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Functional Plant Biology

Supplementary Material

The effects of spring versus summer heat events on two arid zone plant species under field conditions

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The following Supporting Information is available for **The effects of spring versus summer heat events on two arid zone plant species under field conditions**

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Table C1 Air temperature and VPD during heat stress treatments in spring and summer.

Fig. C1 Ambient air temperature and VPD at Australian Arid Lands Botanic Gardens, Port Augusta, South Australia.

Fig. C2 Air temperature (°C) and VPD (kPa) during four replicate heat stress treatments (one replicate per row) imposed in spring (a, c) and summer (b, d).

Supplementary Appendix A. Accompanying data for heat stress responses of *Solanum oligacanthum* and *Solanum orbiculatum*.

Table A1. Main factor means (± SE) of short- and long-term responses to heat stress experiment during spring *versus* summer.

	Species		**Season**		**Nutrient**		**Heat stress treatment**	
	S. oligacanthum	*S. orbiculatum*	Spring	Summer	High	Low	Ambient	Heat stress
D_{PSII}	0.05 ± 0.02	0.06 ± 0.02	0.08 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.02 ± 0.01	0.09 ± 0.02
MSI	0.95 ± 0.01	0.98 ± 0	0.98 ± 0	0.95 ± 0.01	0.95 ± 0.01	0.97 ± 0	0.97 ± 0.01	0.95 ± 0.01
Stem:leaf (g/g)	3.3 ± 0.74	1.58 ± 0.31	1.45 ± 0.18	3.36 ± 0.75	1.45 ± 0.18	3.43 ± 0.77	2.14 ± 0.51	2.79 ± 0.67
GR_{AG} (g/day)	0.01 ± 0.01	0.01 ± 0	0.03 ± 0	-0.01 ± 0	0.02 ± 0.01	0 ± 0	0.02 ± 0.00	0.01 ± 0.01
LMA (g/m^2)	90.37 ± 5.01	134.19 ± 7.44	109.06 ± 6.12	115.29 ± 8.6	108.9 ± 6.1	115.66 ± 8.76	118.58 ± 7.55	105.04 ± 7.32
Flower/day	2.81 ± 0.48	0.9 ± 0.18	2.12 ± 0.46	1.51 ± 0.29	3.09 ± 0.44	0.65 ± 0.16	2.06 ± 0.4	1.59 ± 0.39
Fruit/day	0.1 ± 0.02	0.15 ± 0.03	0.16 ± 0.03	0.1 ± 0.03	0.22 ± 0.03	0.04 ± 0.01	0.15 ± 0.03	0.11 ± 0.02
Flower:AG (g/g)	0.09 ± 0.02	0.03 ± 0	0.09 ± 0.02	0.03 ± 0.01	0.07 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Fruit:AG (g/g)	0.01 ± 0	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Survival (prop.)	0.94 ± 0.03	0.82 ± 0.05	0.91 ± 0.04	0.86 ± 0.04	0.89 ± 0.04	0.88 ± 0.04	0.96 ± 0.02	0.81 ± 0.05
Damage (prop.)	0.31 ± 0.07	0.23 ± 0.05	0.24 ± 0.07	0.3 ± 0.06	0.28 ± 0.06	0.26 ± 0.07	0.1 ± 0.04	0.44 ± 0.07
AG biomass (g)	8.98 ± 1.98	12.98 ± 2.15	8.53 ± 1.4	13.43 ± 2.54	18.64 ± 2.13	3.31 ± 0.34	11.46 ± 2.09	10.43 ± 2.09

	Species		Season		Nutrient		Heat stress treatment	
	S. oligacanthum	*S. orbiculatum*	Spring	Summer	High	Low	Ambient	Heat stress
LA (m^2)	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.01 ± 0	0.05 ± 0.01	0.04 ± 0.01
Pre-Root mass (g)	8.69 ± 3.93	11.28 ± 4.36	0.8 ± 0.08	19.17 ± 4.41	15.85 ± 5.19	4.13 ± 1.24		
Pre-Root:shoot (g/g)	1.24 ± 0.16	1.34 ± 0.19	0.97 ± 0.08	1.61 ± 0.19	1.16 ± 0.18	1.43 ± 0.16		
Pre- AG (g)	5.65 ± 1.94	8.3 ± 3.55	0.85 ± 0.07	13.1 ± 3.14	11.47 ± 3.55	2.48 ± 0.61		
Seed output (mean seed size)	*S. oligacanthum* (7.3 ± 0.7 mg)		0.11 ± 0.02	0.09 ± 0.02	0.17 ± 0.02	0.04 ± 0.01	0.10 ± 0.02	0.10 ± 0.02
	S. orbiculatum (1.5 ± 0.2 mg)		11.97 ± 2.22	6.28 ± 1.87	15.39 ± 2.62	2.866 ± 0.02	11.63 ± 2.25	6.62 ± 1.84

All biomass are dry weights. Parameters are explained in Table 1 with the exception of aboveground (AG) biomass and pre-heat stress harvest of AG biomass (pre-AG), root biomass (Pre-Root mass) and root:shoot ratio (Pre-Root:shoot); Leaf area (LA, m^2), total LA of plant; Seed output, the number of seeds $fruit^{-1}$ normalised to day. Seed output has not been statistically analysed (see Methods)

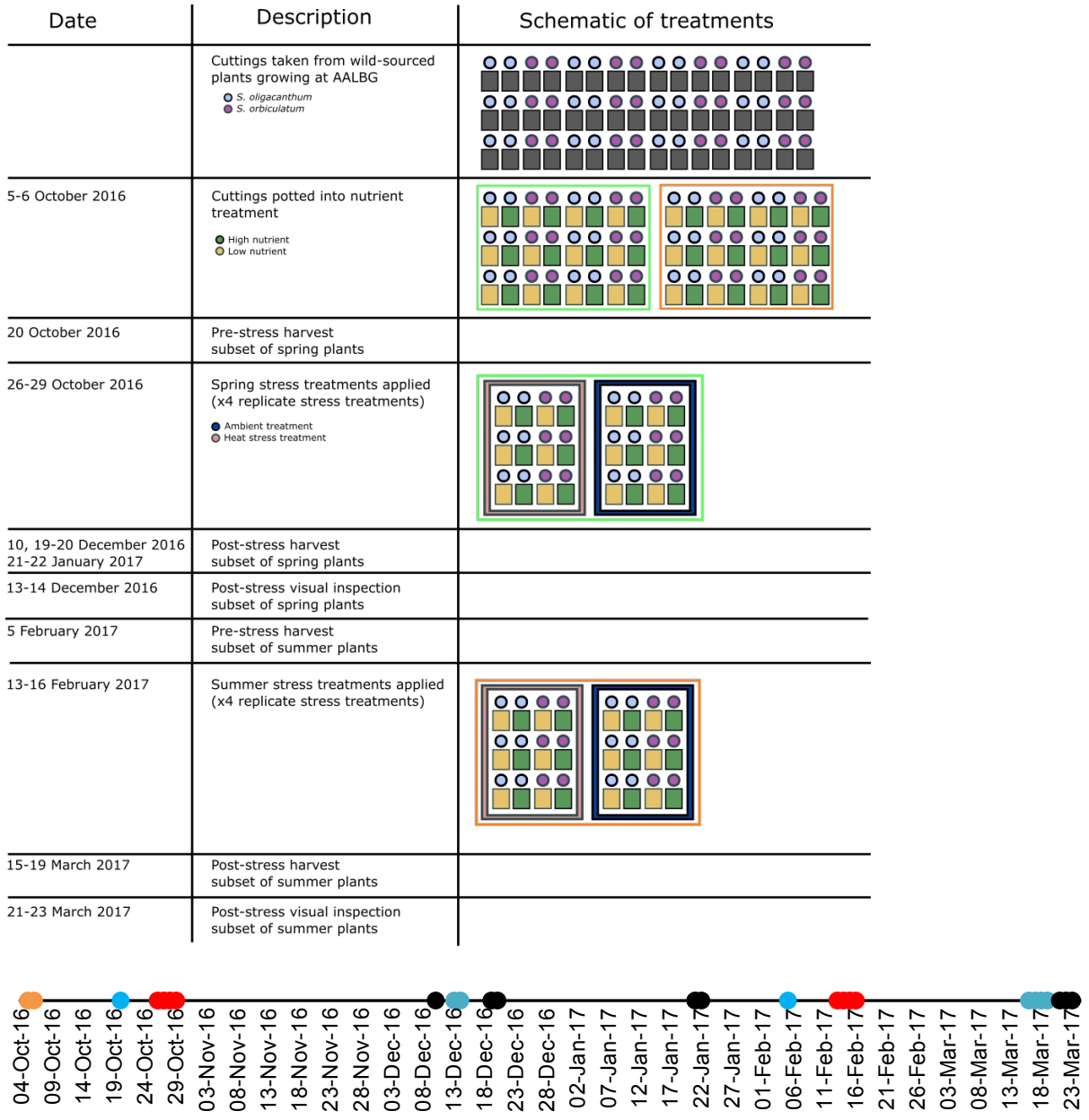

Fig. A1 Schematic of experimental design and timeline of seasonal heat stress experiment. Plants were grown from cuttings and allocated to nutrient treatments (green points); a sub-set of plants were harvested prior to the heat stress treatments (pre-harvest; pale blue points); heat stress treatments were imposed on four consecutive days (red points) in Austral spring (October) and summer (February). After the heat stress treatments, plants were left to grow and a sub-sample was destructively harvested for biomass and fitness (post-harvest; black points). Non-destructive sampling for visible damage, survival and numbers of flowers and fruit of all remaining plants were conducted (dark blue points).

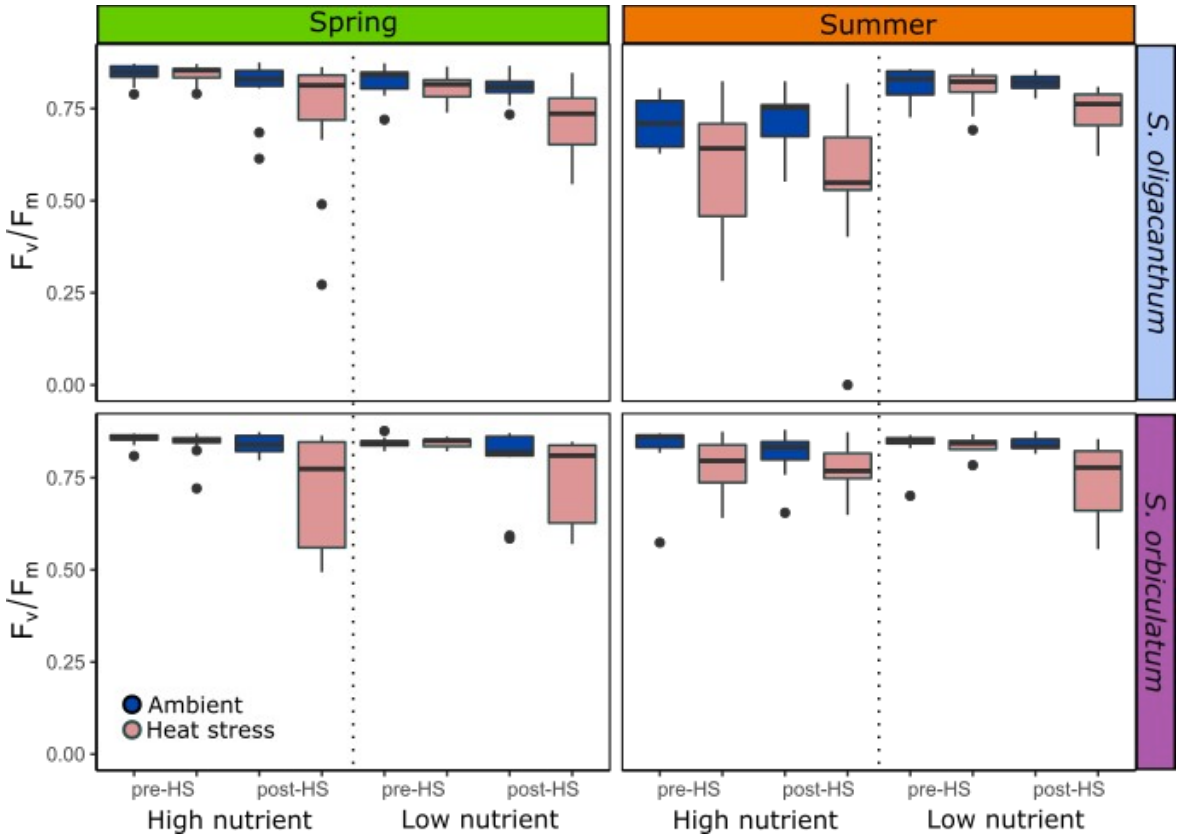

Fig. A2 Maximum quantum yield (F_v/F_m) of *Solanum* plants pre- and post-heat stress. *Solanum oligacanthum* (top panels) and *Solanum orbiculatum* (bottom) plants were grown in high or low nutrients. In spring (left panels) or summer (right) plants were water stressed before exposure to heat stress (red) or ambient conditions (blue). F_v/F_m was measured pre-dawn (dark adapted) on the mornings pre- and post-heat stress. Boxplots include all individual plants (n = 24, except *S. oligacanthum* high nutrient summer = 18). Box and whisker plots (in the style of Tukey: interquartiles with whiskers extending to lowest and highest datum within 1.5*IQR of lower and upper quartiles respectively).

Fig. A3. Resprouting *Solanum oligacanthum* following heat stress.

Supplementary Appendix B. Nutrient conditions of *Solanum oligacanthum* and *Solanum orbiculatum*.

Method B1. Additional methodological details on leaf protein extraction and protein status of leaves

To verify the effect of nutrient status, we determined total leaf protein concentration. The protein extraction protocol was modified from Knight (2010). Frozen leaf samples were ground to a fine power in tubes (Eppendorf™ tubes, Hamburg, Germany) with a 3 mm glass bead. Samples were placed in a tissue homogeniser (MM300, Retsch GmbH, Haan, Germany) for 45 s at 100 Hz, with samples being returned to liquid nitrogen after each round of beating (repeated 10x). A protein extraction buffer (100 mM Tris, 2.5% w/v SDS, 5 mM EDTA, with protease inhibitor cocktail (cOmplete™ ULTRA tablets; Merck, KGaA, Darmstadt, Germany)), was added (740 µL) and samples heated for 5 min before being rested for 1 h at room temperature. The supernatant was collected after centrifugation at 20 000 g for 10 min. The total amount of protein extracted from the samples was determined using BCA assay (Thermo Fisher Scientific, Waltham, MA, USA) run in triplicate using BSA as a standard.

Additional fertiliser increased the leaf nitrogen content of fertlised plants in comparison with plants without fertiliser, although the differences were affected by season and species (Table B1; Fig. **B1**). The leaf protein content of plants sampled in spring did not differ, regardless of nutrient treatment; however, in summer, leaf protein content was signicantly higher in high nutrient than low nutrient grown plants. Leaf protein content was influenced by nutrient status depending upon species, whereby higher leaf protein content was seen in *S. oligacanthum* grown under high than low nutrient conditions, but did not differ with nutrient status in *S. orbiculatum*.

References

Knight, C.A. (2010) Small heat shock protein responses differ between chaparral shrubs from contrasting microclimates. *Journal of Botany*, pp. 7. doi:10.1155/2010/171435

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J. & Postma, J.A. (2012) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology,* **39,** 839-850.

Table B1. ANOVA output of effect of species (*Solanum oligacanthum* and *S. orbiculatum*), season (spring *versus* summer) and nutrient treatment (high *versus* low) on leaf protein content.

	Degrees of freedom	**Sum of Squares**	**F-value**	**p-value**
species	1	1500	3.83	0.05
season	1	3089	7.89	**0.01**
nutrient	1	608	1.55	0.21
species * season	1	1377	3.52	0.06
species * nutrient	1	1874	4.79	**0.03**
season * nutrient	1	2318	5.92	**0.02**
Residuals	146	57154		

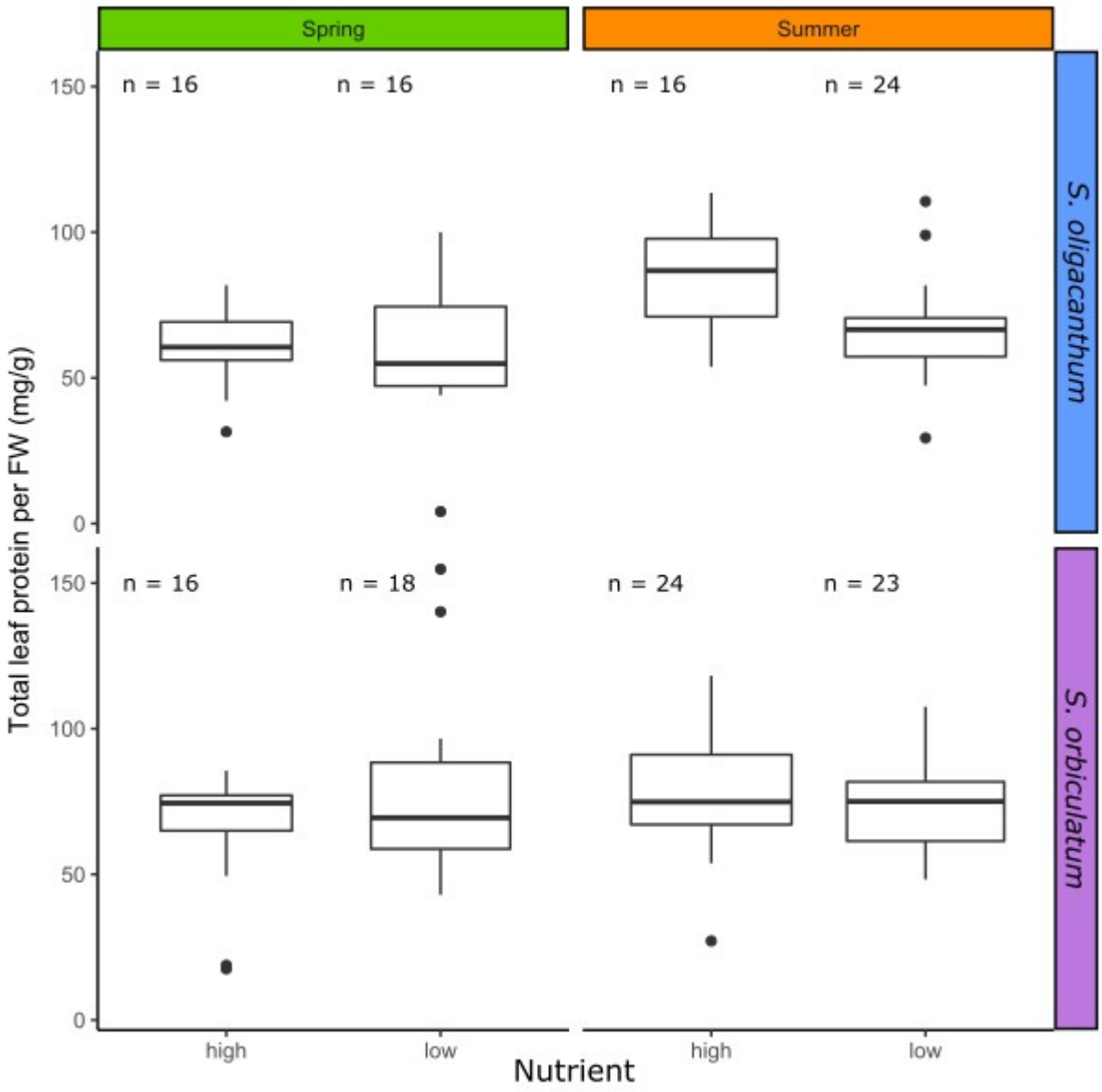

Fig. B1 The effect of fertliser application on nitrogen status of *Solanum oligacanthum* (left) and *Solanum orbiculatum* (right) leaves. Total leaf protein concentration (mg/g FW) from plants following application of fertiliser (high nutrient) or growth in sand and potting mix alone (low nutrient) (Box and whisker plots (in the style of Tukey), with sample size indicated above).

Supplementary Appendix C. Characterising ambient and applied heat stress events in spring and summer

Desert plants naturally experience heatwaves under drought, high light conditions and low wind and humidity, which can result in poor water relations.To confirm that experimental plants were water stressed, pre-dawn leaf water potential (Ψ_L) was compared with that of well-watered plants grown alongside experimental plants. In spring, although non-significant Ψ_L was lower in water stressed (-0.8 (-0.6, -0.9) MPa; bootstrap mean and 95% CI) than well-watered (-0.6 (-0.5, -0.7) MPa) *S. oligacanthum* plants and *S. orbiculatum* plants (-0.7 (-0.5, -0.7) MPa and -0.6 (-0.5, -0.7) MPa of water-stressed and well-watered plants respectively). In summer, Ψ_L was significantly lower in water-stressed than well-watered plants of both *S. oligacanthum* (-0.9 (-0.7, -1.2) and -0.6 (-0.5, -0.6) MPa respectively) and *S. orbiculatum* (-1.2 (-0.9, -1.6) and -0. 7 (-0.5, -0.9) MPa respectively).

During spring, photosynthetically active radiation (PAR) was measured with a Li-190R Quantum Sensor and LI-250A light meter (Li-COR, Lincoln, Nebraska, USA). PAR received in the chambers was ~ 26% lower than light levels outside (independent samples t-test; $t_{14} = 2.57$, $p = 0.02$), however, the mean chamber PAR of 1347 ± 118 mmol m^{-2} s^{-1} was similar to saturating light levels for Australian desert plants (e.g., 1200 mmol m^{-2} s^{-1} PAR for *Acacia anuera* in arid Northern Territory; (Wujeska-Klause *et al.* 2015). Photoperiod in October and February when heat stresses were imposed was approximately 13 h. With an average annual PAR of approximately 1500 mmol m^{-2} day^{-1} (Owen & Griffiths 2013). Heat stress events in nature often occur when wind speed drops, reducing forced convection that would otherwise prevent leaves from overheating (Vogel 2009). To check that experimental heat stress events mimicked such conditions, wind speed inside and outside of the chambers was measured using a digital anemometer (435; Testo, Testo SE & CO.KGaA, Lenzkirch, Germany). Recorded wind speeds were 0.04-1.14 ms^{-1} inside chambers and 0.09-8.9 ms^{-1} outside chambers, with greater variance (SD) outside than in chambers (1.30 and 0.20 ms^{-1} respectively). Wind speed was significantly higher outside than inside chambers (Welch two sample t-test with unequal variance: $t_{390} = 23.527$, $p < 0.001$).

During heat stress treatments, leaf temperature was monitored using a non-contact infrared thermometer (accuracy ± 2.5%°C; IP67; Jaycar, NSW, Australia) and a thermographic camera with emissivity set to 0.95 (accuracy ± 2°C or ± 2% of m.v.; Testo 885-2; Testo SE &

CO.KGaA, Lenzkirch, Germany). Photographs were taken with a number of plants in field of view approximately four times throughout the 3 h heat stress period. For leaf temperature analysis, images taken with the camera were used to find temperatures of three target leaves (0.6 m from heat source) per plant per time point using the manufacturer's software (Testo IRSoft, v4.4). During all heat stress events (except one replicate in spring), ambient air temperature (T_{air}) and humidity in chambers were recorded using climate loggers (DS1923; iButton®, Alfa-Tek Australia) suspended within a double-layer, cup-shaped white plastic shield to maintain air flow around the sensor while reflecting radiation. In addition, air temperature and humidity were constantly monitored, using iButtons®, where potted plants were grown. Vapour pressure deficit was calculated using the formula:

$$VPD = \frac{(100 - relative\,humidity)}{100} \times saturated\,vapour\,pressure$$

Seasonal differences in ambient air temperature and VPD during the experimental period (including the five days prior, during and five days post heat stress treatment) were apparent, with warmer and drier conditions in summer than in spring (Fig. **C1**). In summer, a natural heatwave (three consecutive days exceeding the 90th percentile) occurred two days prior to experimentation (Fig. **C1b**). During the heat stress treatments in spring, air temperatures in the open-top chambers (Table C1; Fig. **C1a**) were generally greater than naturally occurring heatwaves in this region at a similar time of year (three days >33°C, 90th percentile maximum temperature data from Port Augusta Airport 2001-2017; BoM 2018). During summer treatments, imposed heat stress air temperatures in the chambers (Table C1; Fig. **C1b**) were similar to typical summer heatwaves (3 days >40°C). Mean leaf temperatures of heat-stressed plants reached 47°C, in spring and 50°C in summer, which is comparable to a mean maximum leaf temperature of 52°C, measured in other water-stressed native desert plants at this site during early summer (Cook *et al*., unpublished).

References

Bureau of Meteorology (BoM). (2018) Climate Data Online: Port Augusta Aero 2001-2018. Commonwealth of Australia

Owen, N.A. and Griffiths, H. (2014), Marginal land bioethanol yield potential of four crassulacean acid metabolism candidates (*Agave fourcroydes*, *Agave salmiana*, *Agave tequilana* and *Opuntia ficus-indica*) in Australia. GCB Bioenergy, **6**, 687-703.

Vogel, S. (2009) Leaves in the lowest and highest winds: temperature, force and shape. *New Phytologist,* **183,** 13-26.

Wujeska-Klause, A., Bossinger, G. & Tausz, M. (2015) Seedlings of two *Acacia* species from contrasting habitats show different photoprotective and antioxidative responses to drought and heatwaves. *Annals of Forest Science,* **72,** 403-414.

Table C1. Air temperature and VPD during heat stress treatments in spring and summer.

Season	HS treatment	Air temperature (°C)				VPD (kPa)			
		min	max	mean	heat sum	min	max	mean	deficit sum
Spring									
Overall mean	*Ambient*	30.5	32.5	31.4	5692.2	3.6	4	3.8	686.5
	HS	34.6	41.6	39.1	7083.7	4.6	7.2	6.2	1123.5
HS 2	*Ambient*	24.7	28.2	26.4	4781.2	2	2.6	2.3	411.1
	OTC1	32.7	41.1	38.5	6974.7	3.7	6.6	5.6	1010.6
	OTC2	31.1	37.6	34.7	6275.1	3.3	5.2	4.3	781.1
HS 3	*Ambient*	29.7	33.1	31.2	5654.6	3	4.1	3.6	657.6
	OTC1	32.1	38.6	36.2	6546.6	3.8	6	5.1	921.9
	OTC2	34.1	45.1	40.1	7262.1	4.2	8.6	6.5	1184.3
HS 4	*Ambient*	35.6	38.6	36.7	6640.8	5.1	6.1	5.5	991
	OTC1	38.6	46.6	42.8	7738.9	6.1	9.8	7.9	1429.6
	OTC2	37.6	47.1	42.6	7705.1	5.8	9.9	7.8	1413.6
Summer									
Overall mean	*Ambient*	34.5	38.2	36.7	6634.9	4.1	5.4	4.8	872.7
	HS	38.2	44.8	42.8	7746.5	5.3	8.1	7.1	1293.4
HS 1	*Ambient*	28.1	31.6	30.1	5440.1	2.6	6.1	4.8	869.4
	OTC1	30.1	44.1	38.3	6940.6	4.8	6.9	5.8	1047.4
	OTC2	29.7	40.6	37.1	6709.4	5.8	9.2	7.8	1417.3
HS 2	*Ambient*	36.6	41.6	39.2	7095.9	6.2	10.2	8.7	1581.5
	OTC1	40.1	46.6	44	7972	5.5	8.3	6.8	1232.4
	OTC2	41.1	48.6	46.1	8341.4	7.2	11.2	9.6	1737.1
HS 3	*Ambient*	37.6	44.6	41.2	7449.2	6.6	10.4	9	1635.5
	OTC1	42.1	50	47.2	8548.6	3.3	4.7	3.9	705.9
	OTC2	40.6	48.6	46.1	8347.1	4.5	6.9	5.8	1048.3
HS 4	*Ambient*	34.6	38.6	36.2	6554.1	4.6	7	6.1	1099.9
	OTC1	38.1	44.1	41.4	7493.4	2.3	3.2	2.8	505.1
	OTC2	38.1	44.1	42.1	7619.7	2.8	7.5	5.3	958.4

Heat stresses were imposed in open top chambers using infrared lamps. Ambient conditions were measured adjacent to chambers. Minimum, maximum and mean are given for air temperature and VPD. Heat sum and deficit sum are the sum of all readings logged at one min intervals for the 180 min duration of the experiment. No data collected for replicate 1 HS in spring due to non-functional data loggers.

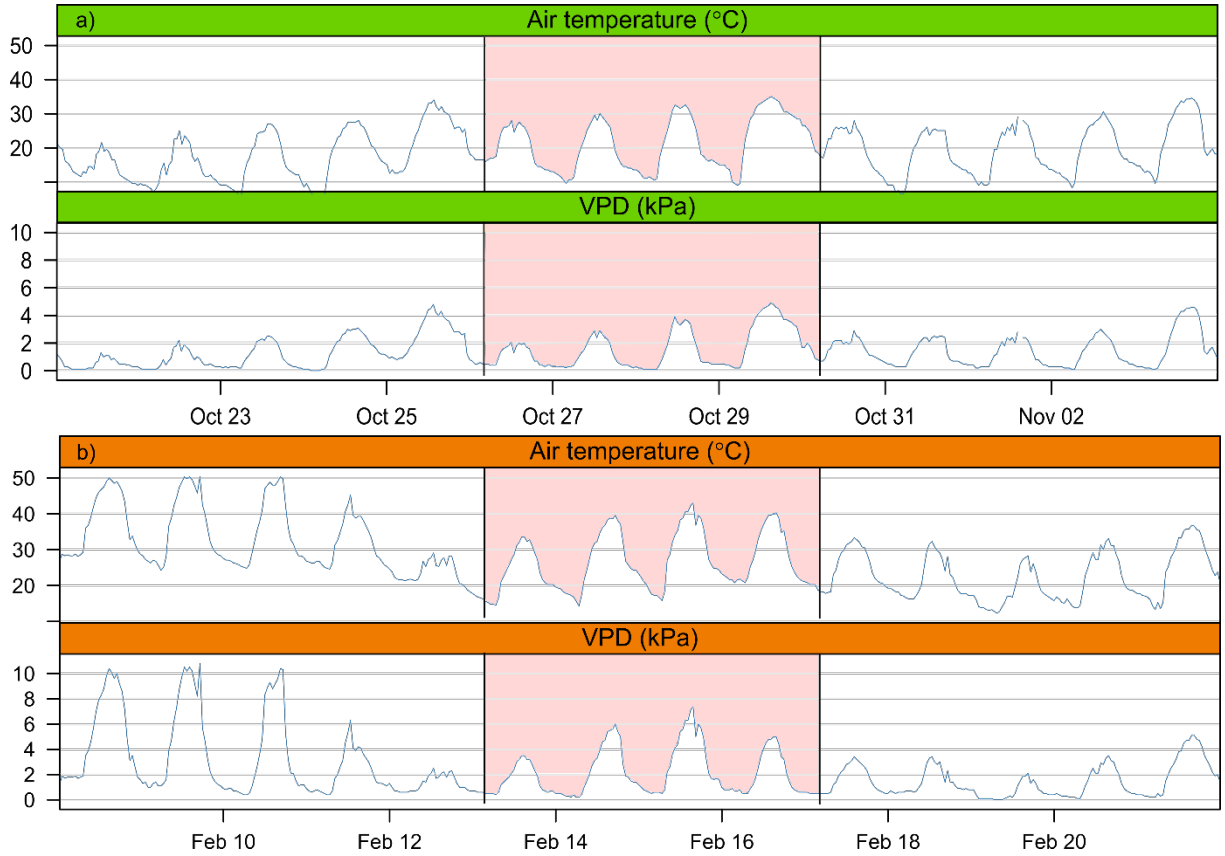

Fig. C1 Ambient air temperature and VPD at Australian Arid Lands Botanic Gardens, Port Augusta, South Australia. Data for the five days preceding, four days during (shaded area) and five days following heat stresses in spring (a) and summer (b).

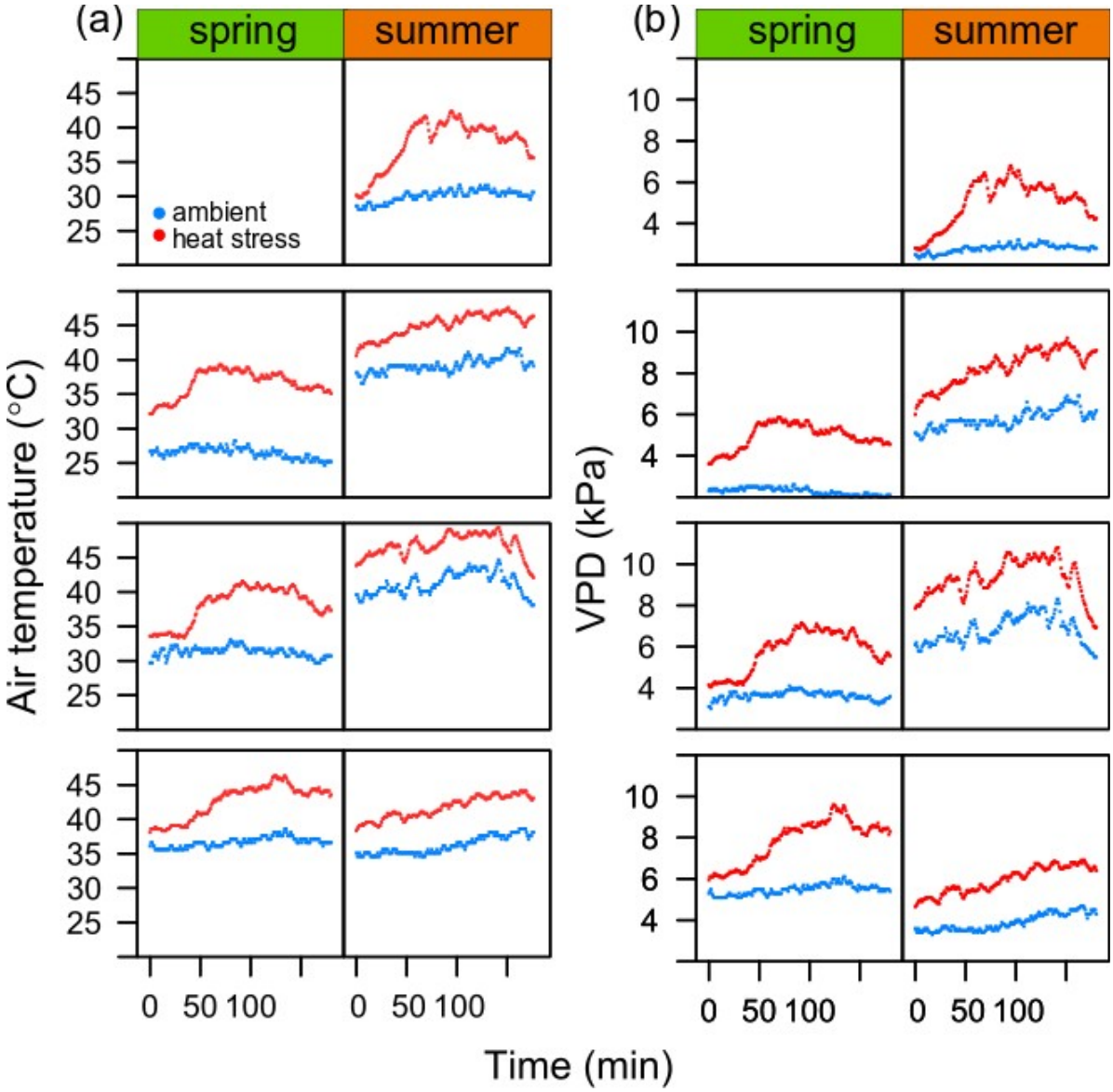

Fig. C2. Air temperature (°C) and VPD (kPa) during four replicate heat stress treatments (one replicate per row) imposed in spring (a, c) and summer (b, d). Heat stress conditions are shown within open top chambers (red lines) and ambient conditions adjacent to chambers (blue lines). No data were collected for the first replicate treatment in spring due to non-functional data loggers.