

*Full Length Research Paper*

# Changes in cotton leaf pigmentation after abnormal exposure to sunlight

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Accepted 22 February, 2013

Leaves are adapted to respond differentially to changing light conditions and intensity. Cotton leaves are normally green during the growing season. However, mechanical manipulation or wind occasionally inverts leaves exposing the abaxial side to direct sunlight resulting in development of red pigmentation in otherwise green leaves. Based on this observation, this study was conducted to determine the effect of exposure of abaxial surface to direct sunlight on leaf pigmentation. Field experiments were conducted in 2007 and 2008. At 92 DAP, the quarter seized (25 cm diameter) main stem leaf (T) and the second leaf (T+2) below that were clamped in an opaque plastic rectangular frame fixed to a bamboo staff supporting the frame. The frame, with the leaf clamped in it, was turned at 180° to completely invert the leaf from its normal position. Inverting the T+2 leaves increased the anthocyanin concentration while causing a reduction in chlorophyll content. Chlorophyll to Anthocyanin ratio was lower in older inverted leaves with a reduced effect in the younger leaves. The abnormal exposure to direct sunlight caused the leaves to actively synthesize more anthocyanin. The data suggest that anthocyanin is synthesized as a photoprotectant that shields leaves from abnormal, excess radiation exposure.

**Key words:** Cotton, anthocyanin, chlorophyll.

## INTRODUCTION

Cotton (*Gossypium hirsutum* L.) leaves have a dark green pigmentation during normal growing conditions. However, some diseases, stresses and the cessation of new growth at the end of the reproductive cycle cause the leaves to lose green color and become reddish to bronze in color due to anthocyanin production (Wells, 2001). Sunlight, bacterial infections (Kangatharalingam et al., 2002), cold night temperature (Neill and Gould, 2003) along with maturity and senescence (Hoch et al., 2003) are some of the factors that cause the development of red color in leaves due to biosynthesis of anthocyanins.

Anthocyanins are abundant in juvenile and senescing leaves and their concentrations increase in response to exposure to ultra-violet radiation, high intensity PAR, drought, and nutrient deficiency (Merzlyak et al., 2008; Steyn et al., 2002). Young and immature leaves of multiple species possess greater anthocyanin content than mature leaves (Hatier and Gould, 2009). In maize seedlings grown under low temperature regime of 18 to 11 °C day/night, anthocyanins absorbed 43% of light energy compared to just 1.4% in plants grown under high temperature of 23 to 30 °C (Pietrini and Massacci, 1998). Anthocyanins are synthesized in the cytosol and are predominantly stored in vacuoles of epidermal cells of cotton leaves (Kangatharalingam et al., 2002). The anthocyanins are low molecular weight photo-protectors which quench the excessive reactive oxygen species in young leaves and acts as an antioxidant to prevent photobleaching of chlorophyll. As the leaf matures and more chlorophyll is produced in the leaves, higher amounts of solar radiation can be utilized for photosynthesis thereby reducing the need of anthocyanin

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**Abbreviations:** Ant (Anthocyanin); Chl (chlorophyll); DAT (Days after treatment); DAP (Days after planting); SLW (Specific leaf weight); DMF (dimethylformamide); PAR (Photosynthetically active radiation); ROS (Reactive oxygen species).

as photo-protector. This change leads to decline in anthocyanin concentration in fully expanded leaves.

During senescence, cotton leaf chlorophyll degrades and leads to increased synthesis of anthocyanin (Wells, 2001). Kar et al. (1993) reported that reduction in the capacity to repair PSII reaction centers during senescence led to greater light sensitivity, resulting in photodamage at relatively moderate sunlight. Plant species that risk photoinhibition during senescence often utilize anthocyanins to protect the leaf (Hoch et al., 2001). Smillie and Hetherington (1999) reported that anthocyanins act as a screen during periods of high radiation stress, thus reducing photodamage to photosynthetic tissues. Anthocyanin synthesis at end of the growing season was mediated by low night temperature and high light intensity in five species (Merzlyak et al., 2008).

Senescing cotton leaves lost chlorophyll and increased in anthocyanin, especially in the presence of a more mature reproductive sink (Wells 2001). However, during peak growth season before boll opening or even cutout, it is commonly observed that parts of cotton leaves inverted by wind or mechanical action and are exposed to direct sunlight on their abaxial surface turn red while rest of the leaf remains green. Kangatharalingam et al. (2002), while investigating the role of anthocyanins in imparting resistance to bacterial leaf blight in cotton, reported the development of anthocyanins on abaxial surface of leaves after exposure to light. It is not known whether abnormal exposure of the abaxial surface to sunlight would increase anthocyanin levels prior to later stages of crop maturity. The experiment contained herein was conducted to determine the effect of exposure of abaxial surface to direct sunlight on leaf pigmentation.

## MATERIALS AND METHODS

Field experiments were conducted in 2007 and 2008 at Central Crops Research Station, Clayton NC on Norfolk Loamy Sand. Cotton variety DP 147 was planted on 29 Apr. 2007 and 14 May 2008 with a two row vacuum planter at 97 cm row spacing. Each plot consisted of four rows. Treatments were imposed on the two interior rows. The experiment was laid out as a randomized complete block design with four replicates.

At 92 DAP, the third main stem leaf from the plant apex (T) and the second leaf (T+2) below that were clamped in a white plastic rectangular frame fixed to a bamboo staff supporting the frame. The frame, with the leaf clamped in it, was turned at 180° to completely invert the leaf from its normal position, with the abaxial side facing upwards and the adaxial side facing the ground (Figure. 1A). Four plants per plot were set up in this manner. The control treatments were enclosed in the frames with the abaxial side facing the ground and the adaxial side facing upwards (normal leaf orientation). The frames were set up in way as to minimize mechanical pressure or damage to the leaves.

At 92, 98, 105, and 111 DAP, leaves (inverted and control) were harvested from one plant per replicate. The leaves were sealed in plastic bags and placed on ice for transportation to

laboratory for analysis. After fresh weight was determined leaf area was measured on LI-COR, LI 3100 area meter (LICOR Inc. Lincoln, NE). For pigment analysis, three leaf disks of 0.3 cm<sup>2</sup> from each leaf were placed in 3 ml dimethylformamide (DMF) in the dark for 2 days at 4 °C. Total chlorophyll and ratio of Chl a/b was determined from the DMF extract spectrophotometrically (Moran, 1982). Concentration of anthocyanin was determined by placing three leaf discs (0.9 cm<sup>2</sup> total) in 3 ml acidified methanol with 10 ml concentrated HCL/L for 2 days at 4 °C. Light absorbance of the methanol extracts were determined at 530 nm (Anthocyanin) and 657 nm (Chl). Anthocyanin concentration was calculated using the following formula of Mancinelli et al. (1988):

$$Ant = A_{530} - 0.25 (A_{657}).$$

The remaining leaves were placed in a dried at 60 °C for 3 days and specific leaf weight (SLW) was obtained by dividing the leaf area by dry weight.

All data was analyzed using Proc Mixed in SAS. Years, leaf position and sampling dates were considered as random effects. Specific error terms were used to determine significance of treatments. Means were separated using Fisher's protected LSD at 5% level of significance.

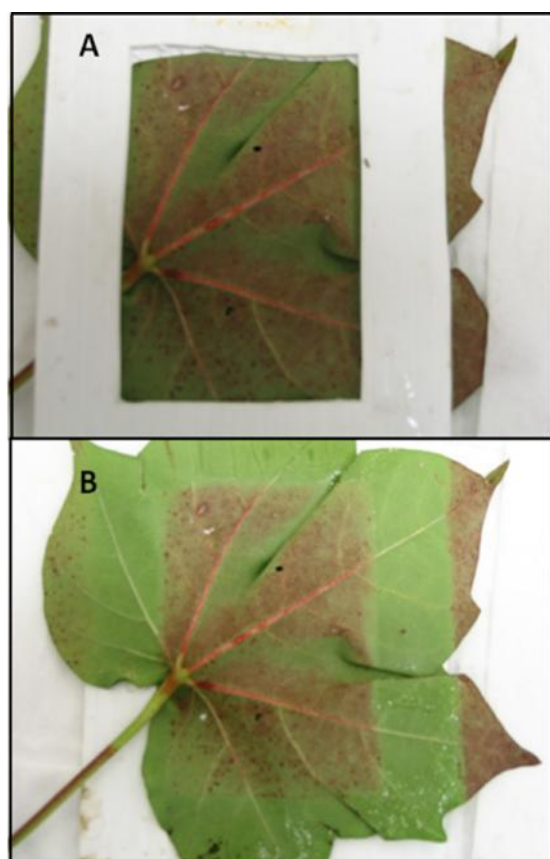
## RESULTS AND DISCUSSION

Inverting the leaves did not have any effect on leaf area, specific leaf weight (SLW), fresh weight or dry weight of the either leaf position (Table 1). The leaf at T+2 had more leaf area, fresh weight, and dry weight while SLW was reduced compared to leaf at position T as expected being approximately six days older. Wells (1988) found that leaves emerging at 62 days after planting (DAP) had more than twice the area of leaves emerging at 89 DAP. The reduction in SLW for larger leaf could be an indication of the negative relationship that exists between leaf area and SLW. Wiebold and Kenworthy (1985) found negative correlations between SLW and leaf area in maturity group IV soybean cultivar terminal trifoliolate leaves at nodes 8 and 10 from the plant apex ( $r = -0.46^*$  and  $-0.69^{**}$ , respectively).

Inverting the T+2 leaves caused a visual reddening of the abaxial surface of the leaf blade (Figure 1A), while the region covered by the plastic frame was unaffected (Figure 1B). The increased reddening of the T+2 leaf was indeed due to an increase in anthocyanin concentration while causing a reduction in chlorophyll concentration (Table 2). Leaves at position T had no significant change in neither anthocyanin nor chlorophyll content (Figures 2 and 3). The abnormal exposure of the abaxial surface to direct sunlight caused the T+2 leaves to actively synthesize more anthocyanin. There was a negative curvilinear relationship between anthocyanin and chlorophyll, especially at greater anthocyanin levels (Figure 4). Wells (2001) reported an increase in anthocyanin synthesis and decline in chlorophyll concentration in cotton leaves undergoing normal maturation during reproductive development. Similarly,

**Table 1.** Analysis of variance summary for leaf area, chlorophyll (Chl), anthocyanin (Ant), fresh, dry and specific leaf weight (SLW) in response to year, treatment, harvest date and leaf position.

Source	Leaf area	Ant	Total Chl	Fresh Weight	Dry Weight	SLW
----- p > F -----						
Year	0.0002	0.7781	0.0611	0.0001	0.0001	0.1324
Treatment (Trt)	0.7123	0.0015	0.0031	0.8153	0.8365	0.7420
Year x Trt	0.5403	0.2701	0.5737	0.8920	0.9598	0.9238
Date	0.7754	0.0744	0.7126	0.6713	0.0292	0.0006
Year x Date	0.3333	0.3834	0.3857	0.2861	0.0331	0.0269
Date x Trt	0.7490	0.3876	0.6838	0.8014	0.3702	0.3827
Year x Date x Trt	0.8043	0.0777	0.7801	0.2583	0.2390	0.3336
Leaf Position (LP)	0.0001	0.0282	0.0001	0.0001	0.0172	<0.0001
Year x LP	0.0363	0.0019	0.2340	0.0592	0.2942	0.0256
Trt x LP	0.1498	<0.0001	<0.0001	0.2136	0.2029	0.6582
Year x LP x Trt	0.2683	0.0810	0.7892	0.2574	0.5811	0.8733
Date x LP	0.7553	0.7484	0.0236	0.2556	0.0572	0.1000
Year x date x LP	0.4035	0.4419	0.8524	0.5556	0.9490	0.4122
Date x LP x Trt	0.9751	0.0230	0.3793	0.9776	0.5224	0.6605
Year x Date x Trt x LP	0.8738	0.0213	0.7850	0.5763	0.5784	0.6329

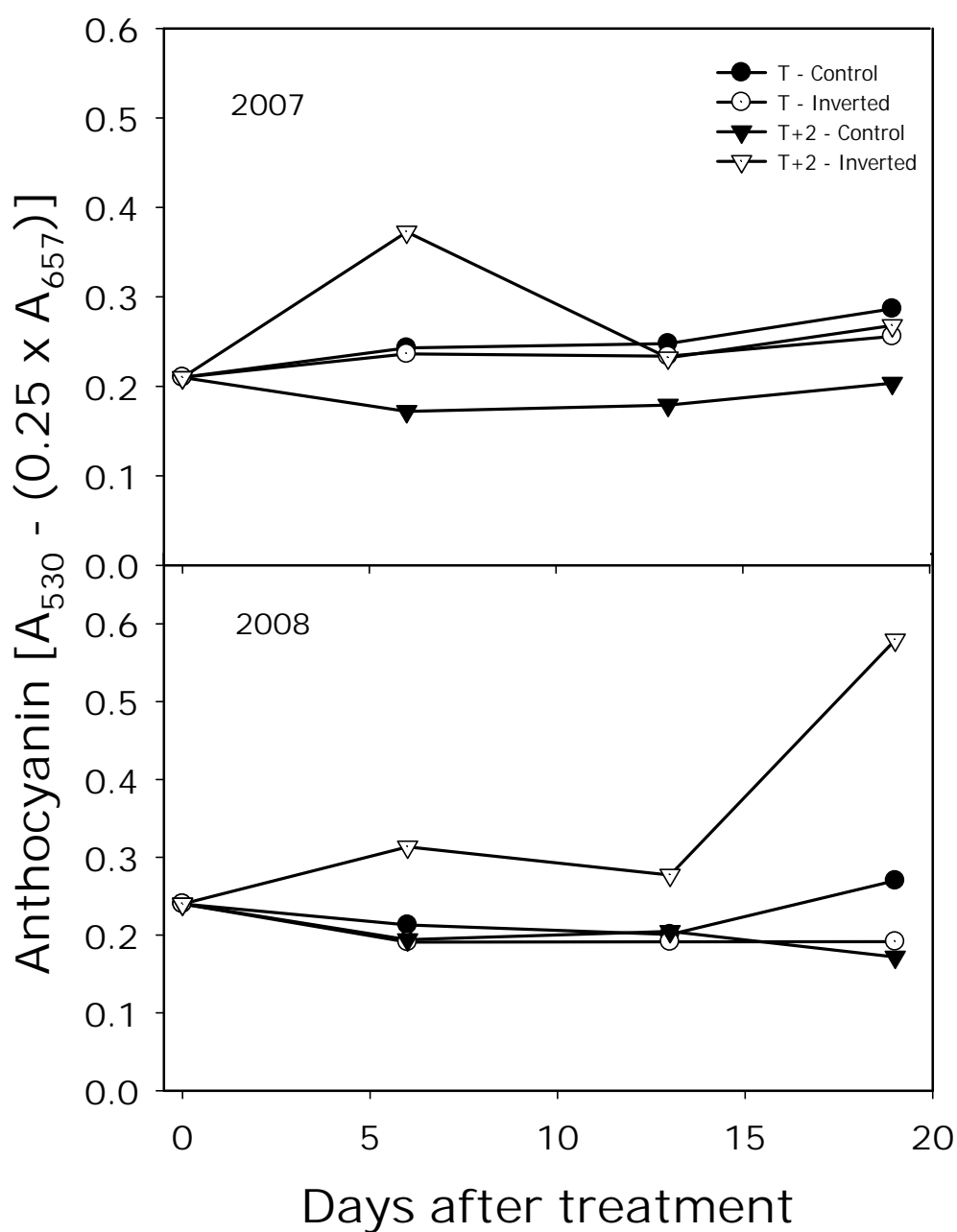
**Figure 1.** Inverted main stem cotton leaf both with (A) and without the plastic frame used to facilitate positioning.

he found a similar negative curvilinear relationship between anthocyanin and chlorophyll. This observation is supported by the decline in chlorophyll to anthocyanin ratio in the T+2 leaf in both years (Figure 5). Wells (2001) reported that the ratio of chlorophyll to anthocyanin generally tracked the patterns in chlorophyll. Gould (2004) called anthocyanin “nature’s Swiss army knife” because it serves multiple roles in plant protection and may, in some instances, be critical for plant survival. The masking of chlorophyll by anthocyanins from harmful radiation has been proposed numerous times (Feild et al., 2001; Steyn et al. 2002), but never empirically tested. The mechanism is confounded by the fact that anthocyanins are also powerful antioxidants (Rice-Evans et al., 1997; Wang et al., 1993). Neill and Gould (2003) proposed that higher incidence of anthocyanins in stress environment is the last line of defense against ROS and photoinhibition after all other mechanisms of protection (xanthophyll cycle and enzymatic antioxidants) have been exceeded.

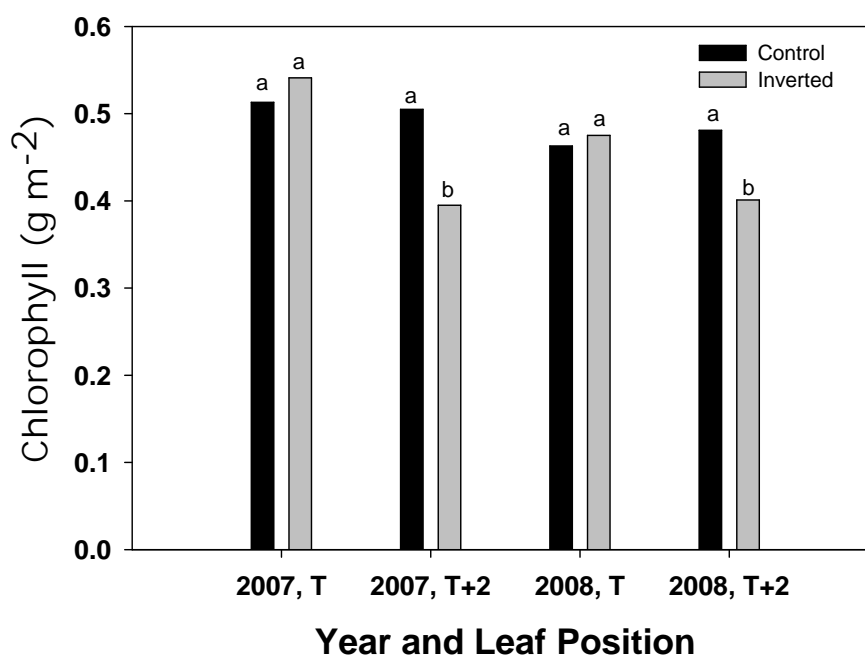
Hoch et al. (2003) theorized that anthocyanins facilitate tree leaf foliar nutrient resorption during senescence by protecting photosynthetic tissues from excess light. Using wild type and anthocyanin-deficient mutants of three deciduous woody species, they found wild type plants maintained higher photochemical efficiencies than mutants and were able to recover more easily from the effects of a high light, low temperature environment. Based on these reports it is possible that the anthocyanin increase in the inverted leaves is induced as a photoprotectant from light directed at tissue that is normally unexposed. It is also possible that

**Table 2.** Treatment means for leaf area, chlorophyll (Chl), anthocyanin (Ant), fresh, dry and specific leaf weight (SLW). Values are averaged for two years.

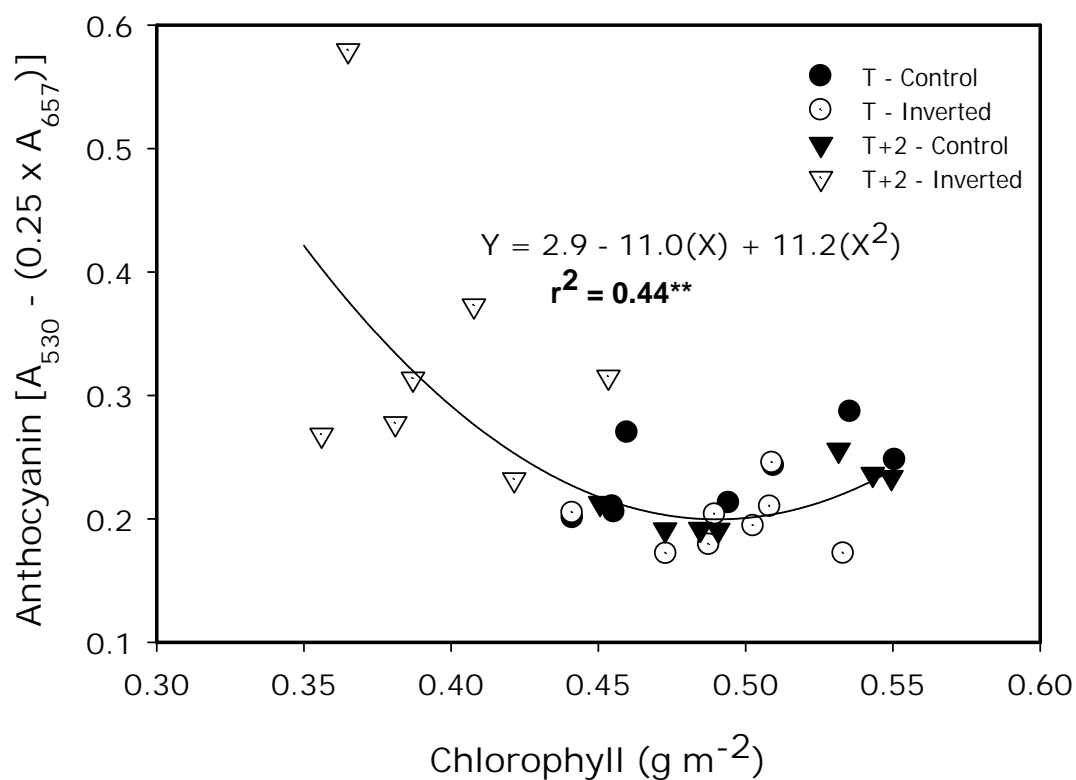
Factor	Leaf Position	Leaf Area cm <sup>2</sup>	Ant cm <sup>-2</sup>	Total Chl g m <sup>-2</sup>	Fresh Weight g	Dry Weight g	SLW g m <sup>-2</sup>
Control	Leaf T	80	0.23	14.64	2.31	0.70	0.0082
	Leaf T+2	110	0.19	14.81	3.17	0.75	0.0066
Inverted	Leaf T	79	0.22	15.11	2.33	0.66	0.0080
	Leaf T+2	114	0.32	11.96	3.28	0.77	0.0065



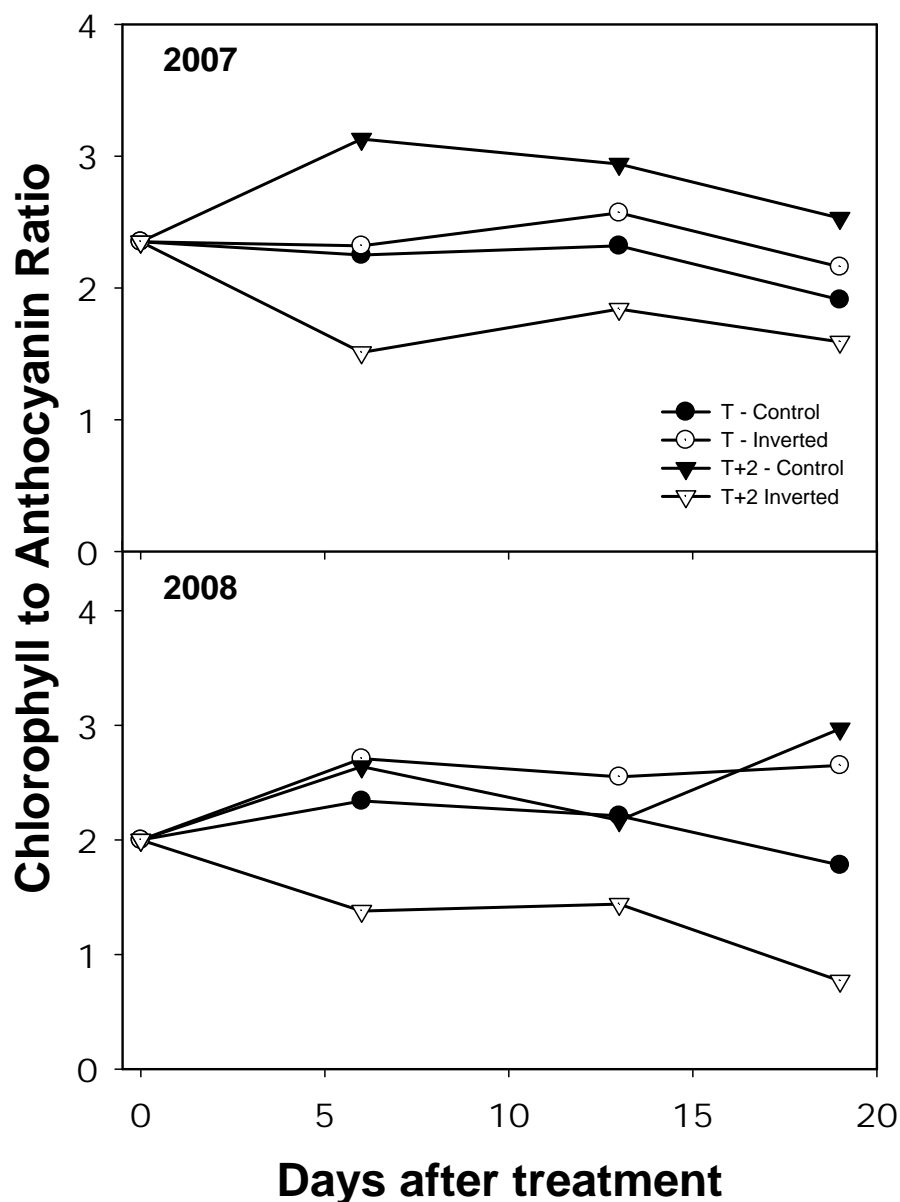
**Figure 2.** Anthocyanin levels of control and inverted main stem cotton leaves at various days after treatment for leaves at two canopy positions (T and T+2) in 2007 and 2008.



**Figure 3.** Chlorophyll concentrations of control and inverted main stem cotton leaves at two canopy positions (T and T+2) in 2007 and 2008. Values are means across harvest dates.



**Figure 4.** The relationship between anthocyanin and chlorophyll. Each symbol represents mean values of four replications within treatment, harvest date, and year.



**Figure 5.** Chlorophyll to anthocyanin ratios of control and inverted main stem cotton leaves at various days after treatment for leaves at two canopy positions (T and T+2) in 2007 and 2008.

anthocyanin plays a similar photoprotectant role as that in deciduous woody species during the period of cutout and prior to the next cycle of reproductive development. This idea is supported by the abatement of main stem anthocyanin synthesis in cotton plants that have had two weeks of flowers removed (Wells, 2001).

The difference in anthocyanin production and chlorophyll loss due to leaf position suggests that there is a leaf age component to the responses. Younger leaves may not have an enhanced capacity for anthocyanin production or may not require it due to the lack of

chlorophyll degradation. It's possible that the younger leaves could better manage the abnormal radiation exposure and anthocyanin production was not required.

Anecdotally, red color development is observed in only older leaves of field-grown plants late in the season.

### Conclusion

Abnormal exposure of lower leaf surface, which normally never receives direct sunlight, caused reduction in

chlorophyll content and increase in anthocyanin content. Anthocyanin was synthesized in response to light exposure possibly for its role in photo-protection and quenching of reactive oxygen species. Albeit a response of individual leaves exposed to an abnormal situation, the reddening of cotton approaching cutout may play a photo-protective role during the period of transition from one cycle of reproductive growth to renewed growth of a subsequent cycle.

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