WEED RESEARCH An International Journal of Weed Biology, Ecology and Vegetation Management

DOI: 10.1111/j.1365-3180.2012.00924.x

Comparison of physiological and antioxidant responses of *Anoda cristata* and cotton to progressive drought

HH RATNAYAKA*, WT MOLIN† & TM STERLING‡

*Department of Biology, Xavier University of Louisiana, New Orleans, LA, USA, †Southern Weed Science Research Unit, Stoneville, MS, USA, and ‡Land Resources and Environmental Sciences, Montana State University, Bozeman, MT, USA

Received 4 August 2011
Revised version accepted 3 April 2012
Subject Editor: Frank Forcella, USDA-ARS, USA

Summary

Knowledge about physiological responses of weed and crop to drought is needed to minimise crop losses caused by weed interference and to improve cropping systems and crop cultivars. In this study, gas exchange, photochemistry and antioxidant defences of two *Anoda cristata* (spurred anoda) accessions from Mississippi and New Mexico and two cotton types (*Gossypium hirsutum* and *Gossypium barbadense*) were evaluated under unstressed conditions and progressive drought. Under no stress, net photosynthesis (P_{net}) and quantum yield (Φ_{PSII}) were similar, but stomatal conductance (g_s) was higher in *A. cristata* than cotton. Glutathione reductase activity was highest in *A. cristata* from Mississippi. Cotton had at least 92% greater α-tocopherol concentration than *A. cristata*. Progressive drought that suppressed gas exchange to near zero decreased biomass and Φ_{PSII}, but increased xanthophyll cycle conversion state in both *A. cristata* and cotton. *Anoda cristata* had prolonged gas exchange at lower leaf water contents than cotton. Air-to-leaf temperature difference was at least 66% greater in *A. cristata*, while α-tocopherol concentration increased only in cotton under progressive drought. *Anoda cristata* appears to use patchy stomatal closure and alternative electron sinks to minimise light damage during severe drought. Furthermore, sustained water-holding capacity and assimilation helped by prolonged stomatal activity, larger pigment pools and lower leaf temperature in *A. cristata* may confer stress tolerance and consequent success in cotton – *A. cristata* interference.

Keywords: spurred anoda, photosynthesis, gas exchange, photochemistry, stress tolerance, oxidative stress, stomatal activity, leaf temperature.

Ratnayaka HH, Molin WT & Sterling TM (2012). Comparison of physiological and antioxidant responses of *Anoda cristata* and cotton to progressive drought. *Weed Research* **52**, 358–366.

Introduction

Anoda cristata (L.) Schlecht. (spurred anoda) was once competitive and difficult to manage in cotton (*Gossypium hirsutum* L. and *Gossypium barbadense* L.) (Arle & Hamilton, 1973; Patterson *et al.*, 1988) and was listed among the 10 most important cotton weeds in the United States (Dowler, 1992). While the use of herbicides, especially glyphosate, has lessened its impact and occurrence in most cotton farms in the United States, it is still a serious problem in other crops/countries (Marzocca, 1976; Puricelli *et al.*, 2003). Furthermore, there are regions where herbicide applications are not feasible, because of reasons such as economics, restrictions and resistance concerns, especially in weeds with persistent seed banks as with *A. cristata* (Puricelli *et al.*, 2002). Thus, studies on mechanisms of stress tolerance of weed and crop continue to be important in the development of cropping systems and crop cultivars that minimise losses because of weeds without the use of herbicides.

Correspondence: HH Ratnayaka, Department of Biology, Xavier University of Louisiana, 1 Drexel Drive, New Orleans, LA 70125, USA.
Tel: (+1) 504 520 5709; Fax: (+1) 504 520 7918; E-mail: hratnaya@xula.edu

Reduced productivity in cotton under *A. cristata* interference is in part due to deprivation of water, caused by interspecific rather than intraspecific interference (Ratnayaka *et al.*, 2003). Like cotton, *A. cristata* is in the *Malvaceae* and has a similar growth habit. Moreover, it has several clearly identifiable biotypes/accessions. Thus, it offers a promising model to understand how drought stress is initiated and tolerated at the physiological level in cotton–weed interactions. Insight into photochemical or stomatal limitations to net assimilation during different stages of a progressive drought may help identify means to mitigate these stress effects. Furthermore, because cultivars of cotton developed with minimum stomatal limitations to photosynthesis may be suitable only for irrigated cropping systems, biochemical limitations may represent effective foci when searching for drought-resistant genotypes (Hutmacher & Krieg, 1983). Thus, clues for developing stress-resistant crops may involve assessments of properties such as non-photochemical energy dissipation of PSII, antioxidants such as α-tocopherol and β-carotene, and enzymes that regulate the redox buffer system, such as catalase, ascorbate peroxidase and glutathione reductase (GR) (Kunert & Foyer, 1993; Secenji *et al.*, 2008), under both stressed and unstressed conditions, along with stomatal regulation.

Assimilation, PSII activity and antioxidant systems of *A. cristata* accessions from arid and humid regions have not been compared under irrigated conditions and progressive drought. The goal of this study was to identify physiological mechanisms of drought tolerance in both weed and cotton types, to help develop cropping systems strategies and drought- and weed-tolerant cotton cultivars. Thus, the hypotheses of this study are (i) constitutive levels of gas exchange, quantum yield and antioxidant systems of two *A. cristata* accessions and two cotton cultivars are different, and (ii) these four plant types will experience different limitations to assimilation and PSII activity and resort to different components of protective antioxidant systems during progressive drought. Changes in leaf bulk water status, relative water content **(**RWC**)**, was used as the reference for the degree of drought.

Materials and methods

Plant material and drought treatment

Seeds of New Mexico (NM, arid) and Mississippi (MS, humid) accessions of *A. cristata* were collected from Leyendecker Research Farm of New Mexico State University and USDA-ARS, Stoneville, MS, respectively. They were scarified by immersion in concentrated sulphuric acid (9.3 N) for 20 min (Solano *et al.*, 1976) and rinsed with water for 30 min before germination. *Anoda cristata* and cotton (Delta and Pine Land cv. 5415 [*G. hirsutum*] and cv. Pima S-7 [*G. barbadense*]) seeds were germinated on wet paper towels. Seedlings were first planted into 120-mL pots containing Terra-Lite Metro Mix 360 (W. R. Grace & Co., Memphis, TN, USA). Plants with the emerging first true leaf were transplanted into 8-L pots containing the same medium and grown in the glasshouse under natural day length in spring. Twelve plants of each *A. cristata* accession were maintained per each of six replications arranged in a randomised complete block design. Temperature was maintained at *c.* 30 and 18°C during day and night, respectively, and relative humidity was between 20 and 40%. Plants were watered daily and fertilised weekly with Technigro 20-18-18 fertiliser (Fisons Horticulture Inc., Warwick, NY, USA) until the progressive drought was imposed at 7 weeks after planting. Watering was withheld from six randomly selected plants of a given plant type in each replication, while the other six plants served as well-watered controls (watered uniformly over the pot to field capacity until dripping). Measurements were taken from a randomly selected water-stressed and control plant of a given plant type in each replication on each of the 6 days between drought initiation and wilt. The experiment was repeated in time.

Gas exchange, leaf temperature and quantum yield

Gas exchange variables and leaf temperature of the fourth leaf from the apex was recorded with an infrared gas analyser-based photosynthesis system (LI-6400, LI-COR Inc. Lincoln, NE, USA). All measurements were taken after net photosynthesis (P_{net}) and stomatal conductance (g_s) stabilised on the graphical display of the instrument's console. Measurement conditions were as follows: internal photon flux density 500 μmol m^{-2} s^{-1} to match the average light level at plant height in the glasshouse, flow rate 400 μmol s^{-1} and internal CO$_2$ concentration 400 μmol mol^{-1}. Quantum yield (Φ_{PSII}) was measured using the same leaf with OS5-FL modulated chlorophyll fluorometer (Opti-Sciences Inc., Tyngsboro, MA, USA). Minimal fluorescence (F'_{o}) was measured after a 3 s pulse of far-red light, and maximal fluorescence (F'_{m}) was measured after a saturation pulse of 0.8 s at 5000 μmol m^{-2} s^{-1}. The measuring beam was set at 360 μmol m^{-2} s^{-1}. Quantum yield was computed as $\left[\left(F'_{\mathrm{m}} - F'_{\mathrm{t}}\right)/F'_{\mathrm{m}}\right]$, where F'_{t} is the fluorescence immediately before saturation pulse.

Antioxidant enzyme activities and pigments

Leaf discs (0.6 cm diam) were taken using a paper hole-puncher from the same leaf used to measure $P_{\mathrm{net.}}$ Discs were frozen immediately on dry ice and stored at –20°C until used for biochemical assays. Ten leaf discs were homogenised in 700 μL of a solution containing 50 mM PIPES buffer, 6 mM cysteine hydrochloride, 10 mM D-isoascorbic acid, 1 mM EDTA, 1% polyvinylpyrrolidone (PVP-10), 0.3% triton X-100, pH 6.8, and centrifuged at 7000 ***g*** for 15 min at 4°C, as modified from Foster and Hess (1980). The supernatant was desalted and used for assaying total protein and enzyme activities (APX, ascorbate peroxidase; CAT, catalase; GR, glutathione reductase), as described previously (Bettmann *et al.*, 2006). Antioxidants and chlorophylls were assayed according to a method modified from Gilmore and Yamamoto (1991), as described previously (Bettmann *et al.*, 2006). Xanthophyll cycle conversion state was computed as the ratio of sum of zeaxanthin and antheraxanthin contents to sum of total xanthophyll content.

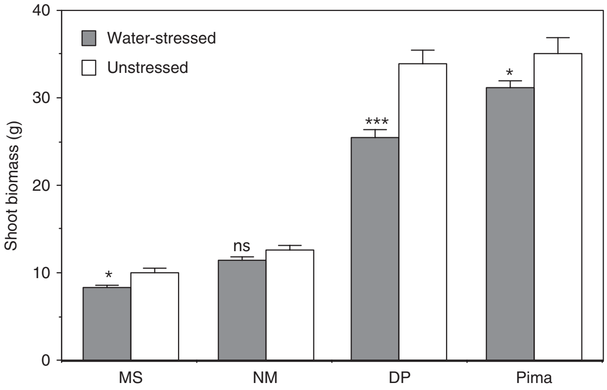

Fig. 1 Shoot biomass of the two *Anoda cristata* accessions (Mississippi and New Mexico) and two cotton cultivars (Delta Pine 5414 and Pima S-7) at the end of experiments. $n = 36$. Error bars are SE; ***, * and ns indicate significant difference at $P < 0.001$, 0.05 and not significant ($P > 0.05$), respectively, compared with unstressed plants within a given plant type. MS = Mississippi accession, NM = New Mexico accession, DP = Delta Pine 5414, Pima = Pima S-7.

Relative water content and biomass

Relative water content was measured using the fifth leaf from the apex as (FW-DW)/(TW-DW), where FW = fresh weight, DW = dry weight and TW = turgid weight (weight after the leaf was kept immersed in de-ionised water overnight). Total biomass of each water-stressed and well-watered plant used for measurements was determined by drying the total shoot mass at 70°C to a constant weight.

Statistical analysis

StatView and JMP (SAS Institute, 1998) were used to analyse the results of the response variables. In well-watered plants, species was considered a treatment. When treatment effects were significant in ANOVA, Fisher's protected LSD was used for mean comparison at $P < 0.05$. For effects of progressive drought in each plant type, inverse hyperbolic regressions were performed separately for each dependent variable vs. RWC. Data from the two experiments were pooled, because no response variable showed a significant interaction with the experiment.

Results

Response under well-watered conditions

Shoot biomass of *A. cristata* was the same in the two accessions (Fig. 1), but less than that of cotton. P_{net} was the same in all four plant types. However, g_s and transpiration (E) were 56% and 35% greater in MS-SA, and 25% and 24% greater in NM-SA, respectively, than either of the cotton cultivars. Similarly, intercellular CO_2 level (C_i) was greater in *A. cristata* than in cotton. Although electron transport rate between photosystem II and I was greater in cotton than in *A. cristata*, Φ_{PSII} was the same in all four plant types (Table 1). Each *A. cristata* accession had lower leaf temperature than cotton with MS-SA maintaining > 50% cooler leaves than either cotton cultivar (Fig. 2).

Chlorophyll concentration (a or $a + b$) in *A. cristata* was more than double that in each cotton cultivar, although the chlorophyll a/b ratio was the same in all four plant types (Table 2). Lutein concentration was 100% and 140% greater in MS accession, and 83% and 120% greater in NM-SA than Delta Pine 5414 and Pima S-7, respectively. MS-SA had 50% and 97% greater β-carotene than Delta Pine 5414 and Pima S-7, respectively, while NM-SA had 57% greater β-carotene content than Pima S-7 (Table 2). However, xanthophyll cycle conversion state was the same in MS-SA and Pima S-7, which was greater than Delta Pine 5414 or NM-SA. Among the antioxidant enzyme activities, GR was greater in *A. cristata*, especially in MS-SA (Table 3). CAT and APX activities in *A. cristata* were either same or lower compared with cotton.

Responses to progressive drought

Progressive drought that caused near complete suppression of gas exchange decreased biomass by 25% in Delta Pine 5414 ($P < 0.001$), 11% in Pima S-7 ($P < 0.05$), 16% in MS-SA ($P < 0.05$) and an insignificant 8% in NM-SA ($P = 0.1$) (Fig. 1). *Anoda cristata* maintained cooler leaves with 100% and 66% greater temperature differential with air temperature in MS-SA and NM-SA,

Table 1 Gas exchange and quantum yield characteristics of *Anoda cristata* (accessions, Mississippi and New Mexico) and cotton (cultivars, Delta Pine 5414 and Pima S-7) under well-watered conditions

Plant	P_{net} (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	C_i (μmol mol^{-1})	WUE (mmol mol^{-1})	Φ_{PSII} $\left[\left(F'_m - F'_t\right)/F'_m\right]$	ETR (μmol s^{-1})
A. cristata							
Mississippi	16.2b	0.50a	7.3a	294a	2.3c	0.71a	22.3c
New Mexico	16.7ab	0.40b	6.7b	276b	2.6b	0.70a	23.8c
Cotton							
Delta Pine 5415	17.2a	0.32c	5.4c	255c	3.2a	0.71a	30.2a
Pima S-7	17.1ab	0.32c	5.4c	258c	3.2a	0.71a	26.8b

Means followed by different letters in a column are statistically different ($P < 0.05$).

P_{net}, net photosynthesis.

g_s, stomatal conductance.

E, transpiration.

C_i, intercellular CO_2 concentration.

WUE, instantaneous water use efficiency.

Φ_{PSII}, quantum yield.

F'_m, maximum light-adapted fluorescence.

F'_t, fluorescence immediately before saturation pulse.

ETR, electron transport rate between photosystem II and I. $n = 72$.

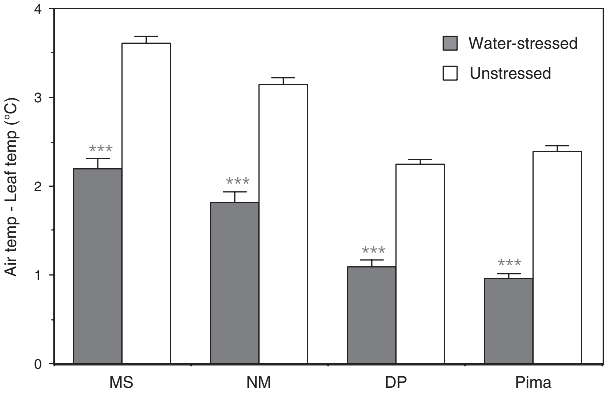

Fig. 2 Air-to-leaf temperature difference of the two *Anoda cristata* accessions (Mississippi and New Mexico) and two cotton cultivars (Delta Pine 5414 and Pima S-7). $n = 36$. Error bars are SE; ***indicate significant difference at $P < 0.001$, compared with another plant type under water stress. MS = Mississippi accession, NM = New Mexico accession, DP = Delta Pine 5414, Pima = Pima S-7.

respectively, than either cotton cultivar ($P < 0.001$) (Fig. 2). Smoothing splines of g_s vs. leaf RWC were associated with r^2 values ($P < 0.001$) ranging from 0.47 in NM-SA to 0.66 in MS-SA (Fig. 3). Although g_s decreased dramatically with declining RWC in all four plant types even during the relatively less severe phase of drought (RWC > 70%), the drop in *A. cristata* tended to be less abrupt compared with cotton. Similarly, the drops in P_{net} (Fig. 4) and E (data not presented) with declining RWC were more gradual in *A. cristata* than cotton. Furthermore, *A. cristata* leaves had higher and more stable FW/DW than cotton over a wide range of RWC (Fig. 5).

The drop in P_{net} towards zero in *A. cristata* occurred without as clear a drop in C_i as in cotton (Fig. 6). Furthermore, Φ_{PSII} (quantum yield) dropped at lower RWC compared with P_{net} in *A. cristata* (Figs 4 and 7). Xanthophyll cycle conversion state increased with

Table 2 Pigment concentrations and xanthophyll cycle conversion state [(Z + A)/(V + A + Z)] of *Anoda cristata* (accessions, Mississippi and New Mexico) and cotton (cultivars, Delta Pine 5414 and Pima S-7) under well-watered conditions

Plant	Chl. *a* (mg g^{-1} DW)	Chl. *a*/*b*	Lutein (HPLC peak area mg^{-1} DW)	β-Carotene (μg g^{-1} DW)	(Z + A)/(V + A + Z)
A. cristata					
Mississippi	24.5a	2.8a	2.4a	10 608a	0.18a
New Mexico	18.7a	2.2a	2.2a	8457b	0.13b
Cotton					
Delta Pine 5415	9.3b	2.3a	1.2b	7085bc	0.14b
Pima S-7	8.4b	2.2a	1.0b	5383c	0.18a

Means followed by different letters in a column are statistically different ($P < 0.05$).

Chl., chlorophyll.

Z, zeaxanthin.

A, antheraxanthin.

V, violaxanthin. $n = 72$.

Table 3 Antioxidant enzyme activities and α-tocopherol concentration of *Anoda cristata* (accessions, Mississippi and New Mexico) and cotton (cultivars, Delta Pine 5414 and Pima S-7) under well-watered conditions

Plant	CAT (Units mg^{-1} protein)	GR (Units mg^{-1} protein)	APX (Units mg^{-1} protein)	α-tocopherol (ng g^{-1} DW)
A. cristata				
Mississippi	31a	0.72a	0.07b	41c
New Mexico	16b	0.47b	0.08b	48c
Cotton				
Delta Pine 5415	23ab	0.35b	0.16a	92b
Pima S-7	22ab	0.31b	0.10ab	132a

Means followed by different letters in a column are statistically different ($P < 0.05$).
CAT, catalase.
GR, glutathione reductase.
APX, ascorbate peroxidase. $n = 72$.

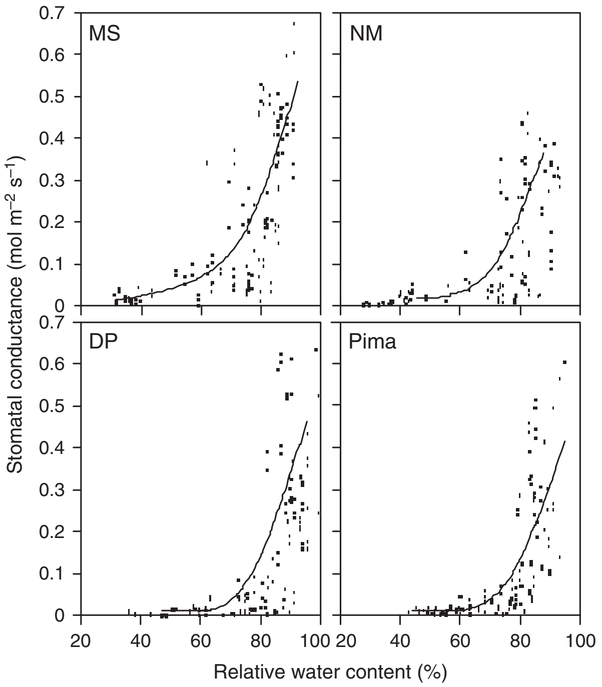

Fig. 3 Stomatal conductance (g_s) vs. relative water content (RWC) of the two *Anoda cristata* accessions (Mississippi and New Mexico) two cotton cultivars (Delta Pine 5414 and Pima S-7) with smoothing spline fits at $\lambda = 0.01$ during progressive drought. MS = Mississippi accession, $r^2 = 0.66$; NM = New Mexico accession, $r^2 = 0.47$; DP = Delta Pine 5414, $r^2 = 0.56$; Pima = Pima S-7, $r^2 = 0.59$.

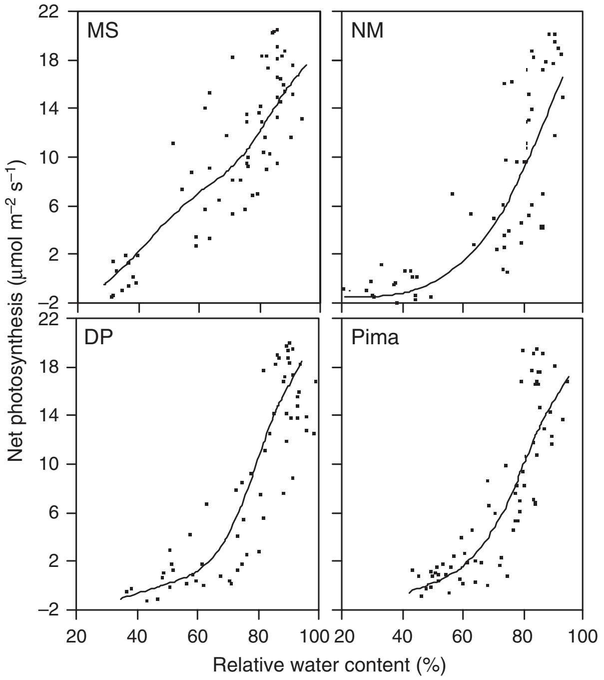

Fig. 4 Net photosynthesis (P_{net}) vs. relative water content (RWC) of the two *Anoda cristata* accessions (Mississippi and New Mexico) two cotton cultivars (Delta Pine 5414 and Pima S-7) with smoothing spline fits at $\lambda = 0.01$ during progressive drought. MS = Mississippi accession, $r^2 = 0.67$; NM = New Mexico accession, $r^2 = 0.70$; DP = Delta Pine 5414, $r^2 = 0.69$; Pima = Pima S-7, $r^2 = 0.68$.

decreasing RWC in all plant types. However, this trend started only at *c.* 50% of leaf RWC in *A. cristata* but at a relatively earlier phase of drought in cotton (Fig. 8). As shown in Fig. 9, α-tocopherol concentration was constitutively lower in *A. cristata* compared with cotton, and it remained unchanged in relation to RWC, especially in MS-SA, during progressive drought. However, α-tocopherol level increased in cotton especially in Pima, during the more severe phase of drought.

Discussion

Response under well-watered conditions

Although P_{net} and Φ_{PSII} were similar in *A. cristata* and cotton, the underlying mechanisms sustaining CO_2 assimilation were different. For instance, *A. cristata* had a greater stomatal activity, indicated by greater g_s, E and C_i than cotton to support the same rate of carbon gain. Greater stomatal activity caused cooler leaves with high CO_2 concentrations, conditions needed to control photorespiration, in *A. cristata* compared with cotton. However, *A. cristata* with greater E will deplete soil moisture in cotton stands more quickly than cotton intraspecific interference alone. Lower E caused greater instantaneous water use efficiency in either cotton type compared with any *A. cristata* accession.

Anoda cristata accessions possess more pigment-rich antennae complexes. In the field, both MS-SA and NM-SA stand higher than cotton canopy at maturity.

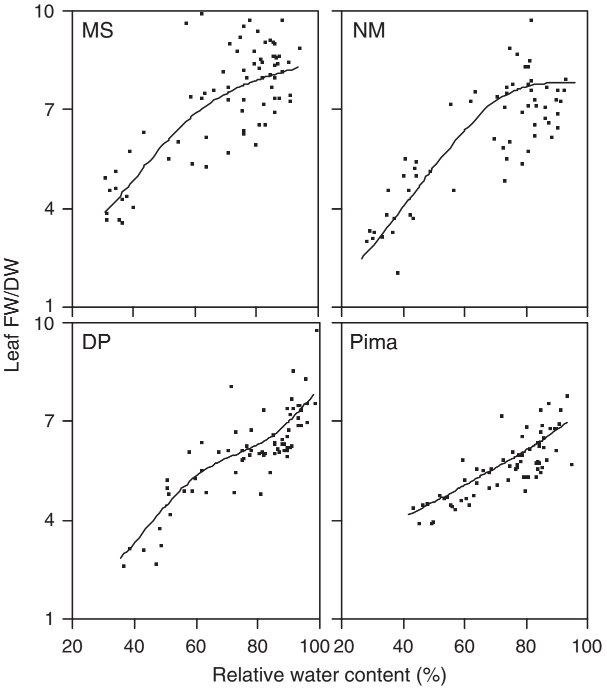

Fig. 5 Leaf fresh weight/dry weight ratio (FW/DW) vs. relative water content (RWC) of the two *Anoda cristata* accessions (Mississippi and New Mexico) two cotton cultivars (Delta Pine 5414 and Pima S-7) with smoothing spline fits at $\lambda = 0.01$ during progressive drought. MS = Mississippi accession, $r^2 = 0.64$; NM = New Mexico accession, $r^2 = 0.77$; DP = Delta Pine 5414, $r^2 = 0.74$; Pima = Pima S-7, $r^2 = 0.66$.

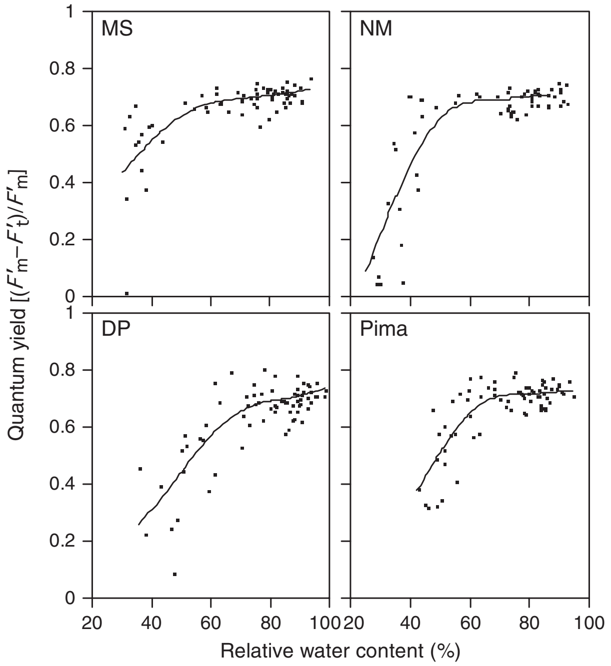

Fig. 7 Quantum yield (Φ_{PSII}) vs. relative water content (RWC) of the two *Anoda cristata* accessions (Mississippi and New Mexico) two cotton cultivars (Delta Pine 5414 and Pima S-7) with smoothing spline fits at $\lambda = 0.01$ during progressive drought. MS = Mississippi accession, $r^2 = 0.56$; NM = New Mexico accession, $r^2 = 0.75$; DP = Delta Pine 5414, $r^2 = 0.65$; Pima = Pima S-7, $r^2 = 0.58$.

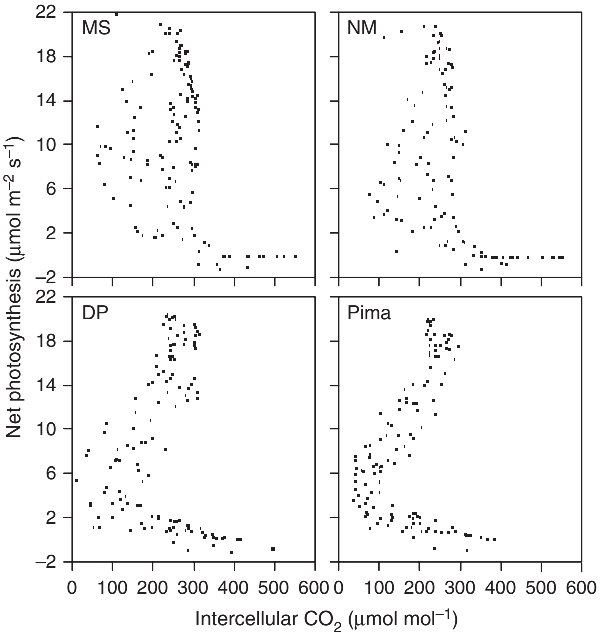

Fig. 6 Association between net photosynthesis (P_{net}) and intercellular CO_2 concentration (C_i) in the two *Anoda cristata* accessions (Mississippi and New Mexico) and two cotton cultivars (Delta Pine 5414 and Pima S-7) during progressive drought. MS = Mississippi accession, NM = New Mexico accession, DP = Delta Pine 5414, Pima = Pima S-7.

Thus, greater pigment content, especially carotenoids including β-carotene and lutein, may be adaptive for avoiding photoinhibition in that high-light environment. Lutein, with its structural similarity to antheraxanthin, an intermediate of heat dissipating xanthophyll cycle, also has been found to be effective in de-excitation of singlet chlorophyll (*a* component of q_E, energy-dependent quenching of excitation energy) under high light (Pogson *et al.*, 1998).

Although GR activities were greater in *A. cristata* than cotton, the difference between NM-SA and either cotton cultivar was insignificant in this study. However, NM-SA had significantly greater GR activity than either Delta Pine 5414 or Pima S-7 cotton in a previous study (Ratnayaka *et al.*, 2003). Higher constitutive levels of GR activities suggest that *A. cristata* maintains higher concentration and rate of metabolism of reduced glutathione, GSH. GSH is a crucial defence metabolite with multiple roles, such as an antioxidant, an intermediate of ascorbate-glutathione cycle and an integral component of the cellular redox balance (Kunert & Foyer, 1993). GSH is also a coenzyme in reactions including DNA synthesis and a signal molecule in gene expression needed for enhanced and sustained defence against environmental stress (Foyer & Halliwell, 1976), another

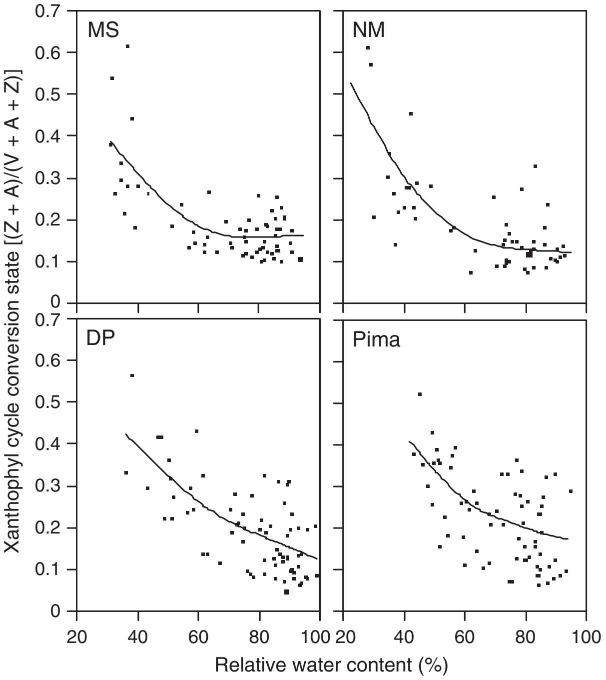

Fig. 8 Xanthophyll cycle conversion state $[(Z + A)/(V + A + Z)]$ vs. relative water content (RWC) of the two *Anoda cristata* accessions (Mississippi and New Mexico) two cotton cultivars (Delta Pine 5414 and Pima S-7) with smoothing spline fits at $\lambda = 0.01$ during progressive drought. MS = Mississippi accession, $r^2 = 0.56$; NM = New Mexico accession, $r^2 = 0.33$; DP = Delta Pine 5414, $r^2 = 0.47$; Pima = Pima S-7, $r^2 = 0.36$.

adaptation to high light above the crop canopy. Furthermore, GSH is involved in detoxification of many herbicides and other xenobiotics (Rennenberg, 1982; Alscher, 1989), which may provide *A. cristata* with an adaptive advantage in agroecosystems where herbicide use is common.

Responses to progressive drought

The more gradual drop in g_s in *A. cristata*, particularly during the relatively more severe phase of drought (RWC < 70%), indicated prolonged stomatal activity compared with cotton. Thus, *A. cristata* maintained the water potential gradient needed to continually uptake and transport water to leaf mesophyll. More stable and higher FW/DW, indicative of more effective mesophyll water-holding capacity in *A. cristata* compared with cotton over a wide range of plant water status, also suggests the presence of such a mechanism. Maintenance of leaf water potential during drought, close to unstressed levels, was found to be competitive in the C_4 weed, *Amaranthus retroflexus* L., by likely causing it to be taller than the crop (Valerio *et al.*, 2011), similar to *A. cristata* in cotton fields.

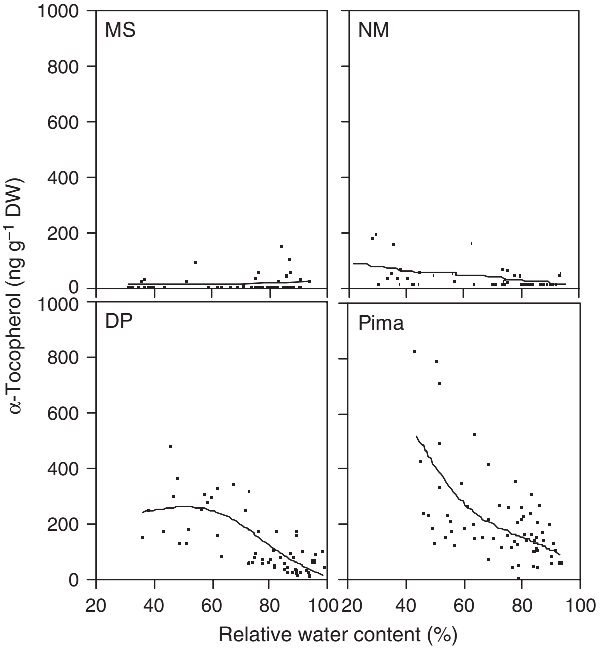

Fig. 9 Concentration of α-tocopherol vs. relative water content (RWC) of the two *Anoda cristata* accessions (Mississippi and New Mexico) two cotton cultivars (Delta Pine 5414 and Pima S-7) with smoothing spline fits at $\lambda = 0.01$ during progressive drought. MS = Mississippi accession, $r^2 = 0.04$; NM = New Mexico accession, $r^2 = 0.16$; DP = Delta Pine 5414, $r^2 = 0.50$; Pima = Pima S-7, $r^2 = 0.40$.

Continued stomatal activity in *A. cristata* also caused greater C_i and lower leaf temperature. However, the discrepancy between patterns of decline in Φ_{PSII} and P_{net} in *A. cristata* suggests that alternative sinks of reducing power, such as photorespiration, also operate under stress to minimise photoinhibition. Thus, the more prolonged P_{net} in *A. cristata* during the severe phase of drought probably occurred under a controlled rate of photorespiration, as well. However, this relatively longer carbon gain in *A. cristata* probably explains the lower percentage reduction in biomass, compared with cotton, during drought. Furthermore, high and variable values for C_i in *A. cristata* during the severe phase of drought suggest a possible heterogeneous stomatal closure (Flexas & Medrano, 2002). Mott and Peak (2007) suggested that stomatal patchiness, groups of stomata operating as locally connected functional units rather than acting individually, may be adaptive for optimising gas exchange under specific environmental conditions.

The effects of drought on the xanthophyll cycle conversion state suggest that violaxanthin deepoxidation-dependent q_E in *A. cristata* occurs during a more severe phase of drought compared with cotton. Thus, thermal energy dissipation through q_E appears to be an important mechanism of prolonging carbon gain in *A. cristata*, and therefore stomatal activity, only under severe drought stress. Havaux *et al.* (2000) showed that α-tocopherol level increases when xanthophyll cycle-dependent energy dissipation is unavailable (mutants). α-Tocopherol is an antioxidant that prevents the formation of $^1O_2$ and lipid peroxy radicals preventing lipid peroxidation and consequent damage to chloroplast membranes (Fryer, 1992; Pogson *et al.*, 1996). Increases in α-tocopherol levels in cotton but nearly unchanged levels in *A. cristata* suggest that energy dissipation through q_E, rather than α-tocopherol, is important in *A. cristata* during severe drought. Furthermore, *A. cristata* maintained its much higher levels of carotenoids and chlorophylls than cotton through the progressive drought (data not presented). Larger pigment pools in *A. cristata*, compared with cotton, also may help sustain the photosystem structure without damage and prolong assimilation during severe stress.

Furthermore, although APX activity, but not CAT or GR, increased in Delta Pine 5114, Pima S-7 and NM-SA under a temporary mild drought (Ratnayaka *et al.*, 2003), no changes in these enzymes were found in this study. The antioxidant enzyme response to water deficit depends on age, severity of stress, degree of ABA biosynthesis, species and cultivar (Tanaka *et al.*, 1990; Mittler & Zilinskas, 1992, 1994; Iturbe-Ormaetxe *et al.*, 1998; Bellaire *et al.*, 2000; Jiang & Zhang, 2001). Oxidative stress tolerance only partly relies on enzyme activity and many factors other than the enzyme activity itself can influence substrate availability (Blokhina *et al.*, 2003). Iturbe-Ormaetxe *et al.* (1998) and Jiang and Huang (2001) reported that decreased enzyme activities under prolonged drought were accompanied by reduced pigment levels, which was also absent in this study. Furthermore, Tausz *et al.* (2004) reported that energy dissipation mechanisms, such as xanthophyll cycle conversion, decrease the oxidative load on the ascorbate-glutathione cycle, regulated by enzymes such as APX and GR.

Conclusions

Different stomatal and assimilatory activities were found to protect photosystems and sustain carbon gain, conferring drought stress tolerance in *A. cristata*, compared with cotton. Although concentrations of antenna pigments were greater in *A. cristata*, photosystems do not seem to be adapted for maximising the generation of reducing power for high assimilation rate, but for maintaining a moderate rate of electron flow and delay photoinhibition during stress. Continuous stomatal activity in *A. cristata*, probably helped by patchy closure, appears to maintain carbon and hydraulic fluxes and low leaf temperature that prolong photosynthesis during severe drought. Antioxidative defence by greater carotenoid concentration may help *A. cristata* survive stress with lower α-tocopherol levels compared with cotton. Our findings show that *A. cristata* may continue to extract soil moisture in a severe drought beyond the tolerance limits of cotton. *Anoda cristata* population threshold counts should therefore be determined depending on the severity of drought expected in the cotton cropping system. High pigment content and low leaf temperature appear promising criteria of cotton cultivar selection for improved weed-competitiveness against *A. cristata*, especially during drought. Furthermore, investigations on whether stress responses observed in *A. cristata* in this research represent common adaptations to drought stress in other weeds may also be useful in cotton-weed management.

Acknowledgements

Authors wish to thank Efren Chavez, Greg Bettmann and Amber Vallotton for technical assistance. This research was supported by the New Mexico State Agricultural Experiment Station, New Mexico State University, and by the USDA/CSREES National Research Initiative Competitive Grants Program (Grant No. 99-35315-7727).

References

Alscher RG (1989) Biosynthesis and antioxidant function of glutathione in plants. *Physiologia Plantarum* **77,** 457–464.

Arle HF & Hamilton KC (1973) Effect of annual weeds on furrow-irrigated cotton. *Weed Science* **21,** 325–327.

Bellaire BA, Carmody J, Braud J *et al.* (2000) Involvement of abscisic acid-dependent and -independent pathways in the up-regulation of antioxidant enzyme activity during NaCl stress in cotton callus tissue. *Free Radical Research* **33,** 531–545.

Bettmann GT, Ratnayaka HH, Molin WT & Sterling TM (2006) Physiological and antioxidant responses of cotton and spurred anoda (*Anoda cristata*) under nitrogen deficiency. *Weed Science* **54,** 641–650.

Blokhina O, Virolainen E & Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany* **91,** 179–194.

Dowler CC (1992) Weed survey-southern states. *Proceedings of Southern Weed Science Society* (January 20–22, 1992, Little Rock, AR, USA) **45,** 392–407.

Flexas J & Medrano H (2002) Drought-inhibition of photosynthesis in C_3 plants:stomatal and nonstomatal limitations revisited. *Annals of Botany* **89,** 183–189.

Foster JG & Hess JL (1980) Responses of superoxide dismutase and glutathione reductase activities in cotton leaf tissue exposed to an atmosphere enriched in oxygen. *Plant Physiology* **66,** 482–487.

Foyer CH & Halliwell B (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbate acid metabolism. *Planta* **133,** 21–25.

FRYER M (1992) The antioxidant effects of thylakoid vitamin E (α-tocopherol). *Plant, Cell and Environment* **15,** 381–392.

GILMORE AM & YAMAMOTO HY (1991) Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C_{18} high-performance liquid chromatographic column. *Journal of Chromatography* **543,** 137–145.

HAVAUX M, BONFILS J-P, LÜTZ C & NIYOGI KK (2000) Photodamage of the photosynthetic apparatus and its dependence on the leaf developmental stage in the *npq1 Arabidopsis* mutant deficient in the xanthophyll cycle enzyme violaxanthin de-epoxidase. *Plant Physiology* **124,** 273–284.

HUTMACHER RB & KRIEG DR (1983) Photosynthetic rate control in cotton, stomatal and nonstomatal factors. *Plant Physiology* **73,** 658–661.

ITURBE-ORMAETXE I, ESCUREDO PR, ARRESE-IGOR C & BECANA M (1998) Oxidative damage in pea plants exposed to water deficit or paraquat. *Plant Physiology* **116,** 173–181.

JIANG Y & HUANG B (2001) Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolism and lipid peroxidation. *Crop Science* **41,** 436–442.

JIANG M & ZHANG J (2001) Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. *Plant and Cell Physiology* **42,** 1265–1273.

KUNERT KJ & FOYER CH (1993) Thiol/disulphide exchange in plants. In: *Sulfur Nutrition and Assimilation in Higher Plants. Regulatory, Agricultural and Environmental Aspects* (eds LJ DE KOK, I STULEN, H RENNENBERG, C BRUNHOLD & W RAUSEN), 139–151. SPB Academic, The Hague, the Netherlands.

MARZOCCA A (1976) *Manual de Malezas*, 3rd edn. 580, Hemisferio Sur, University of Texas, Austin, TX, USA.

MITTLER R & ZILINSKAS BA (1992) Molecular cloning and characterization of a gene encoding pea cytosolic ascorbate peroxidase. *Journal of Biological Chemistry* **267,** 21802–21807.

MITTLER R & ZILINSKAS BA (1994) Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. *Plant Journal* **5,** 397–405.

MOTT KA & PEAK D (2007) Stomatal patchiness and task-performing networks. *Annals of Botany* **99,** 219–226.

PATTERSON DT, HIGHSMITH MT & FLINT EP (1988) Effects of temperature and CO_2 concentration on the growth of cotton (*Gossypium hirsutum*), spurred anoda (*Anoda cristata*), and velvetleaf (*Abutilon theophrasti*). *Weed Science* **36,** 751–757.

POGSON B, MCDONALD KA, TRUONG M, BRITTON G & DELLAPENNA D (1996) *Arabidopsis* carotenoid mutants demonstrate that lutein is not essential for photosynthesis in higher plants. *The Plant Cell* **8,** 1627–1639.

POGSON BJ, NIYOGI KK, BJÖRKMAN O & DELLAPENNA D (1998) Altered xanthophyll compositions adversely affect chlorophyll accumulation and nonphotochemical quenching in *Arabidopsis* mutants. *Proceedings of National Academy of Sciences,* October 27, 1998, **95,** 13324–13329. DOI: 10.1073/pnas.95.22.13324

PURICELLI EC, ORIOLI G & SABBATINI MR (2002) Demography of *Anoda cristata* in wide- and narrow-row soyabean. *Weed Research* **42,** 456–464.

PURICELLI EC, FACCINI DE, ORIOLI GA & SABBATINI MR (2003) Spurred anoda competition in narrow- and wide-row soybean (*Glycine max*). *Weed Technology* **17,** 446–451.

RATNAYAKA HH, MOLIN WT & STERLING TM (2003) Physiological and antioxidant responses of cotton and spurred anoda under interference and mild drought. *Journal of Experimental Botany* **54,** 2293–2305.

RENNENBERG H (1982) Glutathione metabolism and possible biological roles in higher plants. *Phytochemistry* **21,** 2778–2781.

SAS INSTITUTE (1998) *StatView (Ver. 5.0) and JMP (Ver. 2.0.1)*. SAS Institute, Cary, NC, USA.

SECENJI M, BEBES A, HIDEG É & GYÖRGYEY J (2008) Transcriptional changes in ascorbate-glutathione cycle under drought conditions. *Acta Biologica Szegediensis* **52,** 93–94.

SOLANO F, SCHRADER JW & COBLE HD (1976) Germination, growth and development of spurred anoda. *Weed Science* **24,** 574–578.

TANAKA K, MASUDA R, SUGIMOTO T, OMASA K & SAKAKI T (1990) Water deficiency-induced changes I n the contents of defensive substances against active oxygen in spinach leaves. *Agricultural Biology and Chemistry* **54,** 2629–2634.

TAUSZ M, SIRCELJ H & GRILL G (2004) The glutathione system as a stress marker in plant ecophysiology: is a stress-response concept valid? *Journal of Experimental Botany* **55,** 1955–1962.

VALERIO M, TOMECEK MB, LOVELLI S & ZISKA L (2011) Quantifying the effect of drought on carbon dioxide-induced changes in competition between a C_3 crop (tomato) and a C_4 weed (*Amaranthus retroflexus*). *Weed Research* **51,** 591–600.