflected 98–100% of solar irradiance (Thompson *et al.* 2020). Reflectance panel measurements were interpolated to estimate panel reflectance at each timestamp during the field collection:

$$r_{ij} = [(t_{i,j} - t_{0,i}) / (t_{n,i} - t_{0,i})] (r_{n,j} - r_{0,j})$$

where r_{ij} is the time-interpolated, reflectance panel corrected value for the i th timestamp and j th wavelength during the field collection, $r_{0,j}$ and $r_{n,j}$ are the average reflectance values over the reflectance panel for the j th wavelength during the warm-up and cool-down periods respectively, $t_{i,j}$ is the i th timestamp (in seconds from epoch) for the j th reflectance measurement during the field collection, and $t_{0,j}$ and $t_{n,j}$ are the average timestamps for the j th wavelength panel reflectance values during the warm-up and cool-down periods respectively. The corrected reflectance values (r_{ij}) were then used to calculate NDVI as:

$$\text{NDVI} = (r_{ij\text{NIR}} - r_{ij\text{VIS}}) / (r_{ij\text{NIR}} + r_{ij\text{VIS}})$$

where $r_{ij\text{NIR}}$ is the corrected reflectance at 800 ± 5 nm and $r_{ij\text{VIS}}$ is the corrected reflectance at 670 ± 5 nm.

Plant canopy height (H) was calculated using the ultrasonic transducer displacement values and meta notes transcribed during the collection:

$$H = (s - d_i) - z$$

where s is the recorded soil line to sensor boom height, d_i is the displacement data measured by the sensors at the i th timestamp, and z is the known z -offset for each sensor (Thompson *et al.* 2019).

The canopy temperature measured by the infrared thermometers and mean daily ambient air temperature measured by the AZMET weather station were used to calculate the crop water stress index (CWSI) previously described by Jackson *et al.* (1981) as:

$$\text{CWSI} = T_{c_i} - T_a$$

where T_{c_i} is the canopy temperature measured by the sensors at the i th timestamp and T_a is the mean daily air temperature. The CWSI was used for subsequent analysis rather than measured canopy temperature because a previous study (Thompson *et al.* 2018) found CWSI to be more repeatable (broad-sense heritability) than canopy temperature when performing only one collection per day.

Statistical analysis of collected traits: The SAS for Windows software v. 9.3 HPMIXED procedure (SAS Institute, Cary, NC, USA) was used to fit a linear model to each trait collected for each collection day for outlier removal. Each model included one of the collected traits, leaf Chl content, height (H), normalized difference vegetation index (NDVI), or crop water stress index (CWSI) as the dependent variable. The model used was:

Model 1:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \beta(\zeta)_{j(k)} + (\alpha\beta)_{ij} + [\alpha\beta(\zeta)]_{ij(k)} + [\gamma\beta(\zeta)]_{ij(k)} + \varepsilon_{ijkl}$$

where Y_{ijkl} was an individual observation; μ was the grand mean, α_i was the effect of the i th cotton entry; β_j was the effect of the j th planting date; $\beta(\zeta)_{j(k)}$ was the k th irrigation treatment nested within the j th planting date; $(\alpha\beta)_{ij}$ was the interaction effect between the i th cotton entry and the j th planting date; $[\alpha\beta(\zeta)]_{ij(k)}$ was the interaction effect between the i th cotton entry and the k th irrigation treatment nested within the j th planting date; $[\gamma\beta(\zeta)]_{ij(k)}$ was the interaction effect between the l th replicate within the k th irrigation treatment nested within the j th planting date; and ε_{ijkl} was the random error term following a normal distribution with mean 0 and variance σ^2 . The model terms $[\gamma\beta(\zeta)]_{ij(k)}$ and ε_{ijkl} were modeled as random effects with all other terms being treated as fixed effects. Significant outliers were determined by setting an upper and lower limit for the Studentized deleted residuals with a criterion of $\alpha = 0.05$ based on the total number of observations and model terms (Kutner *et al.* 2004). Outliers were removed iteratively.

To determine the significance of each fixed effect and the least squared means (LSMEANS) for each trait the SAS MIXED procedure was used to fit a linear model to the outlier removed data as in Model 1 with the addition of φ_m and $[\alpha\varphi\beta(\zeta)]_{imj(k)}$ terms.

Model 2:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \beta(\zeta)_{j(k)} + (\alpha\beta)_{ij} + [\alpha\beta(\zeta)]_{ij(k)} + \varphi_m + [\alpha\varphi\beta(\zeta)]_{imj(k)} + [\gamma\beta(\zeta)]_{ij(k)} + \varepsilon_{ijklm}$$

where ϕ_m was the effect of the m th day of the year of the collection; $[\alpha\phi\beta(\zeta)]_{ij(k)}$ was the interaction effect between the i th cotton entry and the m th day of the year and the k th irrigation treatment nested within the j th planting date. All other terms were the same as in Model 1. The LSMEANS statement was used on the model term $[\alpha\phi\beta(\zeta)]_{ij(k)}$ to estimate the least squared means for each cotton entry \times irrigation treatment \times planting date \times collection day of the year (DOY).

The leaf area index (LAI) for each cotton entry \times irrigation treatment \times planting date \times DOY collection was then calculated using the corrected NDVI and plant height LSMEANS as:

$$\text{LAI} = \beta (\text{NDVI}_i / \text{NDVI}_{\max}) (H_i / H_{\max})$$

where β is a constant (5.5), NDVI_i is the corrected NDVI value for the i th entry \times treatment \times planting date \times DOY collection, and NDVI_{\max} is the maximum NDVI value collected for that entry \times treatment \times planting date, H_i is the calculated plant height for the i th entry \times irrigation treatment \times planting date \times collection DOY, and H_{\max} is the maximum height collected for that entry \times treatment \times planting date as described by Thorp *et al.* (2015). The LSMEANS were used to develop the figures with the Python 'matplotlib' package and a quadratic interpolation function within the 'scipy.interpolate' package was applied.

To better understand the cumulative relationships amongst the irrigation treatments, planting dates, and mean daily air temperatures and the measured traits, the data was split such that, in 2016, all data collected before the peak L2 heat stress period (described below), were summed and divided by the total number of days ('low heat'), and days falling within the peak L2 heat stress period and after were summed and divided by the total number of days ('high heat') (Fig. 1). In 2017, all data before the peak L2 heat stress period and days falling within the peak heat stress period (described below) were summed and divided by the total number of days ('high heat'), and days after the peak L2 heat stress period were summed and divided by the total number of days ('low heat') (Fig. 1). To understand the relationship between Chl content and cotton yield components, Chl was summed and divided by the total number of days for each year. These variables ('low heat', 'high heat', and 'full year') were then used for structural equation modeling (SEM) to test hypotheses about the relationships between and amongst the variables. SEM uses multiple regression analysis to test designated relationships between one or more independent or dependent variables. The model output uses path diagrams to show the hypothesized relationships in the model (Ullman and Bentler 2013). The 'lavaan' package for R (Rosseel 2012) was used for the SEM analysis with Chl a or b as the dependent variables and all other traits as independent variables. All data were scaled (normalized) using the 'mutate' function to avoid overestimation of relationships between variables. The SEM standardized parameter estimates, and p -values were plotted using the 'semPlot' package for R (Epskamp 2015).

Results

Environmental measurements and data collections:

In 2016, the mean daily air temperatures consistently reached above 40°C from 2 June (DOY 154) to 19 August (DOY 232). The calculated days with Level 1 (L1) heat stress were 43, while the number of Level 2 (L2) d was 21 occurring primarily between late July (DOY 195) and early August (DOY 210). DOY 195–210 was considered the peak L2 heat stress period and used as the demarcation to split the data into 'low heat' and 'high heat' as described above. A total of two Avengers, two Chl, three soil moisture collections, and all nitrogen applications were made during the 'low heat' period from DOY 166–193 (27 d). Additionally, three Avengers, three Chl, and two soil moisture collections were made during the 'high heat' period from DOY 202–243 (41 d) (Fig. 1). In 2017, the mean daily air temperatures reached above 40°C starting in early June (DOY 154) and persisted until 13 September (DOY 256). The calculated days with LI heat stress were 47, and 17 for L2 heat stress days occurring primarily between late June (DOY 172) and early July (DOY 194). DOY 172–194 was considered the peak L2 heat stress period and used as the demarcation period. A total of three Avengers, three Chl, three soil moisture collections, and all nitrogen applications were made during the 'high heat' period from DOY 164–192 (28 d). Additionally, three Avengers, four Chl, and four soil moisture collections were made during the 'low heat' period from DOY 206–248 (42 d) (Fig. 1).

Statistical analysis of experimental parameters on measured traits:

The statistical analysis showed that the irrigation treatments had a significant effect on all the traits measured ($p \leq 0.050$) in both 2016 and 2017. The planting date had a significant effect on all traits measured except for Chl b in both years and plant height in 2017. The cotton entry had a significant effect on all traits except for Chl a in 2016. All model interaction terms were significant except for the entry \times planting date interaction for Chl a in 2016 (Table 1).

Low-soil water and high heat relationships with chlorophyll:

The 60% irrigation treatment for both years and planting dates had higher Chl a and b content [$\mu\text{g cm}^{-2}$] than the other treatments (Fig. 2). In 2016, the 100% irrigation treatment had lower Chl a and b content than the other treatments. In 2017, the lowest content values for Chl b were also found in the 100% treatment, however, the lowest content values for Chl a were found in the 100% treatment for the first planting date and the 80% treatment for the second planting date (Fig. 2). For both plantings across all irrigation treatments in 2016, the lowest content of Chl a occurred during the peak L2 heat stress period (DOY 195–210) (Fig. 2). Chl b showed the lowest content after the peak L2 heat stress period and did not recover to the initial content before the L2 period. In 2017, both Chl a and b had the lowest content after the peak L2 heat stress period (DOY 164–192) across all irrigation treatments and planting dates (Fig. 2).

Table 1. Linear mixed model fixed effect for the planting date by low-soil water trial including F values and probability values (p), where E is the cotton entry term, PD is the planting date term, TRT is the irrigation treatment term, and DOY is the day of year term, for leaf chlorophyll (Chl) a and b content, plant height, normalized difference vegetation index (NDVI), leaf area index (LAI), crop water stress index (CWSI), and fiber yield.

Trait	E		PD		TRT(PD)		DOY		E × PD		E × TRT(PD)		E × DOY × TRT(PD)	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
2016														
Chl a	1.86	0.073	10.46	0.001	7.02	0.000	2,625.97	0.001	1.46	0.178	2.04	0.000	2.23	0.000
Chl b	4.63	0.000	1.63	0.207	5.13	0.000	1,016.28	0.001	1.76	0.002	1.76	0.000	1.82	0.000
Height	15.65	0.000	113.05	0.000	18.80	0.000	2,310.99	0.000	3.34	0.000	2.87	0.000	7.95	0.000
NDVI	14.92	0.000	161.38	0.000	27.85	0.000	3,854.70	0.000	3.37	0.000	4.08	0.000	5.73	0.000
LAI	11.90	0.000	87.86	0.000	6.51	0.043	2,814.26	0.000	2.73	0.000	3.38	0.000	5.21	0.000
CWSI	8.54	0.000	136.20	0.000	35.87	0.000	1,467.51	0.000	13.68	0.000	4.79	0.000	9.13	0.000
Yield	3.60	0.002	5.05	0.041	8.84	0.000	n/a	n/a	5.70	0.000	1.52	0.042	n/a	n/a
2017														
Chl a	2.70	0.009	18.18	0.000	28.56	0.000	367.07	0.001	3.48	0.000	2.46	0.000	3.66	0.000
Chl b	2.74	0.008	1.98	0.161	19.03	0.000	375.24	0.000	4.69	0.000	2.33	0.000	2.49	0.000
Height	32.19	0.000	0.00	0.998	21.69	0.000	7,747.87	0.000	13.73	0.000	7.82	0.000	4.70	0.000
NDVI	7.33	0.000	86.41	0.000	15.74	0.000	5,968.06	0.056	1.98	0.000	3.73	0.000	4.07	0.000
LAI	13.88	0.000	72.13	0.000	12.51	0.000	14,463.50	0.001	3.48	0.000	4.96	0.000	3.72	0.000
CWSI	8.15	0.000	224.53	0.000	13.61	0.000	5,331.64	0.000	5.00	0.003	3.18	0.000	7.84	0.000
Yield	5.10	0.000	57.85	0.000	5.82	0.000	n/a	n/a	3.58	0.002	3.43	0.000	n/a	n/a

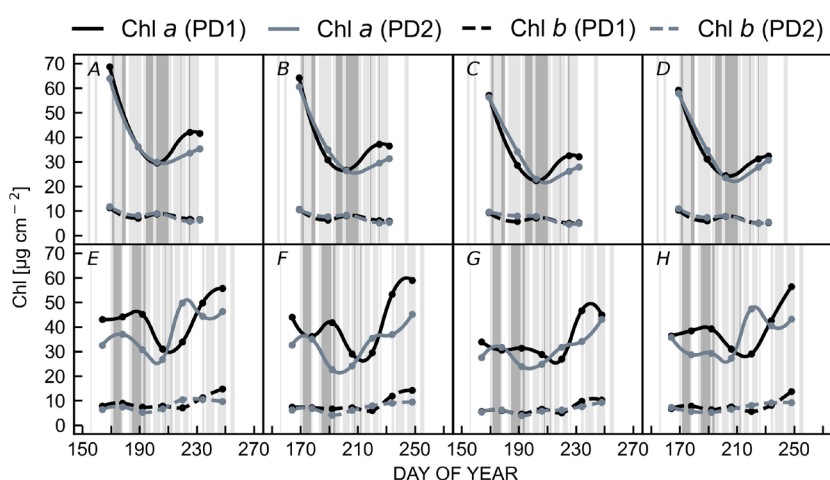


Fig. 2. The extracted leaf chlorophyll a and b content [$\mu\text{g cm}^{-2}$] average ($n = 8$) for each irrigation treatment at 60 (A,E), 80 (B,F), 100 (C,G), and 120 (D,H) planting dates (PD) in 2016 and 2017. The light grey vertical lines represent the Level 1 heat stress days and the dark grey represents the Level 2 heat stress days.

The structural equation modeling showed that during periods of low heat, the total available water to a depth of 120 cm had a significant negative relationship with Chl a content in both years (Fig. 3). The total available water relationship with Chl b was also negative in both years but was only significant in 2016 (Fig. 4). In 2016, the CWSI did not have a significant relationship with Chl a or b during the low heat period but had a significant positive relationship in 2017. For the CWSI, larger index values indicated the plants were water-stressed, so a positive relationship indicated water-stressed plants also had increased Chl content. The mean daily air temperatures during the low heat periods did not have a significant relationship with Chl a in 2016 but did have a significant negative relationship in 2017 (Fig. 3). For

Chl b , a significant positive relationship was seen in 2016 and a significant negative relationship in 2017 (Fig. 4).

During the periods of high heat, the total water available had a significant positive relationship with Chl a and b in 2016 but had no relationship in 2017 (Figs. 3, 4). The CWSI had a significant positive relationship with Chl a and b during the high heat period in 2016 but not in 2017. The mean daily air temperatures had a significant negative relationship with Chl a in 2016 and 2017 but did not have a significant relationship with Chl b (Figs. 3, 4).

Leaf chlorophyll content in cotton entries: Most of the cotton entries followed a somewhat similar pattern of change over the 2016 and 2017 seasons shown in Fig. 2. However, the range of Chl content (largest Chl

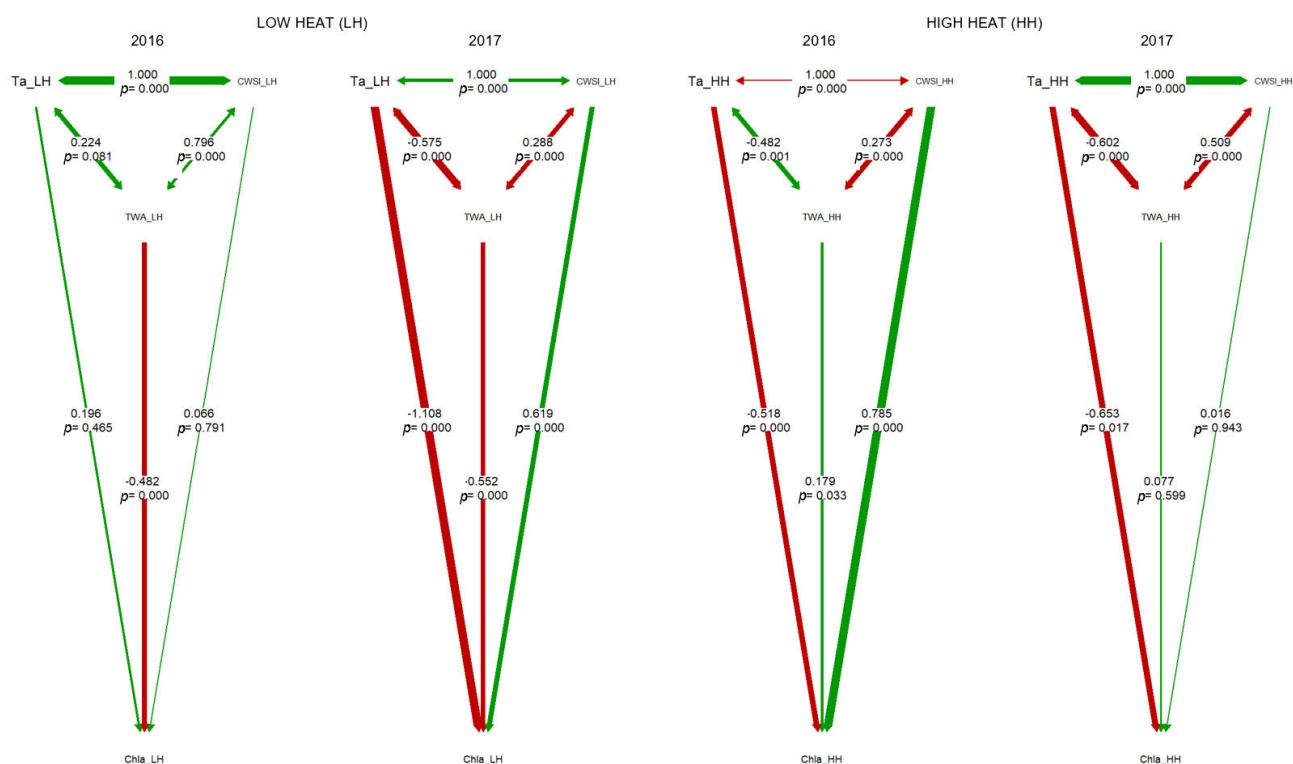


Fig. 3. The structural equation modeling output depicting the impact of mean daily air temperature (TA), total available water (TW), and crop water stress index (CW) on chlorophyll *a* (CA) content during the low and high heat periods in 2016 and 2017. The top values are the SEM standardized parameter estimates, and the bottom values are the *p*-values. *Green lines* indicate positive relationships and *red lines* indicate negative relationships. Line thickness is an indicator of relationship strength. *Double arrows* indicate a covariance between the variables while a *single arrow* indicates a hypothesized relationship with the arrow pointing to the dependent variable.

content value–smallest Chl content value) for each entry within irrigation treatment and planting date was different (Table 1S, *supplement*). Two entries, Siokra L23 and Arkot 9704, consistently had the lowest rates of change across years, irrigation treatments, and planting dates while FM 958 and DP 1549B2XF exhibited some of the highest rates of change (Table 1S).

Leaf chlorophyll content relationships with cotton growth:

The relationship between Chl content (*a* and *b*) and the leaf area index (LAI) was positive during both the high and low heat periods for 2016 and 2017 (Figs. 5, 6). All LAI relationships were significant except for Chl *b* during the high heat period in 2016. The normalized difference vegetative index (NDVI) had negative relationships with Chl *a* and *b* but was only significant during the low heat periods in 2016 and 2017. Plant height and Chl content (*a* and *b*) had significant negative relationships during the low heat period in 2017. The only other significant plant height relationship was with Chl *a* during the high heat period in 2016 (Figs. 5, 6).

Leaf chlorophyll content effect on cotton yield components:

Cotton fiber yield ranged from 1,340 to 2,679 kg ha⁻¹ in 2016 across planting dates and treatments and from 817 to 2,831 kg ha⁻¹ in 2017 (Table 1S). The highest

yields were achieved in either the 100% or 120% irrigation treatments while the lowest yields were in the 60% irrigation treatment. The SEM analysis showed that Chl *a* and *b* had a significant positive relationship with the seed index (an estimate of seed size) and a significant negative relationship with boll size in both 2016 and 2017 (Fig. 7). In 2016, both Chl *a* and *b* had a significant negative relationship with fiber yield but a positive relationship with the number of seeds per boll. In 2017, Chl *a* had a significant positive relationship with fiber yield and no relationship with the number of seeds per boll while Chl *b* had a positive relationship with seeds per boll but had no relationship with cotton fiber yield (Fig. 7).

Discussion

High heat and low-soil water are significant obstacles to the future of sustainable cotton production. The purpose of this field trial was to evaluate the effects of high heat and low-soil water on leaf Chl content in upland cotton (*G. hirsutum* L.) and to identify opportunities for breeding improvement that will lead to sustainable fiber yields under adverse conditions. The unique environment at the Maricopa Agriculture Center, located in Maricopa, Arizona allowed consistent measurements of crop growth and development under fluctuating high temperatures and

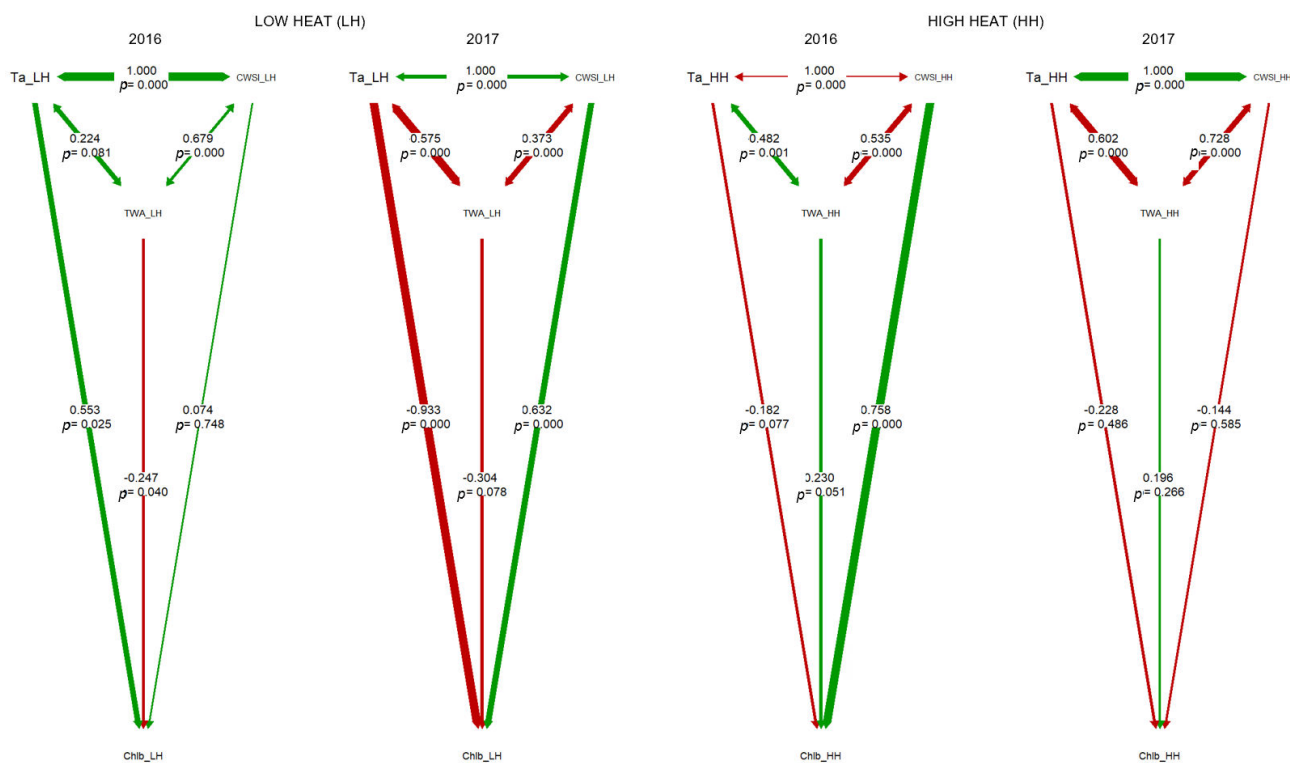


Fig. 4. The structural equation modeling output depicting the impact of mean daily air temperature (TA), total available water (TW), and crop water stress index (CW) on chlorophyll *b* (CB) content during the low and high heat periods in 2016 and 2017. The top values are the SEM standardized parameter estimates, and the bottom values are the *p*-values. *Green lines* indicate positive relationships and *red lines* indicate negative relationships. Line thickness is an indicator of relationship strength. *Double arrows* indicate a covariance between the variables while a *single arrow* indicates a hypothesized relationship with the arrow pointing to the dependent variable.

variable soil-water content. The 2016 and 2017 growing years were very different in terms of the timing and duration of heat stress which enabled the measurement of leaf Chl content under four different temperatures by low-soil water conditions including low heat with low-soil water and high heat with low-soil water in 2016, and high heat with minimal low-soil water and prolonged heat with low-soil water in 2017. The results showed that low-soil water and high heat have opposing impacts on leaf Chl content where low-soil water increased content and high heat reduced content. The results also indicate that irrigation and nitrogen management strategies could play critical roles in achieving Chl stability during prolonged periods of heat stress for sustainable cotton production.

Heat stress has long been identified as a significant factor in reducing leaf Chl content for many crops including cotton (Reddy *et al.* 2004, Hejnák *et al.* 2015). The underlying processes which lead to observed decreases in Chl content are potentially three-fold. First heat stress has been shown to inhibit the Chl biosynthetic pathway preventing plants from creating more Chl (Tewari and Tripathy 1998). Second, high temperatures have been associated with structural changes in the thylakoid membrane leading to heat-induced disorganization and degradation (Havaux *et al.* 1996, Sharkey 2005, Ristic

et al. 2007, Herritt and Fritschi 2020). Third, high heat increases the activity of Chl-degrading enzymes (Wang *et al.* 2018). Hu *et al.* (2020) proposed that Chl degradation during heat stress may be a protective response to prevent more light energy from being adsorbed than can be used, as other photosynthetic machinery, such as Rubisco activase, become inhibited (Law and Crafts-Brandner 1999, Salvucci and Crafts-Brandner 2004). This study revealed that decreases in Chl *a* were much more profound than decreases in Chl *b* in response to low and high heat stress periods. Chl *b* is associated with PSII reaction centers which are involved in the initial step of photosynthesis. Changes in Chl *a* but not *b* would suggest cotton is responding to heat by maintaining the same number of reaction centers (Chl *b*) but reducing the antenna size (Chl *a*) associated with PSII, thereby preventing excess light adsorption. Therefore, it is likely that breeding efforts focused on light absorption efficiency could lead to improved heat stress adaption in cotton.

Low-soil water treatments have been associated with changes in Chl content or density including increases, decreases, and no change for many crops (Sarker *et al.* 1999, Li *et al.* 2006, Arunyanark *et al.* 2008, Akhkhia *et al.* 2011, Hejnák *et al.* 2015). Previous work in peanuts (Arunyanark *et al.* 2008) showed that Chl density

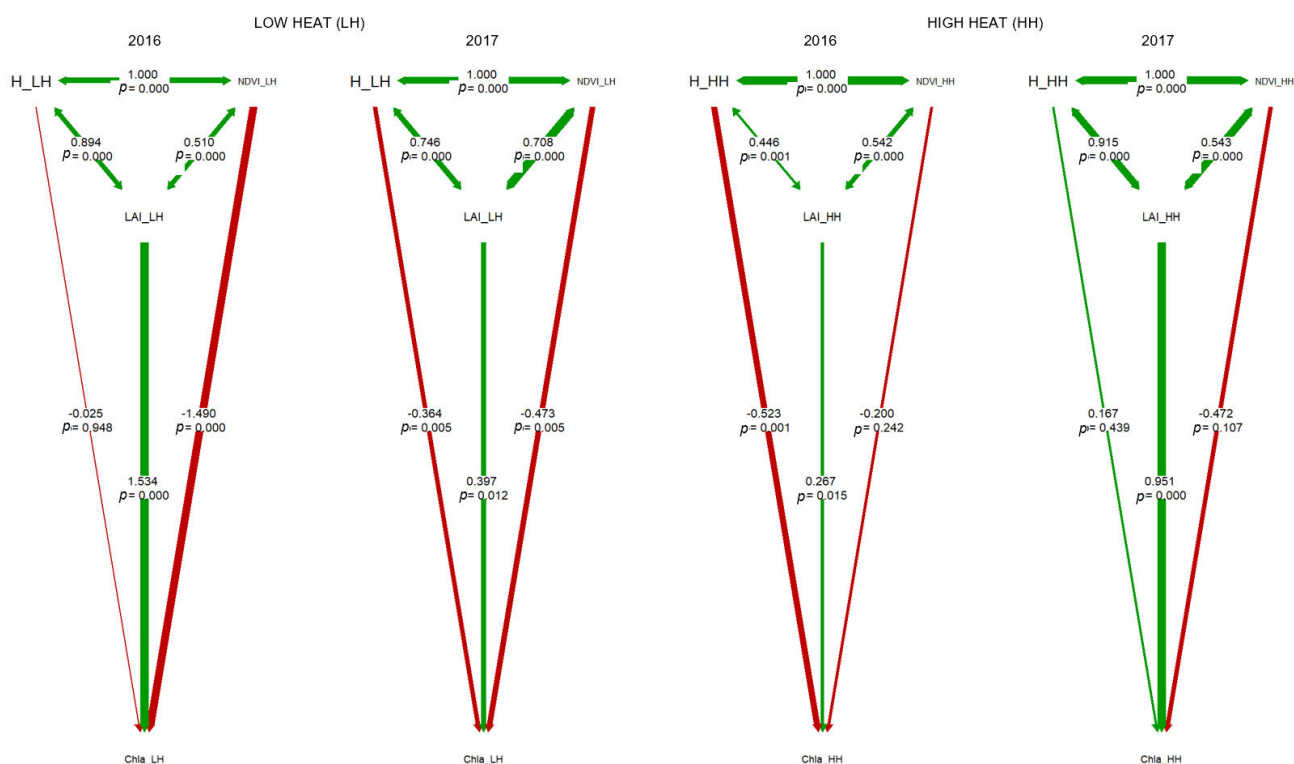


Fig. 5. The structural equation modeling output depicting the impact of chlorophyll *a* (CA) content on the leaf area index (LA), the normalized difference vegetative index (ND), and plant height (HT) during the low and high heat periods in 2016 and 2017. The top values are the SEM standardized parameter estimates, and the bottom values are the *p*-values. *Green lines* indicate positive relationships and *red lines* indicate negative relationships. Line thickness is an indicator of relationship strength. *Double arrows* indicate a covariance between the variables while a *single arrow* indicates a hypothesized relationship with the arrow pointing to the dependent variable.

[$\mu\text{g cm}^{-2}$] increased under water deficit while Chl content [mg per plant] decreased. However, both Chl density and content were positively correlated with plant total dry matter when grown under water-deficit conditions leading the authors to conclude that Chl status in water-stressed peanuts was a good indicator of drought tolerance. Potato varieties, identified as drought-tolerant because they maintained yield under water stress conditions, had no significant changes in Chl *a* content after four weeks with no water (van der Mescht *et al.* 1999). Decreases in Chl *b* were observed after two weeks with no water leading the authors to conclude that total Chl (*a+b*) and Chl ratio (*a/b*) were good indicators of drought tolerance in potatoes. This study showed that the prolonged low-soil water treatment (60%) caused a significant increase in Chl *a* and *b* content compared to the well-watered treatment (100%) in both 2016 and 2017. Increases to both Chl *a* and *b* indicate a compensation mechanism for reduced net photosynthesis in response to low-soil water may have been utilized. Many studies have shown that plants respond to low-soil water by regulating stomatal closure to reduce water loss (Chaves *et al.* 2002) which reduces net photosynthesis. As a result, cytokinin signaling originating from the roots has been hypothesized to increase Chl biosynthesis and stomatal opening to increase photosynthetic capacity

(Farquhar and Sharkey 1982). It is possible that the cotton entries in this study were attempting to preserve net photosynthesis in this manner, however, additional studies that include stomatal conductance and photosynthesis measurements are needed to draw firm conclusions.

It is also important to note that the low-soil water treatments were also accompanied by some measure of heat stress, either L1 or L2. The structured equation modeling (SEM) revealed that only under low heat does low-soil water have a significant relationship with Chl content (less water led to more Chl), under high heat the relationship was reversed (less water led to less Chl). The relationship between Chl and low-soil water was further supported by the calculated crop water stress index (CWSI) ($T_{c_i} - T_a$). When the CWSI was low no significant impacts were found on Chl content, however, when the CWSI increased along with the mean daily air temperatures, significant positive relationships with Chl content were identified. The CWSI relationship with Chl content indicated that heat stress has a much stronger relationship with Chl content than does water stress. The lower CWSI values for the 100% and 120% irrigation treatments indicated the plants were using the available water for evaporative cooling thereby avoiding some measure of heat stress and Chl degradation. These findings are consistent with upland

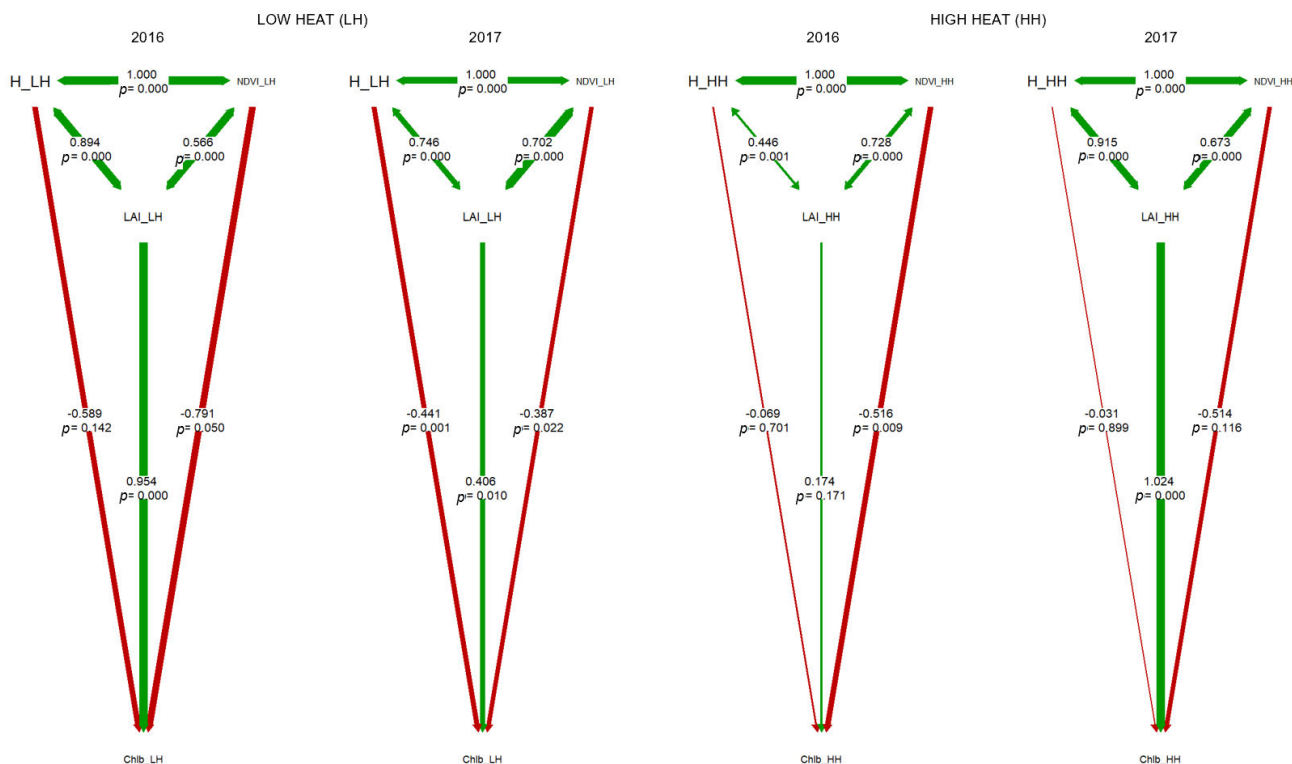


Fig. 6. The structural equation modeling output depicting the impact of chlorophyll *b* (CB) content on the leaf area index (LA), the normalized difference vegetative index (ND), and plant height (HT) during the low and high heat periods in 2016 and 2017. The top values are the SEM standardized parameter estimates, and the bottom values are the *p*-values. *Green lines* indicate positive relationships and *red lines* indicate negative relationships. Line thickness is an indicator of relationship strength. *Double arrows* indicate a covariance between the variables while a *single arrow* indicates a hypothesized relationship with the arrow pointing to the dependent variable.

cotton grown in growth chambers under varying degrees of heat stress and low-soil water treatments (Hejnák *et al.* 2015). These findings also agree with future climate impact models indicating increased air temperatures will result in greater cotton yield loss than changes in cumulative rainfall (Ayankojo *et al.* 2020). The relationship between evaporative cooling and Chl stability highlights the importance of crop water management for sustainable cotton production in high heat environments.

During heat stress, a significant positive relationship between Chl *a* and *b* content was found with the leaf area index but the reverse was generally true for plant height and the normalized difference vegetation index, an estimate of plant biomass. This could indicate that during heat stress periods, cotton will divert available resources to leaf area and Chl content over height and biomass accumulation, presumably to create more source tissue for photosynthate production. Previous studies found that leaf area index and boll carrying capacity were linearly related, leading the authors to hypothesize that the increased leaf area provided more photosynthates to support growing bolls (Ashley *et al.* 1965, Jackson and Gerik 1990). However, this study found that Chl *a* and *b* had either a significant negative relationship or no relationship with boll size and fiber yield. Instead, positive relationships were found between Chl content and the number of seeds

per boll and the seed index. This suggests cotton seed is a stronger sink for available resources than the boll capsule or the fiber. Bondada *et al.* (1997) found that more nitrogen was compartmentalized to the developing seed and fiber than the boll capsule, concluding they were the major sinks, however, they did not look at seed and fiber separately. This study demonstrates that more work is needed to understand the source/sink relationship between developing leaves, bolls, seeds, and fiber regarding photosynthates and nitrogen in cotton.

Many studies have shown that the optimal time to apply nitrogen is during the reproductive phase as developing bolls provide the largest nitrogen sink (Bondada *et al.* 1997, Mullins *et al.* 2003). In this study, all nitrogen applications were made before and during early flowering but before the onset of bolls. Because of the nitrogen timing, some decline in leaf Chl content may be attributed to the translocation of nitrogen to the developing bolls. Bondada *et al.* (1997) found that cotton will translocate available nitrogen to developing bolls independent of leaf age which likely explains why 2016 Chl *a* never recovered to the initial content. However, even during peak heat stress periods in 2016 and 2017, Chl *a* content never dropped below 18 $\mu\text{g cm}^{-2}$ which may be a minimum requirement for the production of photosynthates to maintain fruit load. In 2017, entries maintained some level of Chl *a* stability

2016

2017

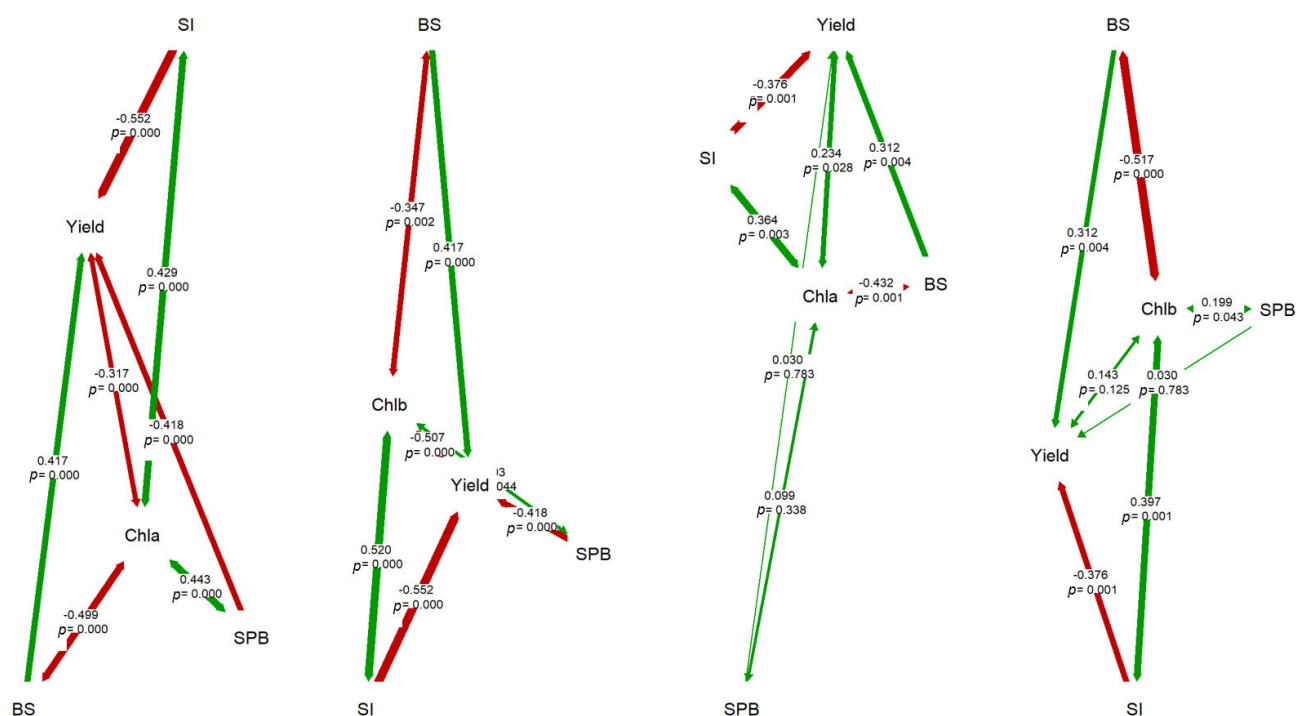


Fig. 7. The structural equation modeling output depicting the impact of chlorophyll *a* (CA) and *b* (CB) content on boll size (BS), the seed index (SI), seeds per boll (SP), and fiber yield (YI) in 2016 and 2017. The top values are the SEM standardized parameter estimates, and the bottom values are the *p*-values. Green lines indicate positive relationships and red lines indicate negative relationships. Line thickness is an indicator of relationship strength. Double arrows indicate a covariance between the variables while a single arrow indicates a hypothesized relationship with the arrow pointing to the dependent variable.

during the peak heat stress period, which may be due to the nitrogen applications that occurred during this time. Even though Chl stability was achieved during peak heat stress and more nitrogen was applied in 2017, fiber yields, on average, were lower in 2017 than in 2016. The early heat stress in 2017 caused a great deal of fruit shed which possibly resulted in less demand for nitrogen. Studies have shown that excess nitrogen will increase vegetative growth leading to smaller bolls with delayed maturity (McConnell *et al.* 1992). This could explain why more nitrogen did not result in higher yields for 2017 and explain why Chl *a* content recovered beyond the initial levels measured. These results suggest that Chl content, particularly Chl *a*, and fruit load could be indicators for timing nitrogen applications.

The findings in this study support the use of leaf Chl to identify high heat and low-soil water adaptive cotton germplasm for future sustainable cotton production. To improve cotton in the short term, breeders should identify germplasm that is light-absorption efficient and that maintains leaf Chl content, either by evaporative cooling or other methods, to use as breeding parents. The entries Siokra L23 and Arkot 9704 were identified as potential candidates. To improve cotton in the long term, more work is needed in three important areas: (1) identifying the rate-limiting steps in light (radiation)-use efficiency,

(2) understanding how nitrogen management strategies impact leaf Chl content during periods of high heat, and (3) understanding the source/sink relationship between leaf Chl content and developing bolls to maximize resource-use efficiency.

Conclusion: The unique environment at the Maricopa Agriculture Center provided the opportunity to measure crop growth and development under fluctuating high temperatures and variable soil-water content. The resulting data revealed that light absorption efficiency and evaporative cooling regulation could lead to improved cotton adaptation for sustainable production in a changing environment. The data also highlight the importance of utilizing crop management strategies, including irrigation and nitrogen management. Novel genotype \times environment \times management studies are needed for effective plant breeding in a changing environment.

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