

2234 Screening Cotton Cultivars for Thermotolerance under Field Conditions

Ms. Nicola S. Cottee , University of Sydney, University of Sydney, Australia
Dr. Daniel K. Y. Tan , University of Sydney, University of Sydney, Australia
Dr. Tom Cothren , Texas A&M University, College Station, TX
Dr. Michael P. Bange , CSIRO Division of Plant Industry, Narrabri, Australia
Dr. Lindsay C. Campbell , University of Sydney, University of Sydney, Australia

Abstract

High temperature stress adversely affects cotton growth, development and ultimately yield. Simple methodologies measuring leaf photosynthesis and chlorophyll fluorescence (F_v'/F_m') have previously been used to differentiate high temperature tolerance in cotton cultivars under controlled conditions. This paper presents an evaluation of these methodologies to measure cotton high temperature stress tolerance under field conditions. In-field ambient temperatures were increased by constructing solarweave tents above the crop canopy in Narrabri, Australia during the 2005-2006 growing season. The experiment was repeated in Texas, USA (2006) to account for environmental variability. Photosynthesis and chlorophyll fluorescence (F_v'/F_m') were measured for two cotton cultivars, Sicot 53 and Sicala 45. A four-day incubation period under the tents gave the best discrimination in leaf photosynthesis and F_v'/F_m' between the two cotton cultivars. Photosynthesis and F_v'/F_m' declined at a greater rate under the tents compared to the control. This trend was evident at both field sites, but only significant for the Texas field site. These results showed that Sicot 53 has a higher degree of thermotolerance than Sicala 45. Hence, there is a potential to screen for heat tolerant cotton genotypes by increasing the ambient temperature under field conditions using solarweave tents and measuring photosynthesis.

Keywords: Cotton, *Gossypium hirsutum*, high temperature tolerance, photosynthesis, F_v'/F_m' , chlorophyll fluorescence

Acknowledgments: This work was partially funded by the Cotton Research and Development Corporation and the Cotton Catchment Communities Co-operative Research Centre.

Introduction

Cotton is generally grown in hot environments. High temperatures ($>35^\circ\text{C}$) throughout the growing season may adversely affect growth and ultimately yield and quality (Hearn and Constable, 1984). Furthermore, high temperature stress is closely related to water deficit and can be further exacerbated by limited and unreliable water availability in cotton production systems (Rizhsky et al., 2004).

Temperature influences the growth and development of a cotton crop. It is a primary factor of both sowing date and seasonal development. Cotton has an optimal thermal window of $23-32^\circ\text{C}$ in which metabolic activity is most efficient (Burke et al., 1988). Maximum daily temperatures exceeding 32°C are common in many cotton growing regions and are presumably limiting growth, development and ultimately crop yield. Effects of high temperature stress include decreased plant growth, development and increased fruit shedding (Hodges et al., 1993). This may be attributed to photosynthetic decline under high temperature stress (Reddy et al., 1991). Specific impacts include decreased electron flow

through the photosystem (Wise et al., 2004), membrane leakage (Sullivan, 1971), decreased mitochondrial viability (de Ronde and van der Mescht, 1997) and/or decreased stability of photosynthetic and respiratory enzymes (Salvucci and Crafts-Brandner, 2004).

Photosynthesis and chlorophyll fluorescence are established methods of screening for high and low temperature tolerance in cotton under greenhouse conditions (Bibi et al., 2004a; Brown and Oosterhuis, 2004; McDowell et al., 2007). The plant specific response to high temperature stress may differ under greenhouse and field conditions due to non-target environmental stress in the field. These screening methods have been extended to the field for water stress studies (Kiato and Lei, 2007; Leidi et al., 1993). In-field high temperature stress has been achieved by staggering planting date (Rahman, 2005), ambient temperature-dependent sampling (Bibi et al., 2004b) and the use of polyethylene shelters (Lopez et al., 2003). This study aims to extend these principles to *in situ* screening for high temperature tolerance in cotton cultivars under field conditions using solarweave tents.

The onset of high temperature stress in cotton production systems may be minimised by selecting higher yielding cotton cultivars under high temperature stress. Hence, methodologies evaluating the ability of cotton to tolerate high temperatures by measuring photosynthesis and chlorophyll fluorescence may be employed to select thermotolerant cotton cultivars for use in future breeding programs (Constable et al., 2001). Screening of cotton cultivars for thermotolerance using various physiological and morphological parameters will enable effective selection of agronomically superior cotton cultivars for production in the warmer cotton growing regions of Australia, and the world.

This study was conducted to determine the effectiveness of measuring leaf photosynthetic rate and chlorophyll fluorescence for evaluation of cotton leaf photosynthetic function under high temperature stress in the field using solarweave tents and to establish whether these methods are suitable for screening cotton cultivars for thermotolerance.

Materials and Methods

Field experiments were conducted over two consecutive cotton growing seasons between October, 2005 and September, 2006. Experiment 1 was conducted at The Australian Cotton Research Institute (149°59'E, 31°12'S), Narrabri, Australia during the 2005-2006 growing season. Experiment 2 was conducted at The Texas Agricultural Experiment Station (30°32'N, 96°26'W), Burleson County, Texas, The United States of America, during the 2006 American cotton growing season. These two locations were selected to account for variability in climatic conditions. Although both sites experienced high temperatures throughout the cotton season (Figure 1), the Texas site had a higher average ambient temperature (Figure 1) and humidity (Figure 2) than the Narrabri site.

Two cotton cultivars (*Gossypium hirsutum* L.) cv. Sicot 53 and Sicala 45 were sown at a rate of 12 plants m⁻² in a Grey Vertosol. Plots measuring 9 rows, 1 m apart by 15 m were used in a randomized complete block design with four replications. Plots were grown under furrow irrigation. Sicot 53 was selected as a relatively thermotolerant cultivar whilst Sicala 45 was thought to have less tolerance to high temperature stress made on the basis of yield data collected in cool and hot cotton growing regions of Australia (Constable 2005 pers. comm., 8 August).

In-field high temperature stress was achieved by erecting a solarweave tent 4 days post irrigation. Solarweave is a clear, radiation permissible fabric. It has a nominal shade value of 18% and may increase the relative proportion of diffuse radiation reaching the canopy,

but was considered an appropriate commercially available fabric for field experiments (Healey and Rickert, 1998; Healey et al., 1998). These tents were erected above the crop canopy to raise temperatures under the fabric, but still facilitate airflow down the rows to permit effective gaseous exchange.

Initial photosynthetic rates and chlorophyll fluorescence were measured at ambient field conditions. The tents were then erected and left over the crop canopy for a defined number of days (ranging from 2 to 5 days) whilst measurements were taken (Table 1). The tents were removed and a recovery measurement was taken two days later. The timing of tent construction and removal was dependent on environmental conditions and therefore, differed slightly between experiments. This treatment was repeated several times throughout the season, between the developmental stages of pinhead square and cut-out. Air temperature and relative humidity were continuously recorded during the treatment period under the tent and in the control plot, using Tinytag Ultra TGX-2580 loggers (Gemini Data Loggers, UK).

Measurements of photosynthesis and lighted-adapted chlorophyll fluorescence (F_v'/F_m') were made using a Li-6400 portable photosynthesis system (Li-Cor Ltd, Lincoln, NE, USA) with a pulse-amplitude modulated (PAM) leaf chamber fluorometer sensor head. Since photosynthesis declines with leaf age, (Guinn and Brummett, 1993) measurements were repeatedly taken on the fourth main-stem leaf of three different plants per replicate, between 1000 and 1230h Initial readings were taken 4-5 days after an irrigation event to ensure sufficient soil moisture, minimise waterlogging and reduce soil moisture variability between the experiments. Light intensity was maintained inside the sensor head at $2000 \text{ mmol m}^{-2} \text{ s}^{-1}$. The reference carbon dioxide concentration was set at $400 \text{ mmol CO}_2 \text{ mol}^{-1}$ using a CO_2 mixer. The leaf chamber block temperature was maintained at 30°C as it is considered the optimal for photosynthesis (Wise et al., 2004). Relative humidity followed ambient conditions. The system flow rate was adjusted to maintain a vapour pressure deficit of 1.5 to 2.5 kPa. Light adapted chlorophyll fluorescence was measured using the fluorometer attachment, immediately following the photosynthesis measurement. Effective quantum yield of photosystem II (PSII) (F_v'/F_m') is an indicator of plant stress with a healthy plant operating at about 80% maximum efficiency ($F_v'/F_m' = 0.83$). Lower values indicate a stress response, particularly photoinhibition (Maxwell and Johnson, 2000). Light adapted chlorophyll fluorescence measurements were taken using the fluorometer attachment, immediately following the photosynthesis measurement.

Analysis of variance was conducted on the Inverse Sine transformed, relative change in photosynthesis and F_v'/F_m' between the control and tent plants for each rep. This analysis was performed using Genstat 9th edition and graphical presentation of data was compiled using SigmaPlot 10.0. Owing to large differences between the means for each cultivar and the least significant difference, statistical analysis of photosynthesis and F_v'/F_m' data was performed according to the following formula:

$$\text{Photosynthesis or } F_v'/F_m' = \text{ArcSin} ((T - C) / C)$$

Where:

T = photosynthesis or F_v'/F_m' under the radiation permissible tents

C = photosynthesis or F_v'/F_m' in the control plots under ambient field conditions

Results & Discussion

Photosynthesis generally decreased in both the control and high temperature treated plants (Figure 3). This decline may be attributed to increasing leaf age (Guinn and Brummett, 1993; Turner et al., 1986) or environmental conditions. The photosynthesis calculations on the Li-6400 are largely influenced by leaf temperature which is further determined by water availability to the leaf for thermal cooling. Hence, a systematic decline in photosynthesis immediately following an irrigation event may also be a result of decreasing soil water availability and subsequently leaf water potential (Turner et al., 1986). In Experiment 1, daily ambient temperature increased between the initial and final treatment measurements (Figure 1). This incremental temperature change may also result in a decline in photosynthesis across the treatments.

Experiment 1

There was a general decrease in photosynthetic rate over time of measurement across all treatments (Figure 3). There was no significant cultivar or treatment effect for this experiment. F_v'/F_m' increased during the high temperature stress period for both plants in the tent and control. This is most likely a function of environmental variability between the measurement days and could be attributed to slightly lower ambient temperature (Figure 1), or a slight increase in relative humidity (Figure 2). Although the results for this experiment are not significantly different (Figure 4), consistent trends may be observed. The absolute change in F_v'/F_m' for Sicot 53 was greater than for Sicala 45 between the initial measurements and measurements taken after one day of high temperature stress (Figure 4). This trend suggests that Sicot 53 was better able to maintain PSII efficiency than Sicala 45 at temperatures of approximately 40°C (Figure 1) and a relative humidity of approximately 80-90% (Figure 2).

F_v'/F_m' was higher in plants after five days of incubation under the tents than in the control (Figure 3). This indicates that the conditions under the tents were more suitable to plant photosynthetic function. This result is more likely to be attributed to a suitable high ambient humidity under the tents (Figure 2), rather than the high temperatures (Figure 1), thereby avoiding a stress response in the plants. F_v'/F_m' was also higher for Sicot 53 than Sicala 45 two days after the removal of the tents. It is speculated that Sicot 53 was better able to maintain efficiency of PSII and was less affected by photoinhibition after returning to ambient growing conditions (Figure 4).

Experiment 2

Photosynthesis remained relatively unchanged for both cultivars between the initial, treatment and recovery measurements in the control plants (Figure 3). This indicates that field-grown plants were acclimated to effectively maintain photosynthesis at temperatures of 35-45°C effectively (Figure 1) under relative humidity of around 60-90% (Figure 2) during the measurement period. This lack of differences between the three measurement days also indicates that the environmental conditions did not differ sufficiently to illicit a stress response during the experimental period.

Photosynthesis decreased for both Sicot 53 and Sicala 45 after four days incubation under the tents. This indicates that field-grown plants were not able to maintain photosynthesis at temperatures of 40 to 50°C during the measurement period. In comparison to control plants, Sicot 53 maintained a higher photosynthetic rate than Sicala 45 after 4 days incubation in the tents ($P < 0.05$) (Figure 4). This suggests a greater degree of

thermotolerance in field-grown Sicot 53 compared to Sicala 45. After tent removal, both Sicot 53 and Sicala 45 plants recovered to a similar level of photosynthesis after 3 days under ambient conditions (Figure 4). Although the photosynthetic rate of both cultivars did not reach the initial rate, they displayed a similar ability to photosynthesize effectively after a period of heat stress.

Chlorophyll fluorescence in the control corroborates with corresponding photosynthesis data. F_v'/F_m' increased throughout the experimental period (Figure 3). Again, this is most likely attributed to changes in ambient temperature and humidity. Under the tents, the relative F_v'/F_m' increased for Sicot 53 and decreased for Sicala 45, compared to the corresponding controls (Figure 4). This suggests that Sicot 53 is likely to have a higher degree of thermotolerance than Sicala 45 during a high temperature stress event. The recovery of both cultivars to similar levels in the control and the tent indicates that the stress event was not sufficient to cause permanent or chronic photoinhibition.

Construction of solarweave tents above a cotton canopy is an effective method of increasing ambient temperature in a field situation. However this increase in temperature is coupled with an increase in relative humidity, and a slight reduction in photosynthetically active radiation (PAR) to the crop. The ability of plants to maintain photosynthesis and F_v'/F_m' may be largely determined by in field acclimation prior to the measurement period and will hence, vary during the crop life cycle. In both experiments, Sicot 53 consistently showed attributes of greater heat tolerance than Sicala 45. Hence, these measurements may be employed for short-term stress screening under field conditions however; further refinement of the methodology will be needed to elucidate a consistent response to high temperatures. Further research into the use of alternative screening methods, such as tissue viability and cell viability tests may complement this research, and also provide a good basis for rapid screening on in field thermotolerance. There is also potential for the development of molecular assays for the detection of thermotolerance in cotton cultivars.

References

- Bibi, A.C., D.M. Oosterhuis, E.D. Gonias, and F.M. Bourland. 2004a. Screening a diverse set of cotton cultivars for high temperature tolerance. D. M. Oosterhuis (ed.). Summaries of Arkansas Cotton Research 2004. University of Arkansas Agricultural Experiment Station Research Series 39-43.
- Bibi, A.C., D.M. Oosterhuis, R.S. Brown, and F.M. Bourland. 2004b. The physiological response of cotton to high temperature for germplasm screening(ed.). Beltwide Cotton Conference, San Antonio, Texas. January 5-9. National Cotton Council of America 2266-2270.
- Brown, R.S., and D.M. Oosterhuis. 2004. High daytime temperature stress effects on the physiology of modern versus obsolete cotton cultivars. D. M. Oosterhuis (ed.). Summaries of Cotton Research in Progress 2004. , Fayetteville, Arkansas. University of Arkansas Agricultural Experiment Station Research Series 63-67.
- Burke, J.J., J.R. Mahan, and J.L. Hatfield. 1988. Crop-specific thermal kinetic windows in relation to wheat and cotton biomass production. *Agronomy Journal* 80: 553-556.
- Constable, G.A., P.E. Reid, and N.J. Thomson. 2001. Approaches utilized in breeding and development of cotton cultivars in Australia, p. 1-15, *In* J. J.N and S. Saha, eds. Genetic Improvement of Cotton. Science Publishers, Inc, Mississippi State.
- de Ronde, J.A., and A. van der Mescht. 1997. 2,3,5-Triphenyltetrazolium chloride reduction as a measure of drought tolerance and heat tolerance in cotton. *South African Journal of Science* 93: 431-433.
- Guinn, G., and D.L. Brummett. 1993. Leaf age, decline in photosynthesis, and changes in abscisic acid, indole-3-acetic acid and cytokinin in cotton leaves. *Field Crops Research* 32: 269-275.
- Healey, K.D., and K.G. Rickert. 1998. Shading material changes the proportion of diffuse radiation in transmitted radiation. *Australian Journal of Experimental Agriculture* 38: 95-100.
- Healey, K.D., K.G. Rickert, G.L. Hammer, and M.P. Bange. 1998. Radiation use efficiency increases when the diffuse component of incident radiation is enhanced under shade. *Australian Journal of Agricultural Research* 49: 665-672.
- Hearn, A.B., and G.A. Constable. 1984. Cotton, p. 495-527, *In* P. R. Goldsworthy and N. M. Fisher, eds. *The Physiology of Tropical Field Crops*. John Wiley & Sons Ltd, Chinchester.
- Hodges, H.F., K.R. Reddy, J.M. McKinion, and V.R. Reddy. 1993. Temperature effects on cotton. Mississippi Agricultural & Forestry Experiment Station, Mississippi State.
- Kiato, M., and T.T. Lei. 2007. 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.

Leidi, E.O., J.M. Lopez, and J.C. Gutierrez. 1993. Searching for tolerance to water stress in cotton genotypes: photosynthesis, stomatal conductance and transpiration. *Photosynthetica* 28: 383-390.

Lopez, M., M.V. Gutierrez, M.A.A. El-Dahan, E.O. Leidi, and J.C. Gutierrez. 2003. Genotypic variation in response to heat stress in Upland cotton(ed.). World Cotton Conference, Capetown, South Africa 104-108.

Maxwell, K., and G.N. Johnson. 2000. Chlorophyll fluorescence - a practical guide. *Journal of Experimental Botany* 51: 659-668.

McDowell, A.J., M.P. Bange, and D.K.Y. Tan. 2007. Cold temperature exposure at 10°C for 10 and 20 nights does not reduce tissue viability in vegetative and early flowering cotton plants. *Australian Journal of Experimental Agriculture* 47: 198-207.

Rahman, H. 2005. Genetic analysis of stomatal conductance in upland cotton (*Gossypium hirsutum* L.) under contrasting temperature regimes. *The Journal of Agricultural Science* 143: 161-168.

Reddy, V.R., D.N. Baker, and H.F. Hodges. 1991. Temperature effects on cotton canopy growth, photosynthesis and respiration. *Agronomy Journal* 83: 699-704.

Rizhsky, L., H.J. Liang, J. Shuman, V. Shulaev, S. Davletova, and R. Mittler. 2004. When defense pathways collide. The response of *Arabidopsis* to a combination of drought and heat stress. *Plant Physiology* 134: 1683-1696.

Salvucci, M.E., and S.J. Crafts-Brandner. 2004. Mechanism for deactivation of Rubisco under moderate heat stress. *Physiologia Plantarum* 122: 513-519.

Sullivan, C.Y. 1971. Mechanisms of heat and drought resistance in grain sorghum and methods of measurement, p. 247-284, *In* N. G. P. Rao and L. R. House, eds. *Sorghum in the Seventies*. Oxford & IBH Publishing Co, New Delhi, India.

Turner, N.C., A.B. Hearn, J.E. Begg, and G.A. Constable. 1986. Cotton (*Gossypium hirsutum* L.): Physiological and morphological responses to water deficits and their relationship to yield. *Field Crops Research* 14: 153-170.

Wise, R.R., A.J. Olson, S.M. Schrader, and T.D. Sharkey. 2004. Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature. *Plant, Cell and Environment* 27: 717-724.

Captions of Figures:

Figure 1 Atmospheric temperature ($^{\circ}\text{C}$) for Experiment 1 (Narrabri) and Experiment 2 (Texas) in the control (ambient conditions) and under the solarweave tents. The horizontal lines represent the mean temperature ($^{\circ}\text{C}$) for the duration of the experiment for the control and under the tents.

Figure 2 Relative humidity (%) for Experiment 1 (Narrabri) and Experiment 2 (Texas) in the control (ambient conditions) and under the solarweave tents. The horizontal lines represent the mean relative humidity (%) for the duration of the experiment for the control and under the tents.

Figure 3 Average net leaf photosynthesis and effective yield of photosystem II (PSII) (F_v'/F_m') for cotton cultivars Sicala 45 and Sicot 53 for Experiment 1 (Narrabri) and Experiment 2 (Texas). Error bars are twice the standard error of the mean.

Figure 4 Average relative rate of net leaf photosynthesis and effective quantum yield of photosystem II (PSII) (F_v'/F_m') for cotton cultivars for Sicot 53 and Sicala 45 for Experiment 1 (Narrabri) and Experiment 2 (Texas) (Significant differences indicated by * = 95% confidence level). Analysis conducted on ArcSin $((T - C) / C)$ transformed data.

Figure 1

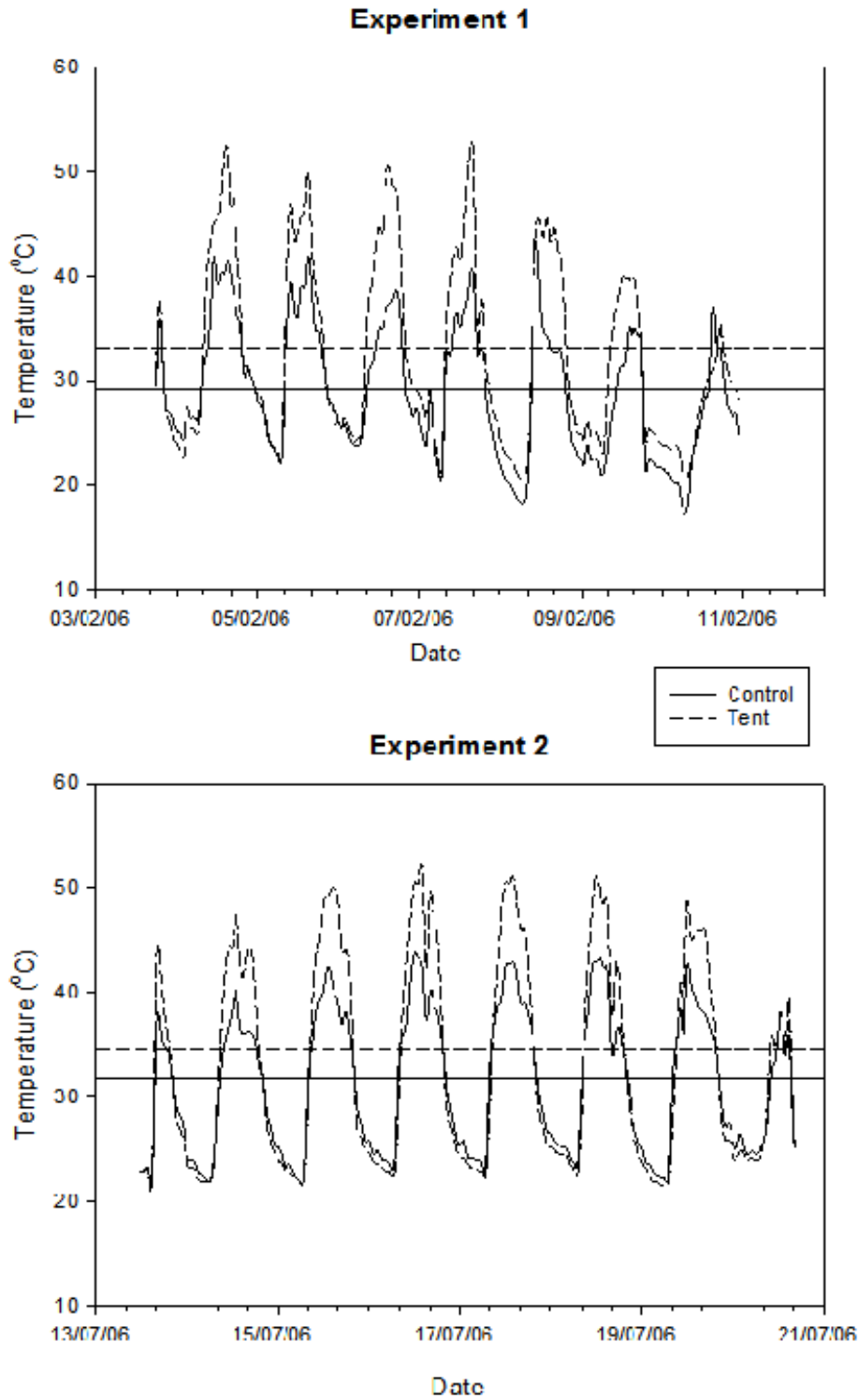


Figure 2

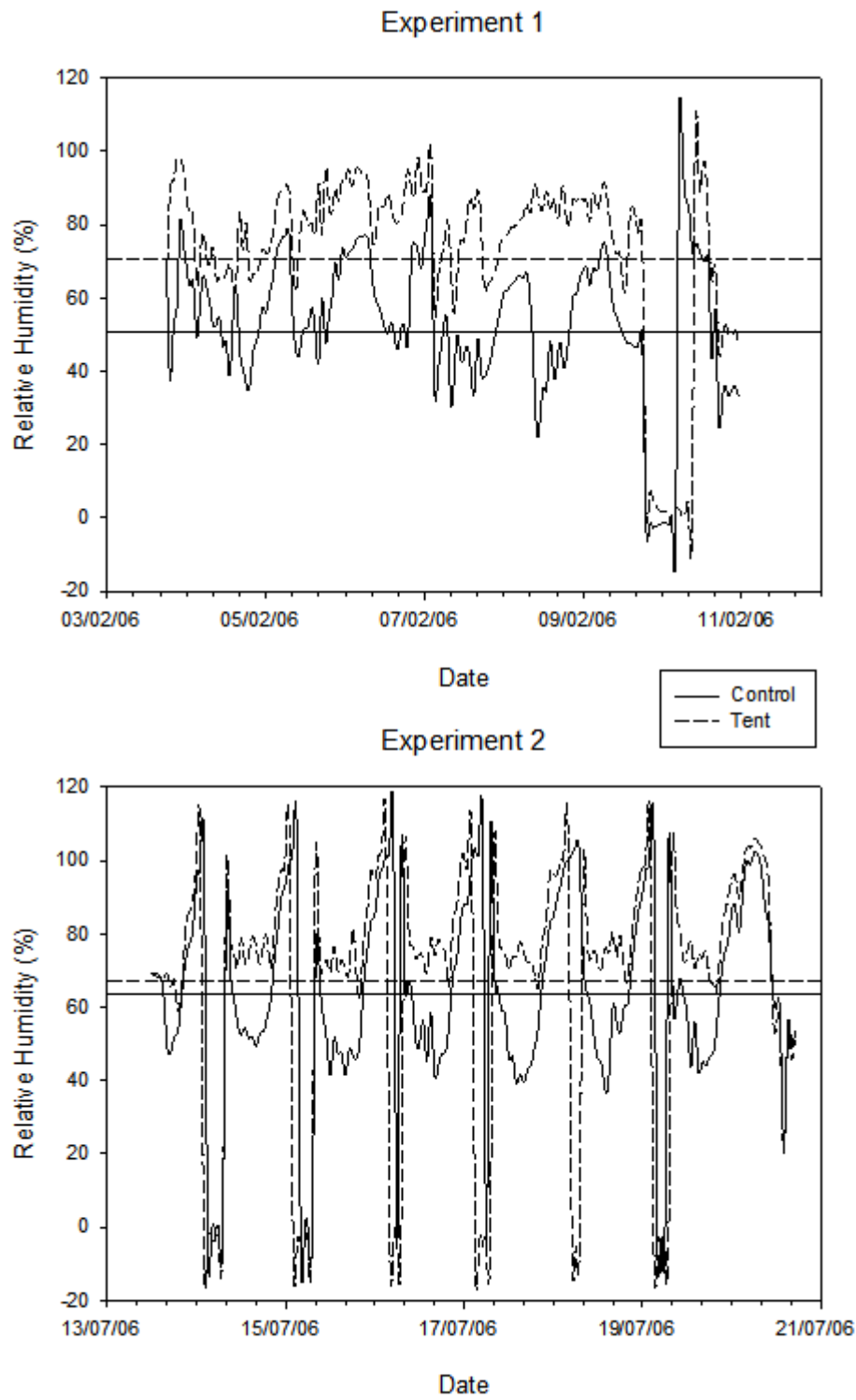


Table 1 Sampling dates and corresponding duration of high temperature stress under the tents for Experiment 1 and Experiment 2.

Experiment	Measurement	Date	Number of days exposure under tents
1	Initial	03/02/2006	0
1	Treatment 1	06/02/2006	2
1	Treatment 2	09/02/2006	5
1	Recovery	11/02/2006	-
2	Initial	13/07/2006	0
2	Treatment 1	19/07/2006	4
2	Recovery	21/07/2006	-

Figure 3

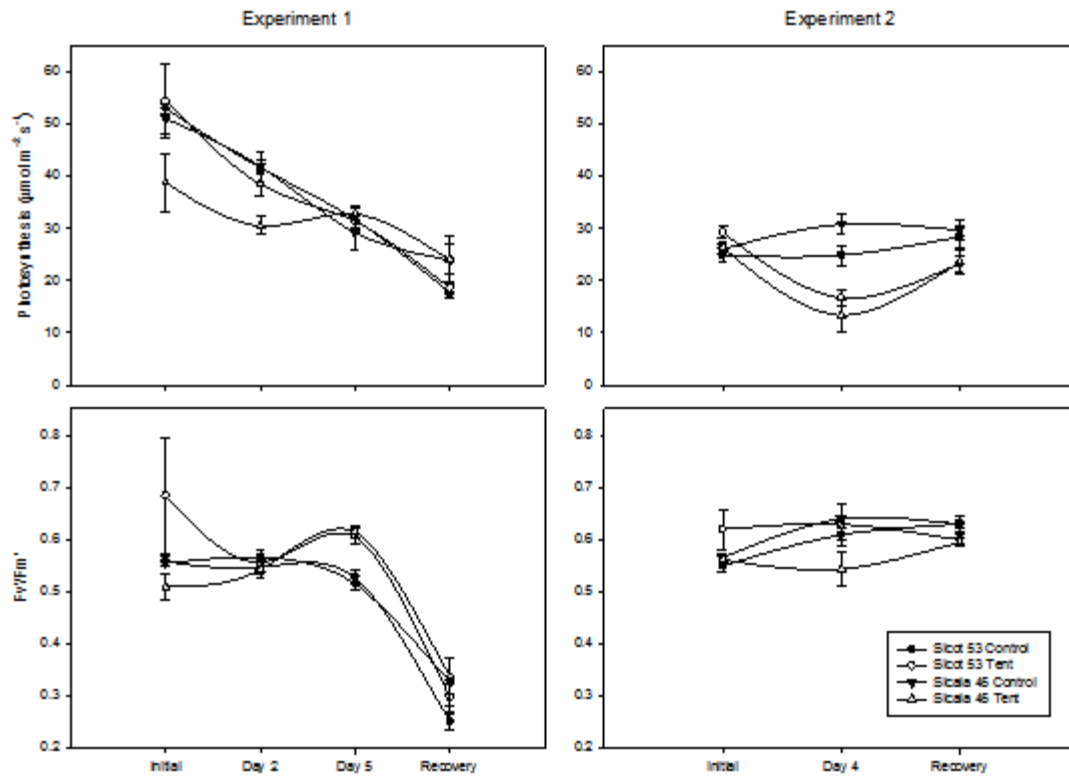


Figure 4

