

# Willow species (genus: *Salix*) with contrasting habitat affinities differ in their photoprotective responses to water stress

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**Abstract.** Although many Mediterranean and xeric plant species enhance their xanthophyll-mediated thermal dissipation under drought conditions, there has been limited research on photoprotective mechanism in droughted plants from other habitats. To investigate whether wetland plants utilise this mechanism under drought conditions, and whether species differ in their responses depending on their habitat affinities, we investigated the response of six willow (*Salix*) species to a short-term drought. In a greenhouse, 40 individuals per species were dried down over 4 weeks. Periodically during the drought, predawn and midday chlorophyll fluorescence measurements were taken and leaf discs were collected for pigment analysis with HPLC. Predawn water potential was also monitored throughout the experiment. All six species increased xanthophyll cycle activity and their capacity to dissipate excess energy during the drought by increasing their total de-epoxidised xanthophyll concentration and the concentration of zeaxanthin in proportion to chlorophyll. In general, habitat generalists had greater photoprotective responses than wetland specialists, while the wetland specialists had higher pre-drought nonphotochemical quenching. These differences are consistent with their contrasting photosynthetic rates. The observed variation in species drought responses suggests that their photoprotective strategies vary with habitat affinity.

**Additional keywords:** nonphotochemical quenching, wetlands, xanthophyll cycle.

## Introduction

Many plant species segregate along soil moisture and larger-scale precipitation gradients based on their drought tolerance, as a result of trade-offs in physiological and life history traits that prevent them from performing well under all environmental conditions (Whittaker 1956; Brodribb and Hill 1999; Silvertown *et al.* 1999; Cavender-Bares *et al.* 2004). Although there has been substantial research investigating the role of xylem trade-offs in determining species distributions (Zimmermann and Brown 1977; Pockman and Sperry 2000; Maherali *et al.* 2004; Hacke *et al.* 2006), there has been less research on potential trade-offs relating to species photoprotective mechanisms. These mechanisms are important in preventing irreversible damage to chloroplast under drought conditions (Demmig *et al.* 1988; Flexas and Medrano 2002), and may play a role in determining species distributions across soil moisture gradients.

Under drought conditions, plants close their stomata, resulting in a build-up of carbon dioxide inside the leaf, and a reduction in photosynthetic activity. This results in an excess of absorbed light that can create reactive oxygen species and lead to chloroplast and photosystem damage. Plants utilise four different mechanisms to

prevent photodamage and safely dissipate excess energy: the xanthophyll cycle (Demmig *et al.* 1987; Adams and Demmig-Adams 1994), photorespiration (Osmond and Grace 1995; Kozaki and Takeba 1996), Mehler reactions (Osmond and Grace 1995; Biehler and Fock 1996) and cyclic electron transport (Katona *et al.* 1992). Of these four mechanisms, the xanthophyll cycle is responsible for dissipating the majority of excess energy under drought conditions (Flexas and Medrano 2002; Demmig-Adams and Adams 2006). It is also used by plants to dissipate energy under high light, low nutrient availability, and during exposure to freezing temperatures (Adams and Demmig-Adams 1992; Demmig-Adams *et al.* 1995; Lovelock *et al.* 1995; Verhoeven *et al.* 1999; Cavender-Bares *et al.* 2005).

There is substantial evidence that Mediterranean (e.g. García-Plazaola and Becerril 2000; Kyparissis *et al.* 2000; Martínez-Ferri *et al.* 2000; Galmés *et al.* 2007) and xeric plant species (e.g. Balaguer *et al.* 2002; Barker *et al.* 2002) increase xanthophyll mediated thermal dissipation under drought conditions and that tropical species utilise these processes under high light conditions (e.g. Lovelock *et al.* 1994; Barker *et al.* 1997; Watling *et al.* 1997; Montgomery *et al.* 2008).

However, there has been limited research on the photoprotective responses of mesic and hydric species to drought conditions. Since photodamage is dependent on stomatal behaviour, not necessarily the severity of a drought, these species may benefit from photoprotective mechanisms if they experience prolonged stomatal closure under high light conditions (Bota *et al.* 2004; Flexas *et al.* 2006). Furthermore, if there is a cost associated with increasing xanthophyll concentrations and maintaining xanthophyll cycle activity, species may vary in their photoprotective capacity depending on the frequency and longevity of water stress they encounter in their native habitats.

To address the importance of xanthophyll mediated thermal dissipation in plants that occur in habitats with different seasonal water availability, we examined the drought responses of six willow species (genus: *Salix*) to a 4 week dry-down. Since willows are highly dependent on water availability (Amlin and Rood 2002; Karrenberg *et al.* 2002), and exhibit significant variation in habitat affinities along a soil moisture gradient (Morley 1969; Gleason and Cronquist 1991), they are an excellent system for this study. The goal of this study was to address two key questions:

- (1) do drought intolerant willow species exhibit enhanced xanthophyll mediated energy dissipation under drought conditions? and
- (2) do species photoprotective responses depend on their ecological habitat of origin?

## Materials and methods

### Species selection

We selected six willow species (genus: *Salix*) native to Minnesota for our study, including three wetland specialists and three broader habitat generalists, which we classified based on habitat descriptions from Morley (1969) and Gleason and Cronquist (1991). The three wetland species (*Salix candida* Flueggé ex Willd., *Salix pedicellaris* Pursh, and *Salix pyrifolia* Andersson) primarily grow in fens, bogs and wet meadows in Minnesota, with *S. candida* inhabiting more alkaline wetlands and *S. pyrifolia* inhabiting more acidic wetlands. The three broader habitat generalists (*Salix bebbiana* Sarg., *Salix discolor* Muhl., *Salix petiolaris* Sm.) occur in a variety of habitats including prairies, moist meadows, alluvial habitats and lakeshores. They tend to occur in habitats that have more seasonal variation in water availability than the three wetland species.

### Growth and dry-down conditions

In the spring of 2004, we propagated six native willow species from seed collected in south-eastern Minnesota at the Cedar Creek Ecosystem Science Reserve. We grew the willows in a greenhouse at the University of Minnesota, which was set to be 20°C year-round and achieved temperatures of 27°C on warm summer days. The plants were kept well watered and fertilised for 2 years. By the summer of 2006, plant height and stem diameter (averaged across 10 individuals per species) were 89.3 ± 3.15 cm (one standard error) and 7.60 ± 0.27 mm (s.e.), respectively. Three weeks before the start of the experiment, we transplanted the plants into 6.25-Le treepots. At this point we

also measured the total leaf area of a subset of eight plants per species.

We began the dry-down treatment in June 2006. Plants were watered to field capacity and allowed to dry out over 4 weeks. We took measurements on six individuals per species, at three points in the experiment: pre-drought (day 0), mid-drought (day 15) and late drought (day 30). Plants were at field capacity during the pre-drought measurements. Plants were illuminated for 12 h per day giving a midday light intensity of ~700–800 μmol on sunny days.

### Predawn water potential measurements

We measured leaf water potential ( $\psi$ ) using a Scholander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) 2 h before dawn each day of our measurements. We removed the leaves with razor blades, put them in plastic bags, and immediately transferred them to the pressure chamber.

### Chlorophyll fluorescence and gas exchange measurements

We measured chlorophyll fluorescence on dark- and light-acclimated leaves for six individuals per species, using a pulse amplitude modulated chlorophyll fluorescence meter (LI-COR 6400-40, Li-Cor Inc., Lincoln, NE, USA). We measured minimal dark-adapted fluorescence ( $F_o$ ), and maximum dark-adapted fluorescence ( $F_m$ ) (using a saturating pulse of 7000 μmol m<sup>-2</sup> s<sup>-1</sup> for 0.8 s), in the 2-h period before dawn on one leaf per plant. We marked the spot on the leaf where the measurement was taken and measured steady-state fluorescence ( $F_s$ ), and maximum fluorescence ( $F_m'$ ) on the same spot on illuminated leaves between 1300 and 1500 hours the same day. A far-red pulse (740 nm) was then applied to excite PSI and thereby oxidised PSII reaction centers for a measurement of  $F_o'$ . During the afternoon measurements, we also measured CO<sub>2</sub> assimilation at a light intensity of 1200 μmol m<sup>-2</sup> s<sup>-1</sup> ( $A_{1200}$ ). We selected this light intensity because it was greater than ambient light levels and is known to saturate photosynthesis in willows (Robinson *et al.* 2004). We measured the first fully-expanded, living leaf on the main stem of each plant but were unable to follow the same leaves throughout the experiment because of leaf senescence.

For our analysis, we calculated: maximum photochemical efficiency of PSII,  $F_v/F_m$  (where  $F_v = F_m - F_o$ ); light-acclimated photochemical efficiency,  $\Delta F/F_m'$  (where  $\Delta F = F_m' - F_s$ ); electron transport, ETR (assuming a leaf absorbance of 0.8 and equal photon excitation of PSII and PSI); photochemical quenching,  $qP$   $[(F_m' - F_s)/(F_m' - F_o)]$ ; nonphotochemical quenching,  $qN$   $[(F_m - F_m')/(F_m - F_o)]$  (Schreiber *et al.* 1986); and Stern-Volmer nonphotochemical quenching,  $NPQ$   $[(F_m - F_m')/F_m']$ .

### Pigment analysis

Immediately after taking each chlorophyll fluorescence measurement, we punched a leaf disc from a nearby leaf with an 8-mm diameter core borer. The discs were put in microcentrifuge tubes and dropped into liquid nitrogen. These samples were kept in a -80°C freezer until they were used for pigment analysis. Pigments were extracted according to Adams and Demmig-Adams (1992). Samples were analysed by HPLC

using an Allsphere ODS-1 (5  $\mu\text{m}$  particle size, 250  $\times$  4.6 mm) column (Alltech Chromatography, Deerfield, IL, USA). Solvents and method used are as described by Gilmore and Yamamoto (1991), however, midway through the analysis of samples the peaks began running together, so the A solvent was adjusted. The two A solvents both consisted of acetonitrile : methanol : 0.1 M Tris, pH 8.0 with a ratio of 78 : 8 : 3 for the first A solvent and 72 : 17 : 5 for the second A solvent. Adjusting the solvent altered the retention time of both chlorophylls, which were calibrated separately for each solvent. We calculated pigment concentrations on an area basis and the de-epoxidation state of the xanthophylls (DPS) as  $(Z + A)/(V + A + Z)$ .

### Statistics

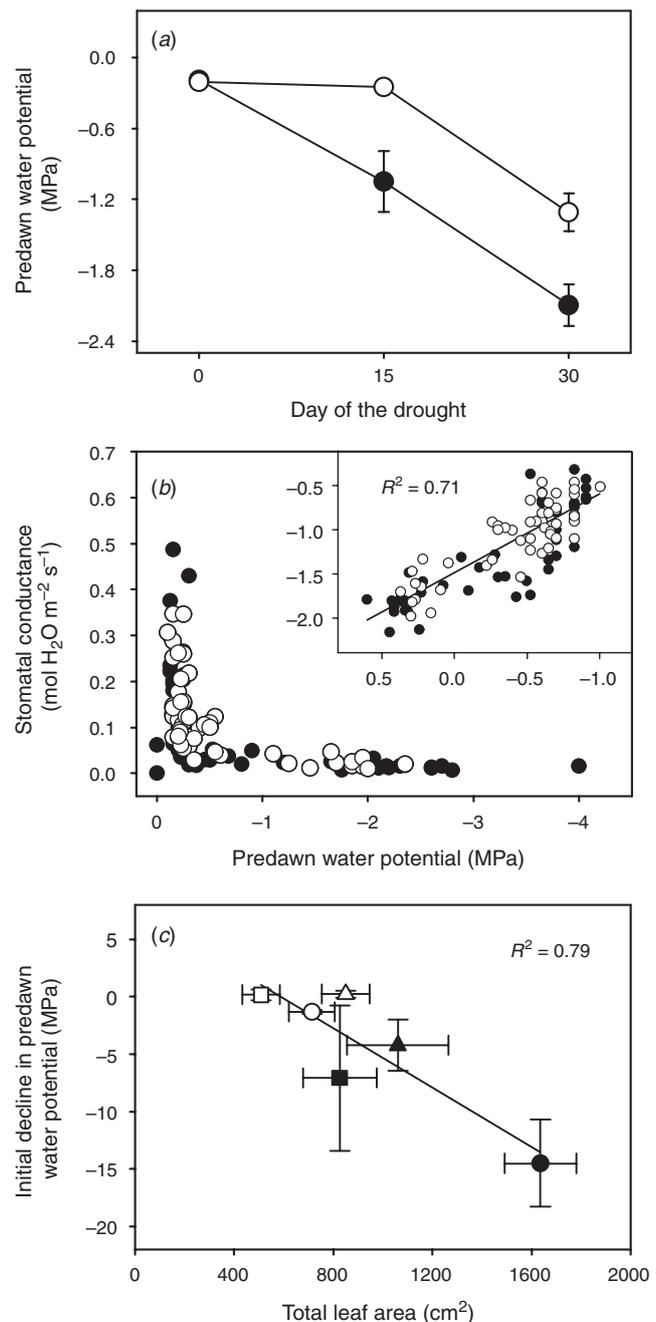
We used repeated-measures ANOVA to analyse pigment, chlorophyll fluorescence and gas exchange measurements overtime across species. Since there were only two individuals of *S. discolor* remaining on the last day of measurement, we presented the ANOVA analysis excluding this species. However, when we conducted the analyses including *S. discolor*, there were no qualitative differences in the results (data not shown). We used a *t*-test to examine the differences between habitat generalists and wetland specialists at each time interval (days 0, 15 and 30). We also used Tukey's multiple comparisons to check for differences between species within the same habitat classification. To investigate differences in species stomatal conductance, we completed a multiple regression analysis on the relationship between stomatal conductance and predawn water potential. Since stomatal conductance is non-linearly related to predawn water potential, we first logged the two axes. We also completed a regression analysis on species total leaf area and their decline in predawn water potential from day 0 to day 15. *P*-values <0.05 were considered significant and values <0.1 were considered slightly significant. All analyses were conducted with JMP 7.0 (SAS Institute, Raleigh, NC, USA).

## Results

### Progression of dry-down

Predawn water potential significantly decreased over time ( $F$ -ratio = 56.5, d.f. = 2, 18,  $P < 0.0001$ , Fig. 1a) during the dry-down (Fig. 1a) and this decrease corresponded with a decrease in stomatal conductance in all the species ( $F$ -ratio = 118, d.f. = 97,  $P < 0.0001$ , Fig. 1b). Across species, there was also a correlation between the decline in water potential from day 0 to 15 and their total leaf area (Fig. 1c,  $F$ -ratio = 14.7, d.f. = 5,  $P = 0.019$ ). By mid-drought, the habitat generalists had significantly lower predawn water potentials than the wetland specialists (Fig. 1a), but there were no significant differences between species within the two groups. Late in the drought, the wetland specialists had achieved predawn water potentials equivalent to the mid-drought measurements of the habitat specialists. There was no habitat effect in the log-log regression of predawn water potential on stomatal conductance.

All six willow species demonstrated drought-induced senescence in response to the dry-down. In several species, the senescence occurred rapidly and multiple plants lost all their leaves by day 30. This resulted in sample sizes of 4, 6, 2, 4, 5 and 6 in the late drought for *S. bebbiana*, *S. candida*, *S. discolor*,



**Fig. 1.** (a) Habitat generalists ( $\bullet$ ) demonstrated a more rapid decline in predawn water potential (MPa) than wetland specialists ( $\circ$ ) during the drought. (b) Predawn water potential correlated with stomatal conductance ( $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ ) in both habitat generalists and wetland specialists. Each point represents measurements taken on one plant, one day during the drought. The inset graph is the log-log graph of the data. (c) The willow species with greater total leaf area ( $\text{cm}^2$ ) demonstrated the greater losses in predawn water potential (MPa) during the drought. Species are indicated by the following symbols:  $\blacktriangle$ , *Salix bebbiana*;  $\bullet$ , *S. discolor*;  $\blacksquare$ , *S. petiolaris*;  $\triangle$ , *S. candida*;  $\circ$ , *S. pedicellaris*;  $\square$ , *S. pyrifolia*. All error bars are  $\pm$  s.e.

*S. pedicellaris*, *S. petiolaris* and *S. pyrifolia*, respectively. After the experiment, we dried-down a subset of individuals from each species until they lost their leaves and then rewatered them.

Almost 60% of the plants resprouted after rewatering, but there was no difference between the resprouting ability of the habitat generalists and the wetland specialists (data not shown).

#### Carbon assimilation

All species demonstrated a significant decrease in light saturated carbon assimilation ( $A_{1200}$ ,  $F$ -ratio = 44.6, d.f. = 2,18,  $P < 0.0001$ ) and stomatal conductance ( $g$ ) over time ( $F$ -ratio = 39.3, d.f. = 2,18,  $P < 0.0001$ ). However, the habitat specialists closed their stomata more rapidly, resulting in significantly lower  $g$ , than the wetland specialists by the mid-drought. This resulted in lower  $A_{1200}$  in the habitat generalists despite their higher initial  $A_{1200}$  (Table 1).

#### Chlorophyll fluorescence

After the onset of the drought, photochemical quenching (qP,  $F$ -ratio = 16.8, d.f. = 2,18,  $P < 0.0001$ ), light quantum efficiency ( $\Delta F/F_m'$ ,  $F$ -ratio = 245.3, d.f. = 2,18,  $P < 0.0001$ ) and electron transport rates (ETR,  $F$ -ratio = 244.2, d.f. = 2,18,  $P < 0.0001$ ) declined in all species. Although habitat generalists had significantly higher ETR predrought, their ETR declined more rapidly than the wetland specialists (Table 1). A similar trend was observed in light quantum efficiency, as  $\Delta F/F_m'$  was higher in habitat generalists than wetland specialists on day 0 but lower on day 15 (Fig. 2c, d). Meanwhile, qP demonstrated no difference between the habitat groups during the drought (Table 1).

The dark quantum efficiency ( $F_v/F_m$ ) of PSII also significantly decreased during the drought ( $F$ -ratio = 79.6, d.f. = 2,18,  $P < 0.0001$ , Fig. 2a, b) but the decrease was small. The predrought average across species was  $0.823 \pm 0.002$  ( $\pm$  s.e.,  $n = 36$ ) and the late drought average was  $0.754 \pm 0.006$  ( $\pm$  s.e.,  $n = 26$ ). Habitat generalists had slightly higher  $F_v/F_m$  before the drought but there were no significant differences between the two groups after the onset of the drought (Fig. 2c, d).

Across all species, there was a significant increase in nonphotochemical quenching calculated as NPQ ( $F$ -ratio = 7.3, d.f. = 2,18,  $P = 0.005$ , Table 1) and as qN ( $F$ -ratio = 11.6, d.f. = 2,18,  $P = 0.0006$ , Fig. 2e, f). Pre-drought, the wetland specialists had significantly higher NPQ and qN than the habitat generalists and this difference remained until the mid-drought in regards to qN ( $\alpha = 0.5$ ). There were no significant differences in qN or NPQ between the species within each group.

#### Pigment analysis

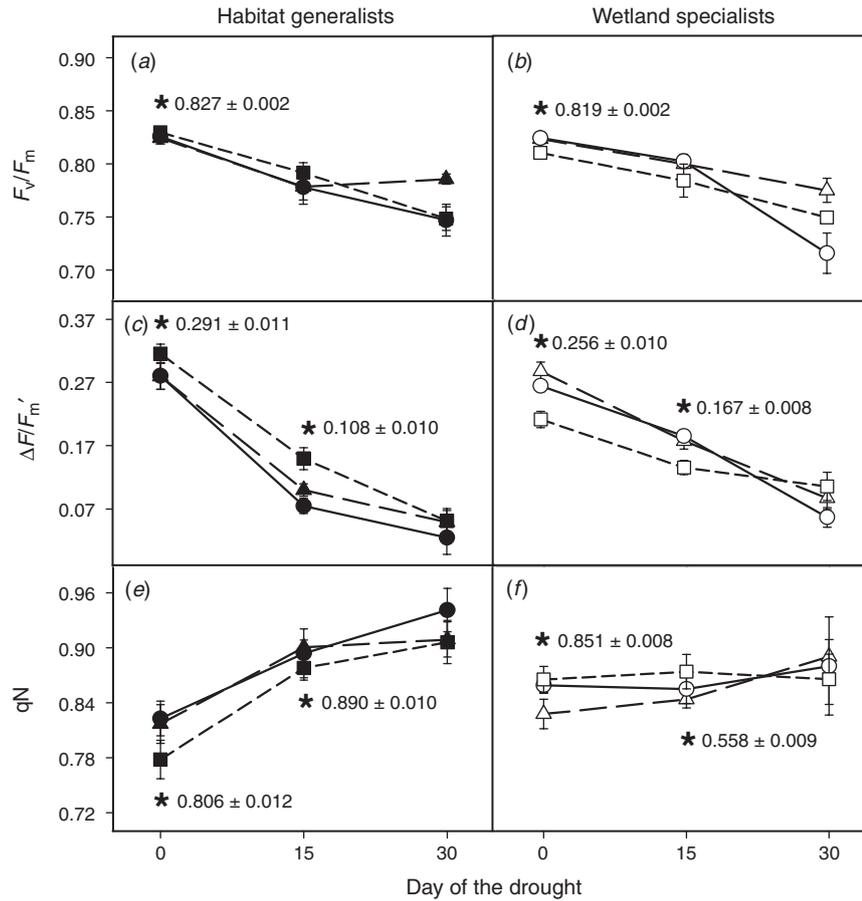
All species exhibited a decline in the measured leaf pigments ( $\alpha = 0.05$ ) during the drought and in the ratio of chlorophyll  $a/b$  (Table 2). Lutein, neoxanthin and chlorophyll were significantly different between habitat generalists and wetland specialists in the late drought. However, these differences disappeared when lutein and neoxanthin were considered in proportion to chlorophyll ( $\mu\text{mol pigment/mol chlorophyll}$ ). The ratio of  $\beta$ -carotene/chlorophyll did not change over time, and the ratio of lutein/chlorophyll increased during the drought ( $F$ -ratio = 14.8, d.f. = 2,18,  $P = 0.0002$ , Table 2). There was no evidence for the presence of lutein epoxide in any of the species.

Across all species, the total xanthophyll concentration (violaxanthin, antheraxanthin and zeaxanthin) in the willow leaves significantly changed over time ( $F$ -ratio = 9.5, d.f. = 2,18,  $P = 0.002$ , Fig. 3). Additionally, both the de-epoxidation state of the xanthophylls (DPS,  $F$ -ratio = 15.5, d.f. = 2,18,  $P = 0.0002$ , Fig. 3) and the ratio of zeaxanthin/chlorophyll significantly increased ( $F$ -ratio = 34.2, d.f. = 2,18,  $P < 0.0001$ , Fig. 4). The increase in DPS correlated linearly with an increase in qN (Fig. 5a) and NPQ (Table 1), and was greater habitat generalists than wetland specialists (Fig. 5b). Habitat generalists also had significantly higher ratios of zeaxanthin/chlorophyll and violaxanthin/chlorophyll (Fig. 4) during the mid-drought. However, the ratio of antheraxanthin/

**Table 1. Average chlorophyll fluorescence and gas exchange parameters of six different willow species during the dry down**

The parameter abbreviations are as follows: electron transport chain (ETR), photochemical quenching (qP), nonphotochemical quenching (NPQ), maximum photosynthetic capacity ( $A_{1200}$ ) in  $\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , and stomatal conductance ( $g$ ) in  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ .  $P$ -values are from  $t$ -tests comparing habitat generalist and wetland specialists for each parameter. Values are reported  $\pm$  s.e.; NS, not significant ( $P > 0.05$ )

	Day	Habitat generalists				Wetland specialists		$P$ -value
		<i>S. bebbiana</i>	<i>S. discolor</i>	<i>S. petiolaris</i>	<i>S. candida</i>	<i>S. pedicellaris</i>	<i>S. pyrifolia</i>	
ETR	0	143 $\pm$ 10.7	143 $\pm$ 10.7	161 $\pm$ 7.67	147 $\pm$ 7.38	136 $\pm$ 4.55	108 $\pm$ 6.50	0.02
	15	51.0 $\pm$ 5.04	38.1 $\pm$ 6.07	76.3 $\pm$ 8.85	91.0 $\pm$ 6.59	95.1 $\pm$ 3.96	69.7 $\pm$ 5.72	<0.0001
	30	37.6 $\pm$ 4.64	37.7 $\pm$ 11.9	39.3 $\pm$ 4.98	44.9 $\pm$ 10.1	35.5 $\pm$ 6.54	65.3 $\pm$ 1.43	NS
qP	0	0.68 $\pm$ 0.04	0.68 $\pm$ 0.03	0.63 $\pm$ 0.04	0.75 $\pm$ 0.03	0.89 $\pm$ 0.07	0.84 $\pm$ 0.12	0.006
	15	0.74 $\pm$ 0.16	0.26 $\pm$ 0.16	0.75 $\pm$ 0.21	0.66 $\pm$ 0.07	1.00 $\pm$ 0.27	0.83 $\pm$ 0.29	NS
	30	0.22 $\pm$ 0.14	0.00 $\pm$ 0.00	0.27 $\pm$ 0.27	0.37 $\pm$ 0.13	0.06 $\pm$ 0.06	0.46 $\pm$ 0.20	NS
NPQ	0	2.29 $\pm$ 0.17	2.33 $\pm$ 0.17	1.92 $\pm$ 0.18	2.41 $\pm$ 0.18	2.94 $\pm$ 0.14	2.81 $\pm$ 0.21	0.001
	15	3.19 $\pm$ 0.21	3.30 $\pm$ 0.43	3.00 $\pm$ 0.23	2.58 $\pm$ 0.12	2.82 $\pm$ 0.28	3.10 $\pm$ 0.33	NS
	30	3.82 $\pm$ 0.57	3.35 $\pm$ 0.00	2.84 $\pm$ 0.37	3.19 $\pm$ 0.34	3.19 $\pm$ 0.57	2.73 $\pm$ 0.23	NS
$A_{1200}$	0	13.52 $\pm$ 2.40	15.9 $\pm$ 1.96	17.1 $\pm$ 1.64	13.9 $\pm$ 1.22	14.0 $\pm$ 1.16	8.89 $\pm$ 1.13	0.03
	15	0.07 $\pm$ 0.27	-0.47 $\pm$ 0.35	2.31 $\pm$ 0.83	5.54 $\pm$ 0.93	6.26 $\pm$ 1.06	4.18 $\pm$ 1.04	<0.0001
	30	0.76 $\pm$ 0.56	-0.10 $\pm$ 0.43	0.81 $\pm$ 0.16	2.00 $\pm$ 1.21	1.66 $\pm$ 0.74	2.46 $\pm$ 0.98	0.01
$g$	0	0.20 $\pm$ 0.05	0.24 $\pm$ 0.04	0.25 $\pm$ 0.05	0.18 $\pm$ 0.02	0.25 $\pm$ 0.04	0.13 $\pm$ 0.03	NS
	15	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.04 $\pm$ 0.01	0.09 $\pm$ 0.01	0.17 $\pm$ 0.04	0.07 $\pm$ 0.01	<0.0001
	30	0.02 $\pm$ 0.01	0.01 $\pm$ 0.00	0.02 $\pm$ 0.01	0.04 $\pm$ 0.02	0.05 $\pm$ 0.02	0.05 $\pm$ 0.02	0.002

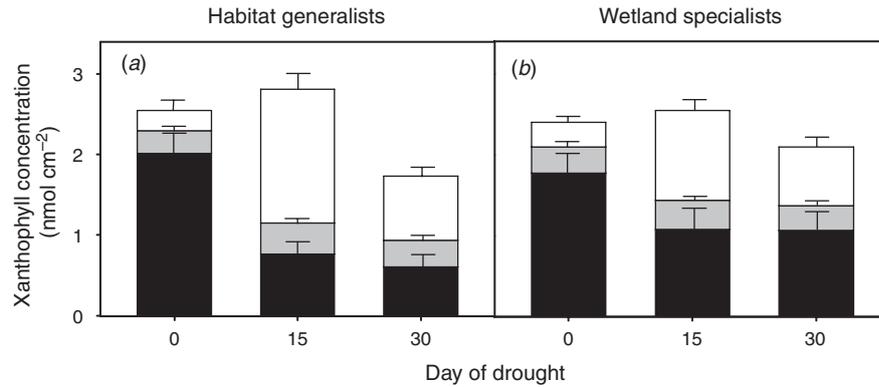


**Fig. 2.** (a, b) Maximum photochemical efficiency of PSII,  $F_v/F_m$ , (c, d) light-acclimated photochemical efficiency,  $\Delta F/F_m'$ , (e, f) and nonphotochemical quenching,  $q_N$ , differed between wetland specialists (open symbols) and habitat generalists (closed symbols) during the drought. Error bars are  $\pm$  s.e.; \* indicates where the habitat generalists significantly differ from the wetland specialists ( $\alpha=0.05$ ). The reported values are the species averages  $\pm$  s.e. Species symbols are the same as Fig. 1.

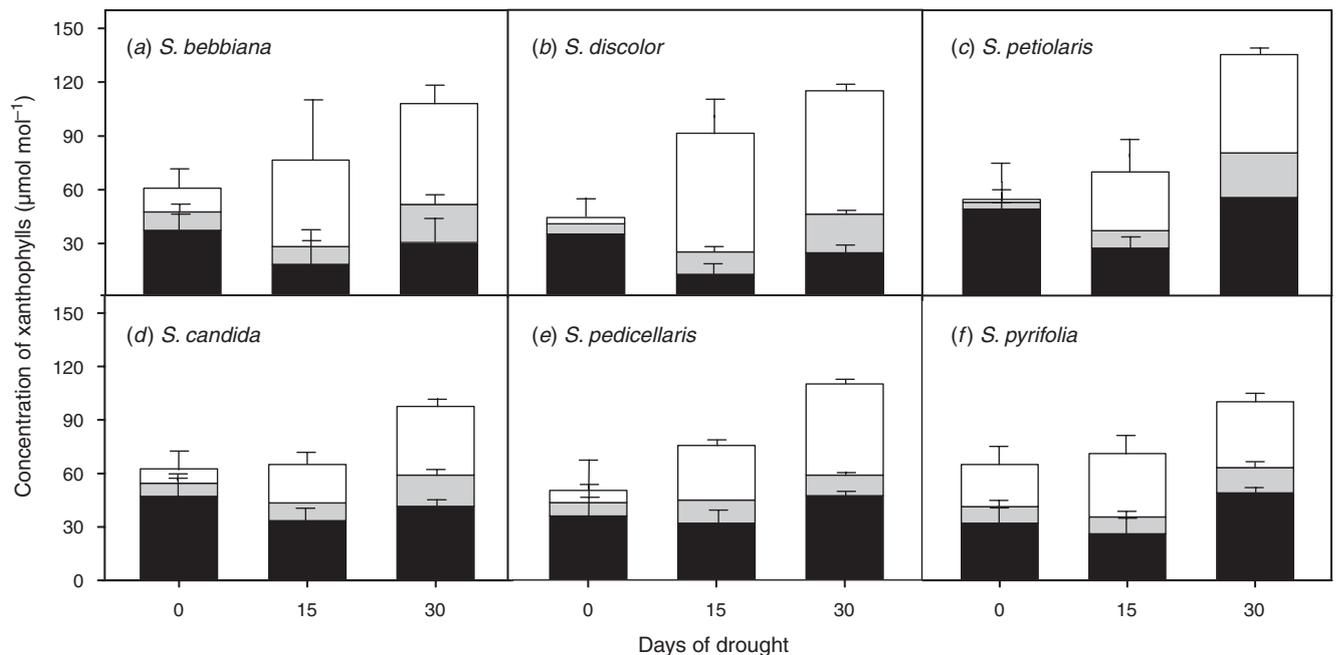
**Table 2.** Average leaf pigment concentrations of six different willow species during the dry down

Pigments are indicated by the following abbreviations: neoxanthin (neo), lutein (lut) and  $\beta$ -carotene ( $\beta$ -c). There were no significant differences between habitat generalists and wetland specialists in these pigment concentrations. Pigments that significantly ( $\alpha=0.01$ ) changed during the drought based on a repeated-measures ANOVA where  $n=26$  are marked, \*. Values are reported  $\pm$  s.e.

	Day	Habitat generalists			Wetland specialists		
		<i>S. bebbiana</i>	<i>S. discolor</i>	<i>S. petiolaris</i>	<i>S. candida</i>	<i>S. pedicellaris</i>	<i>S. pyrifolia</i>
neo/chl ( $\mu\text{mol mol}^{-1}$ )	0	34.0 $\pm$ 16.5	33.0 $\pm$ 5.16	35.5 $\pm$ 8.05	40.4 $\pm$ 7.63	38.0 $\pm$ 13.8	33.8 $\pm$ 5.81
	15	41.4 $\pm$ 3.50	36.2 $\pm$ 5.60	40.0 $\pm$ 3.44	35.2 $\pm$ 9.76	43.7 $\pm$ 6.61	42.3 $\pm$ 9.86
	30	53.8 $\pm$ 15.3	51.0 $\pm$ 9.01	59.7 $\pm$ 13.2	46.1 $\pm$ 4.52	59.5 $\pm$ 27.4	55.1 $\pm$ 17.0
lut/chl* ( $\mu\text{mol mol}^{-1}$ )	0	116 $\pm$ 19.5	85.7 $\pm$ 9.11	91.8 $\pm$ 15.7	86.3 $\pm$ 17.7	98.9 $\pm$ 14.6	101 $\pm$ 13.7
	15	125 $\pm$ 11.0	119 $\pm$ 6.96	111 $\pm$ 16.3	103 $\pm$ 13.1	124 $\pm$ 34.7	121 $\pm$ 9.85
	30	131 $\pm$ 24.1	121 $\pm$ 10.4	170 $\pm$ 35.7	139 $\pm$ 52.1	159 $\pm$ 53.5	154 $\pm$ 38.5
$\beta$ -c/chl ( $\mu\text{mol mol}^{-1}$ )	0	94.9 $\pm$ 8.16	79.2 $\pm$ 5.09	89.6 $\pm$ 7.84	87.5 $\pm$ 4.00	84.6 $\pm$ 5.29	87.7 $\pm$ 2.34
	15	89.5 $\pm$ 3.43	87.8 $\pm$ 3.67	74.1 $\pm$ 3.52	82.4 $\pm$ 7.18	82.3 $\pm$ 4.54	81.2 $\pm$ 11.6
	30	92.4 $\pm$ 7.50	73.7 $\pm$ 2.2	103 $\pm$ 11.7	91.2 $\pm$ 16.4	81.7 $\pm$ 12.3	88.5 $\pm$ 6.33
chl* ( $\text{nmol cm}^{-2}$ )	0	36.6 $\pm$ 6.90	57.3 $\pm$ 7.51	55.9 $\pm$ 6.92	45.1 $\pm$ 4.09	49.0 $\pm$ 7.61	32.7 $\pm$ 4.05
	15	37.2 $\pm$ 7.67	34.4 $\pm$ 2.90	48.2 $\pm$ 5.97	47.6 $\pm$ 2.29	40.7 $\pm$ 7.32	29.6 $\pm$ 3.65
	30	13.7 $\pm$ 4.6	27.8 $\pm$ 8.25	17.2 $\pm$ 2.07	28.7 $\pm$ 4.60	27.8 $\pm$ 8.25	17.2 $\pm$ 2.07
chl a/b*	0	2.66 $\pm$ 0.20	2.77 $\pm$ 0.08	2.67 $\pm$ 0.38	2.78 $\pm$ 0.35	2.77 $\pm$ 0.12	3.18 $\pm$ 0.16
	15	3.32 $\pm$ 0.29	2.75 $\pm$ 0.17	3.44 $\pm$ 0.21	3.14 $\pm$ 0.18	3.38 $\pm$ 0.12	2.85 $\pm$ 0.18
	30	3.89 $\pm$ 0.40	3.55 $\pm$ 0.01	3.37 $\pm$ 0.14	3.63 $\pm$ 0.011	3.27 $\pm$ 0.17	2.91 $\pm$ 0.18



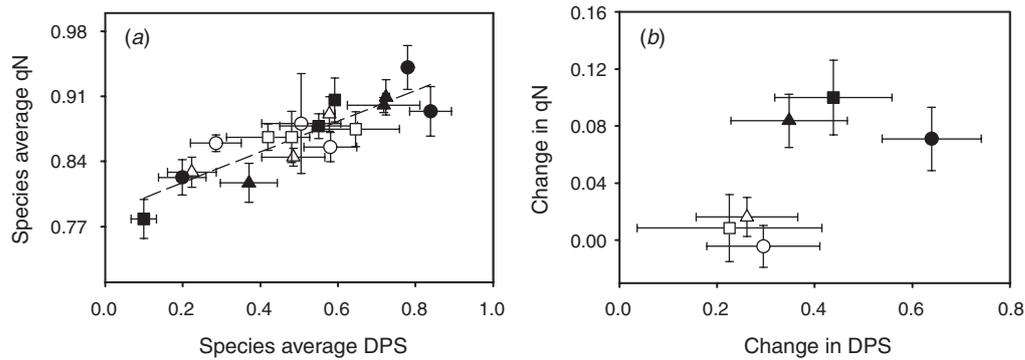
**Fig. 3.** The total de-epoxidation state of the xanthophylls increased during the drought in all species. There was no significant difference between the xanthophyll pool size ( $\text{nmol cm}^{-2}$ ) in the habitat generalists and the wetland specialists, although the generalists had slightly higher DPS values. The xanthophyll epoxidation states are labelled as follows: violaxanthin (black), antheraxanthin (grey) and zeaxanthin (white). Error bars are  $\pm$  s.e.



**Fig. 4.** The concentrations of violaxanthin, antheraxanthin and zeaxanthin measured as  $\mu\text{mol pigment per mol chlorophyll}$  changed during the drought in all species. (a–c) Zeaxanthin concentrations per unit chlorophyll were significantly higher in habitat generalist (d–f) than wetland specialists on day 15 ( $P=0.03$ ). The xanthophyll epoxidation states are marked the same as in Fig. 3. Error bars are  $\pm$  s.e.

chlorophyll was only significantly higher in the habitat generalists late in the drought ( $P=0.03$ , Fig. 4). The greater DPS values of the habitat generalists in both the mid- ( $P=0.07$ ) and late drought ( $P=0.08$ , Fig. 4) were slightly significant, indicating that the habitat generalists may have greater xanthophyll cycle activity than wetland specialists. This relationship was stronger when species were compared at similar predawn water potentials (habitat generalists, day 15 and wetland specialists, day 30) ( $P=0.02$ ).

When considered individually, the six willow species demonstrated distinct responses to the imposed drought treatment. While *S. discolor* exhibited the greatest increase in zeaxanthin both in absolute concentration and concentration in proportion to total chlorophyll, *S. pyrifolia* and *S. pedicellaris* demonstrated relatively small changes in zeaxanthin concentration (Fig. 4). Three of the species (*S. bebbiana*, *S. discolor* and *S. candida*) also increased their total xanthophyll concentrations by mid-drought (Table 2).



**Fig. 5.** (a) Nonphotochemical quenching (qN) correlated with the de-epoxidation state of the xanthophylls (DPS) across all the species. Each point represents the average qN and DPS values for each species on each day of measurement. (b) Habitat generalists exhibited a greater increase of qN and DPS from day 0 to day 15 than the wetland specialists. Species symbols are the same as Fig. 1. Error bars are  $\pm$  s.e.

## Discussion

### *Photoprotection and the xanthophyll cycle*

This experiment demonstrated that six willow species which are relatively drought intolerant can dissipate excess energy through the xanthophyll cycle in response to drought conditions. During a 4 week dry down, these species exhibited both an increase in their de-epoxidated xanthophyll concentrations (DPS) and their nonphotochemical quenching (qN, Figs 2, 3, 5). Further, some of the species increased their capacity to dissipate excess energy by upregulating xanthophyll production. This is significant because leaf light absorption declines with chlorophyll level and an increase in the proportion of zeaxanthin to chlorophyll (Fig. 4) enhances a plant's photoprotective capacity.

All six of the species in this study were able to minimise photodamage and maintain high maximum photosynthetic efficiency ( $F_v/F_m$ , Fig. 2a, b) during the drought in some of their leaves. However, most of the species also exhibited significant leaf loss and senescence. This is important to note because high light is known to shorten leaf life span in many species (Williams *et al.* 1989; Cavender-Bares *et al.* 2000), and there is evidence that photodamage may play a role in leaf senescence (Lovelock *et al.* 1994). It is, therefore, possible that the species in this study did experience photodamage during the dry down but it went undetected because we do not have measurements immediately before senescence on individual leaves.

### *Leaf chlorophyll loss*

All six species in this study demonstrated a decline in leaf chlorophyll content during the 4 week dry-down. Chlorophyll loss is common under drought conditions in other deciduous species (Munné-Bosch *et al.* 2001), and also some perennial grasses (Balaguer *et al.* 2002) and evergreen species (Martínez-Ferri *et al.* 2000; Munné-Bosch and Alegre 2000). Although chlorophyll loss can result from oxidative damage, there is increasing evidence that it can also result from enzyme-mediated processes (Matile *et al.* 1999). These processes may be advantageous as they reduce light absorption and can limit the amount of damaging excess energy in the leaf (Adams *et al.* 1990; Munné-Bosch *et al.* 2001). We note that all six willow

species exhibited a slower degradation of chlorophyll *a* than chlorophyll *b* over time. This pattern has been observed in several other senescing species (Biswal 1995; Suzuki and Shioi 2004) but is not ubiquitous (Munné-Bosch *et al.* 2001).

### *Drought deciduousness and photoprotection*

Xanthophyll cycle activity and chlorophyll loss played an important role in the photoprotective responses of these six willow species during the dry-down, but these processes were only effective in preventing leaf damage under mild to moderate drought conditions. After 4 weeks of drought, many of the plants began to senesce. Although this senescence was not lethal, and many plants resprouted after rewatering, it did indicate that these species rely on other mechanisms to survive more severe droughts.

Plant drought response strategies are generally broken into two categories: drought avoidance and drought tolerance. Some plants 'avoid' drought conditions and high xylem tensions by rapidly closing their stomata, and other plants 'tolerate' drought conditions by maintaining function at low water potentials. The six willows in this experiment are drought avoiders, which is similar to other early successional species (Martínez-Ferri *et al.* 2000). Consistent with other drought avoiders, they minimise photodamage by reducing their leaf chlorophyll levels, and limit water loss by dropping their leaves (Martínez-Ferri *et al.* 2000; Munné-Bosch and Peñuelas 2003; Munné-Bosch and Alegre 2004). Willows are also effective drought avoiders because they are avid resprouters (Newsholme 1992; Karrenberg *et al.* 2002), and some species can refill cavitated vessels (Utsumi *et al.* 1998).

### *The ecological significance of species responses*

The habitat generalists (*S. bebbiana*, *S. discolor*, and *S. petiolaris*) and the wetland specialists (*S. candida*, *S. pedicellaris*, and *S. pyrifolia*) demonstrated significant differences in their function even before the initiation of the drought treatment. The wetland specialists, on average, had lower predrought photosynthetic activity ( $A_{1200}$ ), light-acclimated photochemical efficiency ( $\Delta F/F_m'$ ) and overall leaf area, and higher qN and DPS than the habitat generalists (Table 1, Figs 1, 2). These differences

may be indicative of contrasting growth strategies in the species. In general, plants from nutrient limited systems such as wetlands are known to have more conservative growth strategies than species in more productive environments (Grime and Hunt 1975; Chapin 1980; Reich 1993). If this is the case with willows, then differences in species predrought and drought physiology could be related to their distinct growth strategies. It is also possible that wetland plants specifically benefit from higher levels of qN and DPS under well watered conditions because of the greater chance of waterlogging in their native habitats. Since waterlogging causes a reduction in photosynthesis, it can also lead to photodamage in some plants (Close and Davidson 2003).

After the initiation of the drought, the habitat generalists and wetland specialists continued to diverge in physiological function. The habitat generalists greater leaf areas likely contributed to their large declines in predawn water potential by mid-drought (Fig. 1c). At that point, the habitat generalists were demonstrating more conservative water use and lower rates of stomatal conductance ( $g$ ) than the wetland specialists. This resulted in lower  $A_{1200}$  and electron transport (ETR) in these species (Table 1). Although the wetland specialists maintained greater photosynthetic function into the mid-drought, it is possible that under drought conditions, willows benefit from dormancy. In general, the habitat generalists senesced before the wetland specialists and by the end of the dry-down, 39% of the habitat generalists had lost all of their leaves. When the plants were rewatered, there were no significant differences in the resprouting ability of the wetland specialists and the habitat generalists, however, we only investigated resprouting immediately after senescence and it is possible that species exhibit different responses after longer periods of dormancy.

Another difference in the drought responses of the habitat generalists and the wetland specialists involved their photoprotective activity. The habitat generalists, on average, exhibited the greatest photoprotective responses to the drought, as indicated by their larger increases in both qN and DPS (Fig. 5). Although the habitat generalists' lower predawn water potentials in the mid-drought can partially explain their higher photoprotective response, the wetland specialists still failed to increase their xanthophyll activity in the late drought when they had achieved comparable predawn water potentials. These results indicate that the wetland specialists exhibit smaller or slower photoprotective responses to drought than the habitat generalists, suggesting that there is a relationship between species photoprotective ability and their habitat affinity.

The six willow species in the study also demonstrated differences in their carotenoid concentrations during the drought, but these differences did not correspond with their habitat classifications. Since many carotenoids such as lutein and  $\beta$ -carotene are involved in thermal quenching and scavenging of reactive oxygen species (Dall'Osto *et al.* 2006), differences in their concentrations may be important to species drought tolerance. Furthermore, some plants use other antioxidants including salicylic acid, to minimise photodamage under drought conditions (Yang *et al.* 2004; Abreu and Munné-Bosch 2007). Therefore, it is possible other pigments and antioxidants besides those measured are important to these

species drought response, but further research is needed to better understand these processes in willows.

## Conclusion

Willows generally inhabit mesic and hydric habitats and are considered drought intolerant compared with many other plant species. Although willows rarely encounter severe droughts in their native habitats, they often encounter changes in water availability that can reduce their stomatal conductance (Pockman and Sperry 2000; Amlin and Rood 2002), making them susceptible to photodamage under conditions of excess light. This experiment demonstrated that willows are capable of increasing their xanthophyll mediated thermal dissipation under drought conditions, and that the rate and extent of their response appears to vary with their habitat affinity.

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## References

- Abreu ME, Munné-Bosch S (2007) Photo- and antioxidant protection and salicylic acid accumulation during post-anthesis leaf senescence in *Salvia lanigera* grown under Mediterranean climate. *Physiologia Plantarum* **131**, 590–598. doi: 10.1111/j.1399-3054.2007.00985.x
- Adams WW III, Demmig-Adams B (1992) Operation of the xanthophyll cycle in higher-plants in response to diurnal changes in incident sunlight. *Planta* **186**, 390–398. doi: 10.1007/BF00195320
- Adams WW III, Demmig-Adams B (1994) Carotenoid composition and down-regulation of photosystem-II in 3 conifer species during the winter. *Physiologia Plantarum* **92**, 451–458. doi: 10.1111/j.1399-3054.1994.tb08835.x
- Adams WW III, Winter K, Schreiber U, Schramel P (1990) Photosynthesis and chlorophyll fluorescence characteristics in relationship to changes in pigment and element composition of leaves of *Platanus occidentalis* L. during autumnal leaf senescence. *Plant Physiology* **92**, 1184–1190. doi: 10.1104/pp.92.4.1184
- Amlin NM, Rood SB (2002) Comparative tolerances of riparian willows and cottonwoods to water-table decline. *Wetlands* **22**, 338–346. doi: 10.1672/0277-5212(2002)022[0338:CTORWA]2.0.CO;2
- Balaguer L, Pugnaire E, Martínez-Ferri E, Armas C, Valladares F, Manrique E (2002) Ecophysiological significance of chlorophyll loss and reduced photochemical efficiency under extreme aridity in *Stipa tenacissima* L. *Plant and Soil* **240**, 343–352. doi: 10.1023/A:1015745118689
- Barker DH, Logan BA, Adams WW I, Demmig-Adams B (1997) The response of xanthophyll cycle-dependent energy dissipation in *Alocasia brisbanensis* to sunflecks in a subtropical rainforest. *Australian Journal of Plant Physiology* **24**, 27–33. doi: 10.1071/PP96059
- Barker DH, Adams WW, Demmig-Adams B, Logan BA, Verhoeven AS, Smith SD (2002) Nocturnally retained zeaxanthin does not remain engaged in a state primed for energy dissipation during the summer in two *Yucca* species growing in the Mojave Desert. *Plant, Cell & Environment* **25**, 95–103. doi: 10.1046/j.0016-8025.2001.00803.x

- Biehler K, Fock H (1996) Evidence for the contribution of the Mehler-peroxidase reaction in dissipating excess electrons in drought-stressed wheat. *Plant Physiology* **112**, 265–272.
- Biswal B (1995) Carotenoid catabolism during leaf senescence and its control by light. *Journal of Photochemistry and Photobiology B: Biology* **30**, 3–13. doi: 10.1016/1011-1344(95)07197-A
- Bota J, Medrano H, Flexas J (2004) Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? *New Phytologist* **162**, 671–681. doi: 10.1111/j.1469-8137.2004.01056.x
- Brodribb T, Hill RS (1999) The importance of xylem constraints in the distribution of conifer species. *New Phytologist* **143**, 365–372. doi: 10.1046/j.1469-8137.1999.00446.x
- Cavender-Bares J, Potts M, Zacharias E, Bazzaz FA (2000) Consequences of CO<sub>2</sub> and light interactions for leaf phenology, growth, and senescence in *Quercus rubra*. *Global Change Biology* **6**, 877–887. doi: 10.1046/j.1365-2486.2000.00361.x
- Cavender-Bares J, Kitajima K, Bazzaz FA (2004) Multiple trait associations in relation to habitat differentiation among 17 Floridian oak species. *Ecological Monographs* **74**, 635–662. doi: 10.1890/03-4007
- Cavender-Bares J, Cortes P, Rambal S, Joffre R, Miles B, Rocheteau A (2005) Summer and winter sensitivity of leaves and xylem to minimum freezing temperatures: a comparison of co-occurring Mediterranean oaks that differ in leaf lifespan. *New Phytologist* **168**, 597–612. doi: 10.1111/j.1469-8137.2005.01555.x
- Chapin FS (1980) The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* **11**, 233–260. doi: 10.1146/annurev.es.11.110180.001313
- Close DC, Davidson NJ (2003) Long-term waterlogging: nutrient, gas exchange, photochemical and pigment characteristics of *Eucalyptus nitens* saplings. *Russian Journal of Plant Physiology: a Comprehensive Russian Journal on Modern Phytophysiology* **50**, 843–847. doi: 10.1023/B:RUPP.0000003284.25827.95
- Dall'Osto L, Lico C, Alric J, Giuliano G, Havaux M, Bassi R (2006) Lutein is needed for efficient chlorophyll triplet quenching in the major LHCII antenna complex of higher plants and effective photoprotection *in vivo* under strong light. *BMC Plant Biology* **6**, 32.
- Demmig B, Winter K, Kruger A, Czygan FC (1987) Photoinhibition and zeaxanthin formation in intact leaves – a possible role of the xanthophyll cycle in the dissipation of excess light energy. *Plant Physiology* **84**, 218–224. doi: 10.1104/pp.84.2.218
- Demmig B, Winter K, Kruger A, Czygan FC (1988) Zeaxanthin and the heat dissipation of excess light energy in *Nerium-oleander* exposed to a combination of high light and water-stress. *Plant Physiology* **87**, 17–24. doi: 10.1104/pp.87.1.17
- Demmig-Adams B, Adams WW III (2006) Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. *New Phytologist* **172**, 11–21. doi: 10.1111/j.1469-8137.2006.01835.x
- Demmig-Adams B, Adams WW, Logan BA, Verhoeven AS (1995) Xanthophyll cycle-dependent energy-dissipation and flexible photosystem-II efficiency in plants acclimated to light stress. *Australian Journal of Plant Physiology* **22**, 249–260. doi: 10.1071/PP9950249
- Flexas J, Medrano H (2002) Energy dissipation in C<sub>3</sub> plants under drought. *Functional Plant Biology* **29**, 1209–1215. doi: 10.1071/FP02015
- Flexas J, Ribas-Carbo M, Bota J, Galmes J, Henkle M, Martinez-Canellas S, Medrano H (2006) Decreased Rubisco activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO<sub>2</sub> concentration. *New Phytologist* **172**, 73–82. doi: 10.1111/j.1469-8137.2006.01794.x
- Galmés J, Abadía A, Cifre J, Medrano H, Flexas J (2007) Photoprotection processes under water stress and recovery in Mediterranean plants with different growth forms and leaf habits. *Physiologia Plantarum* **130**, 495–510. doi: 10.1111/j.1399-3054.2007.00919.x
- García-Plazaola JI, Becerril JM (2000) Effects of drought on photoprotective mechanisms in European beech (*Fagus sylvatica* L.) seedlings from different provenances. *Trees – Structure and Function* **14**, 485–490.
- Gilmore AM, Yamamoto HY (1991) Resolution of lutein and zeaxanthin using a non-encapped, lightly carbon-loaded C-18 high-performance liquid-chromatographic column. *Journal of Chromatography. A* **543**, 137–145. doi: 10.1016/S0021-9673(01)95762-0
- Gleason HA, Cronquist A (1991) 'Manual of vascular plants of Northeastern United States and adjacent Canada.' (The New York Botanical Gardens: New York)
- Grime JP, Hunt R (1975) Relative growth rate: its range and adaptive significance in a local flora. *Journal of Ecology* **63**, 393–422.
- Hacke UG, Sperry JS, Wheeler JK, Castro L (2006) Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiology* **26**, 689–701.
- Karrenberg S, Edwards PJ, Kollmann J (2002) The life history of Salicaceae living in the active zone of floodplains. *Freshwater Biology* **47**, 733–748. doi: 10.1046/j.1365-2427.2002.00894.x
- Katona E, Neimanis S, Schonknecht G, Heber U (1992) Photosystem I-dependent cyclic electron-transport is important in controlling photosystem-II activity in leaves under conditions of water-stress. *Photosynthesis Research* **34**, 449–464. doi: 10.1007/BF00029818
- Kozaki A, Takeba G (1996) Photorespiration protects C<sub>3</sub> plants from photooxidation. *Nature* **384**, 557–560. doi: 10.1038/384557a0
- Kyparissis A, Drilias P, Manetas Y (2000) Seasonal fluctuations in photoprotective (xanthophyll cycle) and photoselective (chlorophylls) capacity in eight Mediterranean plant species belonging to two different growth forms. *Australian Journal of Plant Physiology* **27**, 265–272. doi: 10.1071/PP99037
- Lovelock CE, Jebb M, Osmond CB (1994) Photoinhibition and recovery in tropical plant species: response to disturbance. *Oecologia* **97**, 297–307.
- Lovelock CF, Jackson AE, Melick DR, Seppelt RD (1995) Reversible photoinhibition in Antarctic moss during freezing and thawing. *Plant Physiology* **109**, 955–961.
- Maherali H, Pockman WT, Jackson RB (2004) Adaptive variation in the vulnerability of woody plants to xylem cavitation. *Ecology* **85**, 2184–2199. doi: 10.1890/02-0538
- Martínez-Ferri E, Balaguer L, Valladares F, Chico JM, Manrique E (2000) Energy dissipation in drought-avoiding and drought-tolerant tree species at midday during the Mediterranean summer. *Tree Physiology* **20**, 131–138.
- Matile P, Hortensteiner S, Thomas H (1999) Chlorophyll degradation. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 67–95. doi: 10.1146/annurev.arplant.50.1.67
- Montgomery RA, Goldstein G, Givnish TJ (2008) Photoprotection of PSII in Hawaiian lobeliads from diverse light environments. *Functional Plant Biology* **35**, 595–605. doi: 10.1071/FP08031
- Morley T (1969) 'Spring flora of Minnesota.' (The University of Minnesota Press: Minneapolis, MN)
- Munné-Bosch S, Alegre L (2000) Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. *Planta* **210**, 925–931. doi: 10.1007/s004250050699
- Munné-Bosch S, Alegre L (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. *Functional Plant Biology* **31**, 203–216. doi: 10.1071/FP03236
- Munné-Bosch S, Peñuelas J (2003) Photo- and antioxidative protection during summer leaf senescence in *Pistacia lentiscus* L. grown under Mediterranean field conditions. *Annals of Botany* **92**, 385–391. doi: 10.1093/aob/mcg152
- Munné-Bosch S, Jubany-Mari T, Alegre L (2001) Drought-induced senescence is characterized by a loss of antioxidant defenses in chloroplasts. *Plant, Cell & Environment* **24**, 1319–1327. doi: 10.1046/j.1365-3040.2001.00794.x

- Newsholme C (1992) 'Willows: the genus *Salix*.' (Timber Press: Portland, OR)
- Osmond CB, Grace SC (1995) Perspectives on photoinhibition and photorespiration in the field – quintessential inefficiencies of the light and dark reactions of photosynthesis. *Journal of Experimental Botany* **46**, 1351–1362.
- Pockman WT, Sperry JS (2000) Vulnerability to xylem cavitation and the distribution of Sonoran desert vegetation. *American Journal of Botany* **87**, 1287–1299. doi: 10.2307/2656722
- Reich P (1993) Reconciling apparent discrepancies among studies relating life span, structure and function of leaves in contrasting plant life forms and climates: 'the blind men and the elephant retold'. *Functional Ecology* **7**, 721–725. doi: 10.2307/2390194
- Robinson KM, Karp A, Taylor G (2004) Defining leaf traits linked to yield in short-rotation coppice *Salix*. *Biomass and Bioenergy* **26**, 417–431. doi: 10.1016/j.biombioe.2003.08.012
- Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis Research* **10**, 51–62. doi: 10.1007/BF00024185
- Silvertown J, Dodd ME, Gowing DJG, Mountford JO (1999) Hydrologically defined niches reveal a basis for species richness in plant communities. *Nature* **400**, 61–63. doi: 10.1038/21877
- Suzuki Y, Shioi Y (2004) Changes in chlorophyll and carotenoid contents in radish (*Raphanus sativus*) cotyledons show different time courses during senescence. *Physiologia Plantarum* **122**, 291–296. doi: 10.1111/j.1399-3054.2004.00401.x
- Utsumi Y, Sano Y, Fujikawa S, Funada R, Ohtani J (1998) Visualization of cavitated vessels in winter and refilled vessels in spring in diffuse-porous trees by cryo-scanning electron microscopy. *Plant Physiology* **117**, 1463–1471. doi: 10.1104/pp.117.4.1463
- Verhoeven AS, Adams WW III, Demmig-Adams B (1999) The xanthophyll cycle and acclimation of *Pinus ponderosa* and *Malva neglecta* to winter stress. *Oecologia* **118**, 277–287. doi: 10.1007/s004420050728
- Watling JR, Robinson SA, Woodrow IE, Osmond CB (1997) Responses of rainforest understorey plants to excess light during sunflecks. *Australian Journal of Plant Physiology* **24**, 17–25. doi: 10.1071/PP96074
- Whittaker RH (1956) Vegetation of the Great Smoky Mountains. *Ecological Monographs* **26**, 1–80. doi: 10.2307/1943577
- Williams K, Field CB, Mooney HA (1989) Relationships among leaf construction cost, leaf longevity, and light environment in rain-forest plants of the genus *Piper*. *American Naturalist* **133**, 198–211. doi: 10.1086/284910
- Yang YN, Qi M, Mei CS (2004) Endogenous salicylic acid protects rice plants from oxidative damage caused by aging as well as biotic and abiotic stress. *The Plant Journal* **40**, 909–919. doi: 10.1111/j.1365-313X.2004.02267.x
- Zimmermann MH, Brown CL (1977) 'Trees: structure and function.' (Springer-Verlag: New York)

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