

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

3 K.V. Milner<sup>1</sup>, K. French<sup>2</sup>, D.W Krix<sup>1</sup>, S.M. Valenzuela<sup>1</sup>, A. Leigh<sup>1</sup>

4 <sup>1</sup>School of Life Sciences, University of Technology Sydney, Ultimo 2007 Australia;

5 <sup>2</sup>Centre for Sustainable Ecosystem Solutions, School of Earth, Atmospheric and Life  
6 Sciences, University of Wollongong, Wollongong 2522 Australia

7 **Author for correspondence**

8 *Dr Kirsty Milner,*

9 Tel: +61 (02) 9514 1765

10 Email: [Kirsty.Milner@uts.edu.au](mailto:Kirsty.Milner@uts.edu.au)

11

12 **Abstract**

13 Heatwaves are increasingly occurring out-of-season, which may affect plants not primed for  
14 the event. Further, heat stress often coincides with water and/or nutrient stress, impairing short-  
15 term physiological function and potentially causing downstream effects on reproductive  
16 fitness. We investigated the response of water-stressed arid-zone *Solanum oligacanthum* and  
17 *S. orbiculatum* to spring vs summer heat stress under differing nutrient conditions. Heat stress  
18 events were imposed in open-topped chambers under *in situ* desert conditions. To assess short-  
19 term impacts, we measured leaf photosystem responses ( $F_v/F_M$ ) and membrane stability; long-  
20 term effects were compared via biomass allocation, visible damage, flowering and fruiting.  
21 Plants generally fared more poorly following summer than spring heat stress, with the  
22 exception of  $F_v/F_M$ . Summer heat stress caused greater membrane damage, reduced growth  
23 and survival compared with spring. Nutrient availability had a strong influence on downstream  
24 effects of heat stress, including species-specific outcomes for reproductive fitness. Overall,  
25 high temperatures during spring posed a lower threat to fitness than in severe arid summer  
26 conditions of high temperature and low water availability, which were more detrimental to  
27 plants in both the short and longer term. Our study highlights the importance of considering  
28 ecologically relevant, multiple-stressor events to understand different species responses to  
29 extreme heat.

## 30 **Keywords**

31 Chlorophyll fluorescence ( $F_v/F_m$ ), desert species, fitness, heat stress, heatwaves, membrane  
32 stability, *Solanum* species, thermal tolerance.

## 33 **Introduction**

34 To understand the effects of climate change on plants, many studies have focused on imposing  
35 a projected CO<sub>2</sub> and/or average temperature rise (Nijs *et al.* 1996; Hovenden *et al.* 2006; Crous  
36 *et al.* 2018). These studies provide information on species responses to predicted changes in  
37 long-term averages. Yet, the increased frequency of extreme events is potentially more  
38 influential. The effects of extreme events last beyond their short duration (Jentsch *et al.* 2007)  
39 and have greater influence on plant physiology than mean climate (Jump & Penuelas 2005;  
40 Reyer *et al.* 2013). Observations following heatwaves have reported reduced gross primary  
41 productivity of forests (Ciais *et al.* 2005; Bauweraerts *et al.* 2014) and increased mortality  
42 (Allen *et al.* 2010; Harris *et al.* 2018; Hoffmann *et al.* 2019). During heatwaves, high air  
43 temperatures, when coupled with drought conditions (often the case; Vicente-Serrano *et al.*  
44 2014; Zscheischler & Seneviratne 2017), contribute to reduced photosynthesis through  
45 stomatal closure (Berry & Bjorkman 1980; Tang *et al.* 2007). Continued high leaf temperatures  
46 affect photosynthesis directly by damaging photosynthetic proteins (Havaux 1993a; Sharkey  
47 2005) or indirectly, through leakiness of and oxidative damage to thylakoids and other  
48 membranes (Daniell *et al.* 1969; Djanaguiraman *et al.* 2018). As photosynthesis declines and  
49 respiration rates increase (Atkin *et al.* 2005; O'Sullivan *et al.* 2013), there is less available  
50 energy for other processes, such as protection, repair, growth and reproduction (Parsons 1990).  
51 With high temperature, the stress response of plants is stimulated in order to protect from  
52 damage and return cells to normal metabolic function (Mittler *et al.* 2012). Therefore, in  
53 addition to directly impairing productivity and function, heat stress also presents a cost in the  
54 energy required for protection and repairing damage. This raises the question of how plants  
55 allocate resources between protection and survival *versus* growth and reproduction.

56 Heatwaves are now more likely to occur out-of-season and the heatwave season is starting  
57 earlier (Steffen *et al.* 2014; Reddy *et al.* 2021). An important implication of a-seasonal  
58 heatwaves is that they are potentially more damaging because they occur when organisms are  
59 not physiologically primed for high temperatures. With priming—a sub-lethal stress event (for  
60 example, Havaux 1993b)—plants are able to acclimatise into warmer seasons (Atkin *et al.*  
61 2000; Atkin & Tjoelker 2003; Aspinwall *et al.* 2017). Out-of-season heatwaves are likely to

62 leave some species ill-prepared, but evidence for the impacts of timing of high temperature  
63 events is scarce. In a rare exception under a benign climate characterised by ‘cool summer’,  
64 spring heatwaves actually benefitted plants because heated temperatures approached a  
65 physiological optimum for growth (De Boeck *et al.* 2011). It is not known whether the same  
66 conclusion would be drawn in a more severe climate, such as a desert, where a sudden spring  
67 heat stress event can exceed optimal temperatures.

68 Heatwaves shape arid ecosystems (Holmgren *et al.* 2006), with plants already living close to  
69 thermal limits and exceeding thresholds during extreme events (O'Sullivan *et al.* 2013). Poor  
70 water availability during these times is likely to exacerbate the effects of high temperature  
71 alone (Valladares & Pearcy 1997; Cook *et al.* 2021). Thus, an ecologically relevant  
72 investigation of plant response to heat stress in arid systems should be done in combination  
73 with seasonally appropriate water stress. Furthermore, many deserts are typified by poor access  
74 to nutrients, due either to water scarcity (Erskine *et al.* 1996; Handley *et al.* 1999) or through  
75 low nutrient soils (Stafford Smith & Morton 1990). Physiological responses to protect plants  
76 against heat damage are activated quickly but can direct resources away from downstream  
77 processes such as growth and reproduction (Begcy *et al.* 2018; Rajametov *et al.* 2021). Limited  
78 access to soil nutrients, particularly nitrogen, means that the effects of heat stress events may  
79 be compounded (Heckathorn *et al.* 1996a; Heckathorn *et al.* 1996b), especially when coupled  
80 with drought (Teskey *et al.* 2015; Ward *et al.* 2015; Harris *et al.* 2018).

81 Here we sought to compare the immediate and longer-term responses of desert plants to an  
82 early heatwave *versus* late heatwave. Unlike many controlled heat wave experiments, and to  
83 capture desert conditions, plants for this study were grown and treated *in situ*, incorporating  
84 the naturally high light and low humidity conditions that are difficult to reproduce in growth  
85 chambers. To water-stressed plants under two nutrient treatments, we imposed a heat stress  
86 event of ecological relevance in spring and summer against a background of the naturally  
87 occurring weather in each season. We addressed the following questions: 1) Is a spring or  
88 summer heat stress more detrimental to growth and fitness outcomes for desert plants? 2) How  
89 does nutrient availability influence downstream effects of heat stress, i.e., growth and fitness?  
90 We applied these questions to two Australian arid zone *Solanum* species grown under two  
91 nutrient treatments and followed the plants through to fruiting.

## 92 **Materials and Methods**

### 93 *Site description and species*

94 This study was conducted at the Australian Arid Lands Botanic Garden, Port Augusta, South  
95 Australia (32°28'4.35" S, 137°44'36.99" E), where mean maximum monthly temperatures  
96 reach 34.2°C in January and mean monthly precipitation is highest in December at 25.7 mm  
97 (BoM 2018). Two perennial arid zone *Solanum* species with differing microhabitat preferences  
98 were selected. *Solanum orbiculatum* (Dunal ex Poir.) subsp. *orbiculatum* is found in drier  
99 microhabitats (sandplains and dunes, rocky hills and outcrops; FloraNT 2013) and *Solanum*  
100 *oligacanthum* (F. Muell.), which typically grows in wetter microhabitats (sandy or clayey soils  
101 in soaks and creek lines; Bean 2004). Both are perennial herbs with hair-covered leaves and  
102 long flowering and fruiting periods (Bean 2004; FloraNT 2013).

### 103 *Plant growth and treatments*

104 Plants for this experiment were grown from cuttings collected from at least five plants from  
105 the living collection at the AALBG, originally sourced from the wild, and established in 75  
106 mm tubes for 33 weeks. Cuttings were transferred to 4.5 L pots filled with 3:1 local sandy soil  
107 to standard potting mix for four weeks before experiments began (Fig. A1, Appendix A).  
108 Plants from each species were randomly assigned to either the low nutrient (potting medium  
109 (Van Schaik's Bio Gro, Mt Gambia SA) a mix of composted bark and scoria, containing trace  
110 elements, but no controlled release fertiliser) or high nutrient (potting medium with Nutricote  
111 Standard Pink slow-release fertiliser; Yates, NSW, Australia, N:P:K 19.1:0.0:11.9) group,  
112 applied at time of potting. The effect of nutrient treatment is shown in Appendix B. Growing  
113 plants in pots was necessary to maximise controlled conditions; however, pot-binding can  
114 occur if plants are left in undersized pots for too long. During a pilot trial, where plants were  
115 left in pots until after their shoot growth had slowed, there was evidence of plants becoming  
116 pot-bound. Therefore, plants for this experiment were grown for a shorter duration, in the phase  
117 between newly established cuttings to peak vegetative growth, which minimised pot-binding,  
118 as confirmed at harvest. Within each nutrient treatment, plants were randomly divided into two  
119 groups relating to season of the heat stress: austral spring (October 2016) or summer (February  
120 2017) heat stress (Fig. A1, Appendix A). These groups were further subdivided into heat stress  
121 treatments: ambient plants, receiving no heat stress, and plants subjected to a heat stress event  
122 in open-top chambers. Plants were grown outdoors in full sun.

### 123 *Heat stress application*

124 Our focus was on single extreme high temperature days, periodically recorded in these regions  
125 (e.g., 45.4°C recorded in South Australia in October; BoM 2019). To best represent prevailing

126 desert conditions, plants were grown and treated *in situ*, incorporating the naturally high light  
127 and low humidity conditions that are difficult to reproduce in growth chambers and heat  
128 stresses were imposed against a background of the natural weather. We aimed to replicate what  
129 plants would experience during a heat stress event, of equivalent length and severity to the  
130 afternoon of a heatwave in the desert, when wind speed drops and the leaves of water stressed  
131 plants are likely to reach dangerously high temperatures (Leigh *et al.* 2012). Because natural  
132 heatwaves often are coupled with low soil water availability (Teskey *et al.* 2015), irrigation  
133 was withheld from all plants six days prior to heat stress to ensure that the heatwave was  
134 associated with ecologically relevant conditions. Daily checks of plants for appearance of water  
135 stress (wilting) were conducted and pre-dawn leaf water potential ( $\Psi_L$ ) was measured on the  
136 day of the heat stress treatment using a pressure chamber (Model 1505D; PMS Instrument  
137 Company, OR, USA). Leaf water potential was more negative in water-stressed than well-  
138 watered plants, significantly so only in summer (see **Appendix C**). However, although the  
139 difference in  $\Psi_L$  for spring was not significant, there were visual signs of leaf wilting for plants  
140 that had water withheld, particularly in *S. orbiculatum*.

141 The heat stress treatment was applied in two open-top chambers (0.72 m (h) x 0.49 m (w) x  
142 1.09 m (l), with sides enclosed using PVC plastic sheeting). Two ceramic infrared lamps with  
143 reflectors (1000 W, 230 V full trough element, 60 kW/m<sup>2</sup>,  $\lambda$  range 2-10  $\mu$ m; Ceramicx, Ireland,  
144 fitted with aluminised steel reflectors and solid state relays) were hung 0.3 m above each  
145 chamber and tilted at  $\sim 40^\circ$ , similar to Kimball *et al.* (2008). Heat stress events were imposed  
146 to chambers for 3 h between 13:00-16:00. As leaf temperature can vary considerably from air  
147 temperature, our goal was to monitor leaf temperature within chambers, maintaining the  
148 temperature of target leaves close to 45°C (in line with BoM 2018 data; **Appendix C**), which  
149 we did by gradually ramping up IR radiation from the lamps over the first hour, then holding  
150 temperature for the remaining two hours (Fig. 1). Due to chamber size, and to maximise  
151 replication, four heat stress events were run on consecutive afternoons, each on a separate set  
152 of plants. Shortly before commencing each event, at least one plant from each treatment group  
153 was placed in one of two chambers and ambient plants were placed adjacent to the chambers.

#### 154 *Evaluating effects of heat stress*

155 To evaluate short-term response of plants to heat stress, and to match the height at which leaf  
156 temperature was measured, PSII function and membrane stability were evaluated from three  
157 leaves on each of three plants per treatment group. Leaves were sampled at a height of 0.2 m

158 from the base of the plant (for heat-stressed plants this height was a distance of 0.6 m from the  
159 heat source). The longer-term energetic cost of a heat stress event was estimated from growth  
160 and fitness metrics at the whole plant scale, that is the whole plant was sampled regardless of  
161 distance from the heat source.

162 Photosystem II (PSII) efficiency was assessed via maximum quantum yield ( $F_v/F_m$ ) of PSII,  
163 which was measured pre-dawn using a chlorophyll fluorometer (mini-PAM, Heinz Walz  
164 GmbH, Effeltrich, Germany) on the morning of, and the day after, heat stress treatment. Many  
165 plants had a pre-heat stress  $F_v/F_m$  values below 0.83 (Fig. A2, Appendix A), likely due to the  
166 water stress preceding the heat stress. For this reason, an estimate of damage to PSII was  
167 calculated using the equation:

$$D_{PSII} = 1 - \frac{Post F_v/F_m}{Pre F_v/F_m} \quad \text{Eqn 1}$$

168

169 from Curtis et al. (2014). This metric accounts for the starting point of the plants prior to the  
170 heat stress, including background damage that may be caused by water stress or seasonal  
171 differences.

172 Membrane stability was assessed with a conductivity meter (TetraCon 925; WTW, Weilheim,  
173 Germany) to measure the electrolyte leakage from ~ 0.5 g of fresh leaf placed in 15 mL of de-  
174 ionised water for 90 min (modified from French *et al.* (2019)). A membrane stability index  
175 (MSI) was then calculated:

$$MSI = 1 - \left( \frac{EC_{90} - EC_0}{EC_{max} - EC_0} \right) \quad \text{Eqn 2}$$

176

177 where  $EC_0$  was the conductivity of the water 5 min after leaves were placed in the water,  $EC_{90}$   
178 was the conductivity measured after 90 min and  $EC_{max}$  was the conductivity measured the  
179 morning after leaves were heat treated at 100°C to rupture cell walls.

180 Following the heat stress treatments, plants were returned to irrigated, full sun growth  
181 conditions. Approximately 1-2 months after each seasonal stress event, a single replicate plant  
182 from each treatment group was randomly selected for harvest. Visual damage to all plants was  
183 assigned based on estimated percentage of dead or discoloured leaves on the plant; plant  
184 survival also was recorded. At harvest, leaves and stems were separated from roots. Roots were

185 washed of soil and all organs were oven dried at 60°C until weight was stable. Biomass values  
186 reported are dry weights.

187 The influence of heat stress on plant growth was assessed using growth rate of aboveground  
188 biomass ( $GR_{AG}$ ;  $g\ day^{-1}$ ), the instantaneous rate of increase, which takes into account the  
189 relative size of the plant (referred to as relative growth rate in Pérez-Harguindeguy *et al.* 2013),  
190 calculated as:

$$GR_{AG} = \frac{(\ln M_2 - \ln M_1)}{(t_2 - t_1)} \quad \text{Eqn 3}$$

191

192 where  $M_1$  and  $M_2$  are aboveground biomass, harvested at  $t_1$  and  $t_2$  or pre- and post-heat stress,  
193 respectively. Aboveground biomass was used, as root samples in summer were lost. For  
194 estimating  $M_1$ , a subset of plants was harvested pre-heat stress and the mean dry mass was  
195 used.

196 To investigate relative aboveground resource allocation, the ratio stem:leaf was calculated. In  
197 addition, as an indicator of environmental tolerance and competitive ability (Poorter *et al.*  
198 2009) leaf mass per area (LMA,  $g\ m^{-2}$ ) was determined. At the time of harvesting, all leaves  
199 per plant were scanned and leaf area measured using ImageJ software (National Institutes of  
200 Health; Bethesda, MD). Plant level LMA was calculated by dividing total LA by total dry leaf  
201 biomass.

202 Prior to each heat stress treatment, flowers and fruits were removed from the plants so that only  
203 those that developed after the heat stress were recorded. To estimate the fitness cost of heat  
204 stress, flowers and fruits of all plants were counted and flowers and fruit from harvested plants  
205 were weighed (including peduncle as accessory costs). Due to many fruit not being fully  
206 developed at harvest the mean number of seeds was recorded from a subset of fruit ( $n = 9-12$   
207 for *S. oligacanthum* and  $n = 12$  for *S. orbiculatum*). Estimates of seed output per plant were  
208 calculated based on the mean number seeds per fruit multiplied by the number of fruit on a  
209 plant, normalised to per day output due to differences in harvest times. In order to assess  
210 whether plants altered the resources directed to flowering or fruiting with respect to treatment,  
211 the ratio of flower or fruit mass to aboveground biomass was calculated (flower:AG,  
212 fruit:AG (g:g)).

213 *Statistical analyses*

214 This experiment used a four-factor design, each factor with two levels. Species, season, nutrient  
215 treatment, and heat stress treatment were fixed effects. The heat stress treatment was  
216 administered over four consecutive days, with three new plants from every treatment  
217 combination used each day. Total number of plants was 2 species x 2 seasons x 2 nutrient  
218 treatments x 2 heat stress treatments x 4 replicate heat stress days x 3 replicate plants + pre-  
219 heat stress harvest: 2 species x 2 seasons x 2 nutrient treatments x 3 replicate plants = 216. As  
220 mean air temperature within the two chambers was equivalent and significantly higher than  
221 ambient temperature (ANOVA;  $F_{2,18} = 5.27$ ,  $p = 0.0158$ ), we did not consider chamber in the  
222 experimental design and the values for any parameter for the three plants was averaged for  
223 each of the four replicate stress days, except high nutrient *S. oligacanthum* in summer, or where  
224 deaths occurred. Variables of growth (LMA,  $GR_{AG}$ , stem:leaf, flower and fruit:AG) were  
225 sampled from one plant per heat stress treatment day. Analysis of visible damage and  $GR_{AG}$   
226 included dead plants, but dead plants were removed from analysis of stem:leaf and LMA.

227 Visible damage and survival were analysed using binomial logistic regression using R (R Core  
228 Team 2018). The proportion of plants per heat stress treatment day that survived or recorded  
229 visible damage (where visible damage  $>10\% = 1$ , and  $<10\% = 0$  or no damage) were used in  
230 analyses. All other variables were analysed using ANOVA using the “lm” function, after  
231 assumptions were tested and relevant transformations made (see Table 1). Models were  
232 simplified by hierarchical removal of non-significant interactions (Crawley 2013). Order of  
233 removal used AIC values using the ‘drop1’ function of the ‘car’ package (Fox & Weisber  
234 2011). Interaction terms were dropped until the model with the lowest AIC value that did not  
235 deviate significantly from the maximal model was found. Due to the unbalanced nature of the  
236 experiment, Type II sums of squares were used (Langsrud 2003) to preserve the marginality  
237 principle. Where there were significant interactions, Tukey HSD in the emmeans package were  
238 used to find differences (Lenth 2018).

## 239 **Results**

### 240 *Heat stress on leaf temperatures*

241 The recorded maximum leaf temperatures reached in chambers during imposed heat stresses  
242 did not differ between spring and summer ( $F_{1,56} = 7.52$ ,  $p = 0.0008$ ; Fig. 2), despite seasonal  
243 differences in ambient conditions between spring and summer (Fig. B1, 2, Appendix B. There  
244 was a significant season by temperature effect, whereby ambient leaf temperatures in summer  
245 were higher than those of leaves in spring (Fig. 2).



246 *Short-term responses of PSII damage and membrane stability to heat stress*

247 There was significantly more damage to PSII in plants that were exposed to heat stress than in  
248 ambient plants (Fig. **3a**) and more damage to PSII in summer than in spring (Fig. **3b**, Table 1),  
249 irrespective of species or nutrient levels. In terms of membrane damage, there was a significant  
250 season x nutrient x heat stress effect (Table 1), whereby, plants with reduced access to nutrients  
251 incurred little damage to membranes in spring or summer, with only a small increase in damage  
252 between ambient and heat-stressed plants (Fig. **3c**). For high nutrient plants, there was no  
253 difference between ambient and heat-stressed plants in spring, but following a summer heat  
254 stress, more membrane damage was recorded (Fig. **3c**). The effect of season on membrane  
255 damage differed between species (Table 1). There was little membrane damage to  
256 *S. orbiculatum* in either season, while *S. oligacanthum* recorded similarly low damage in  
257 spring, but greater membrane damage in summer (Fig. **3d**).

258 To summarise and compare responses of these desert annuals, we produced a heat map of  
259 normalised severity of response for each variable (Table 2). With regards to the short-term  
260 responses, generally there was greater damage to MSI in summer, and in heat-stressed rather  
261 than ambient treatment plants (Table 2). The exception to this trend was damage to PSII, where  
262 plants in spring were more negatively affected than plants in summer.

263 *Growth and allocation of resources*

264 In relation to resources allocated to leaves, *S. orbiculatum* had higher LMA than  
265 *S. oligacanthum* (Table 1; Fig. **4a**). There were no effects of season, nutrient or heat stress  
266 treatment on LMA (Table 1). *Solanum oligacanthum* allocated greater mass to stems than  
267 leaves compared to *S. orbiculatum* (Table 1; Fig. **4b**). Overall, more mass was allocated to  
268 stems than leaves in summer compared with spring (Table 1; Fig. **4c**) and stem:leaf was higher  
269 in low nutrient plants than high nutrient plants (Table 1; Fig. **4d**). There was no effect of heat  
270 stress treatment on the stem:leaf ratio. Growth rate of aboveground biomass differed with the  
271 seasons, but depended upon both species and nutrients, with significant interactions with these  
272 factors (Table 1). Growth of aboveground biomass was higher in spring than summer in both  
273 *S. oligacanthum* and *S. orbiculatum* (Fig. **4e**). Aboveground growth was higher in high nutrient  
274 plants than low nutrient plants in spring, and significantly reduced in overall summer relative  
275 to spring, such that there was no difference between nutrient treatments in summer (Fig. **4f**).  
276 GR<sub>AG</sub> was reduced in plants that were heat-stressed compared with their ambient counterparts  
277 (Table 1; Fig. **4g**). Overall, GR<sub>AG</sub> was reduced in summer compared with spring, with low

278 nutrient plants faring more poorly than their high-nutrient counterparts (Table 2).

### 279 *Visible damage and survival*

280 Both species showed visible damage to leaves and stems and experienced mortality following  
281 heat stress treatment. The influence of nutrient availability on the proportion of plants showing  
282 visible damage was determined by heat stress treatment (Table 1). Generally, greater damage  
283 was observed in heat-stressed plants than plants under ambient conditions. Low-nutrient, heat-  
284 stressed plants incurred more damage than their ambient-grown counterparts (Fig. 5a). There  
285 were three significant interactions explaining the proportion of plants that survived heat stress  
286 (Table 1). First, survival was determined by species, nutrient availability and heat stress  
287 treatment. When *S. oligacanthum* had access to nutrients and was heat-stressed, survival was  
288 comparable to ambient plants, however, under low nutrient conditions and after heat stress  
289 survival was marginally reduced compared with ambient counterparts (Fig. 5b). On the other  
290 hand, survival of *S. orbiculatum* plants was reduced in heat-stressed plants compared with  
291 ambient plants, regardless of nutrient availability (Fig. 5b). Second, survival was influenced  
292 by heat stress depending upon the season in which the heat stress occurred, but post hoc  
293 differences could not be determined (Table 1; Fig. 5c). Within a season, survival was largely  
294 reduced in heat-stressed plants compared with ambient plants and fewer ambient plants  
295 survived in summer compared with their spring equivalents. Finally, species differences were  
296 apparent in relation to season, but post hoc differences could not be determined (Table 1; Fig.  
297 5c). Typically, *S. orbiculatum* had the poorest survival rates, irrespective of season, and  
298 *S. oligacanthum* survival was lower in summer than in spring (Fig. 5d). Visible damage was  
299 generally greatest in high nutrient plants in summer, compared with other treatment groups  
300 (Table 2). The influence of nutrient status on survival was converse to its influence on visible  
301 damage; a greater proportion of high nutrient plants survived than their low nutrient  
302 counterparts, and *S. oligacanthum* appeared to survive better than *S. orbiculatum* (Table 2).

### 303 *Fitness*

304 The number of flowers produced following heat stress treatment was determined by season and  
305 influenced by both nutrient and species (Table 1). For plants with access to high nutrients,  
306 flower production was high and there was no effect of season; for low nutrient plants, however,  
307 the number of flowers produced was reduced in summer compared with spring (Fig. 6a).  
308 Species differences were observed in flower production but dependent on season (Table 1): the  
309 number of flowers produced by *S. oligacanthum* was reduced from spring to summer (Fig. 6b).

310 Generally, *S. orbiculatum* produced fewer flowers than *S. oligacanthum* but unlike  
311 *S. oligacanthum*, the number produced increased from spring to summer (Fig. 6b). Plants that  
312 were heat-stressed produced fewer flowers than plants under ambient conditions (Table 1; Fig.  
313 6c). The biomass allocated to flowering was affected by an interaction of species with season  
314 and heat stress (Table 1). Regardless of season or heat stress treatment, *S. orbiculatum* allocated  
315 similarly low biomass to flowers compared *S. oligacanthum* (Fig. 6d). *Solanum oligacanthum*  
316 had notably high flower: aboveground biomass ratios in spring, which then dropped to being  
317 significantly lower in summer (Fig. 6d). Generally, there was a small, non-significant,  
318 reduction in fruit production in heat-stressed plants compared with ambient treatment plants;  
319 however, the greatest effect on fruit production was nutrient availability (Table 1). Plants with  
320 access to additional nutrients produced more fruit per plant than those in low nutrient soils (Fig.  
321 6e). The allocation of biomass to the fruit production was affected by a species by season  
322 interaction (Table 1), such that *S. oligacanthum* allocated low amounts of biomass to fruit,  
323 irrespective of season, but the amount of biomass allocated to fruit in *S. orbiculatum* was  
324 reduced in summer compared with spring (Fig. 6f). Comparison of proportional reproductive  
325 output of each species under different treatments clearly shows that *S. orbiculatum* has greater  
326 seed output per plant than *S. oligacanthum* (Fig. 6g,h). Seed production of both species was  
327 higher when plants had access to high nutrients, compared to low nutrient conditions. Overall,  
328 access to nutrients appeared to be the most important factor influencing reproductive fitness,  
329 with the contrast between species being greatest in summer, where the difference between low  
330 and high nutrient *S. orbiculatum* fitness was less defined than that of *S. oligacanthum* (Table  
331 2).

## 332 Discussion

333 In this study, we investigated the impacts on two desert *Solanum* species of nutrient level and  
334 timing of heat stress events on plants' short-term tolerance and their reproductive fitness in the  
335 longer term. There are a myriad of ways that heat stress can be experimentally applied; most  
336 often in growth cabinets and glasshouses under highly controlled conditions; less commonly  
337 in open top chambers under field conditions (De Boeck *et al.* 2012; Marchin *et al.* 2022). We  
338 deliberately applied our heat stress events under ambient weather conditions. This necessarily  
339 meant that plants experienced our experimental heat treatments in the context of natural  
340 environmental conditions, which varied both within and across seasons. Overall, we found that  
341 1) a heat stress event in summer is worse than a heat stress event in spring and 2) the influence  
342 of nutrient availability on downstream effects of heat stress is species-specific.

343 *Summer is harsh for desert plants and the worst time for a heat stress event*

344 Based on the reported importance of seasonal priming for coping with a subsequent stress event  
345 (Aspinwall *et al.* 2017), our initial expectation was that imposed heat stress might result in  
346 greater damage to plants in spring, which are not primed for high temperature extremes.  
347 However, our findings did not support this idea (Table 2). We suggest that summer was worse  
348 because firstly, these desert species maintain a high basal tolerance to high temperatures in  
349 spring (44 – 45°C for both study species, Milner *et al.* unpublished). Recent records of spring  
350 temperatures in these regions have included spikes of up to 45.4°C (BoM 2019). So, it follows  
351 that basal thermal tolerance for species adapted to this environment must be high to maintain a  
352 thermal safety margin (O'Sullivan *et al.* 2013; Drake *et al.* 2018). Therefore, despite not being  
353 primed for their early heat stress, our study species generally incurred less damage in spring  
354 than in summer, suggesting an inherent resilience to a-seasonal high temperatures.

355 The second reason plants experiencing a summer heatwave fared worse than in a spring  
356 heatwave, is likely due to the combined severity of heat stress imposed on the already stressful  
357 conditions typical of desert summer. In mesic environments, differential responses to the  
358 timing of stress events have been attributed to event severity. Whereas a heat spike in spring  
359 can be beneficial if temperatures rise to those optimal for photosynthesis (Marchand *et al.* 2005;  
360 De Boeck *et al.* 2011), even average temperatures in summer might be supra-optimal. Similarly  
361 in desert environments, a brief warm period in spring might stimulate photosynthesis, but not  
362 be sufficiently sustained to cause long-term damage. By contrast, summer desert conditions  
363 can exacerbate other stresses like water limitation; in fact, drought alone has a greater effect on  
364 plant health than heat stress alone (De Boeck *et al.* 2011; Davies *et al.* 2018). In our study, all  
365 our plants experienced some water stress, potentially exacerbated by an element of root  
366 crowding and certainly by higher VPD (Fig. C1; Appendix C), such that even in the absence  
367 of heat stress, our ambient treatment plants often did more poorly in summer than in spring.  
368 Therefore, even though leaf temperatures of heat-stressed plants were similar in spring and  
369 summer (Fig. 2), the additional severity of background conditions including a natural heatwave  
370 in summer will have compounded the effects of temperature stress, something that may not  
371 have been captured under glasshouse conditions. Our findings thus provide realistic insight into  
372 the susceptibility of desert plant species as the frequency of these kinds of events continues to  
373 increase as predicted (Cowan *et al.* 2014; Reddy *et al.* 2021).

374 Not only are conditions during a summer heat event more severe, but supra-optimal conditions  
375 surrounding the event also are likely to hamper recovery in the long term. Physiological  
376 recovery from heat stress occurs when benign temperatures (Drake *et al.* 2018; Guha *et al.*  
377 2018), including cooler nights (Atkin *et al.* 2005) and/or access to water (Wang & Huang  
378 2004), return. Although our plants were returned to water immediately following heat stress,  
379 long-term effects of drought on tissue damage, survival and fitness can manifest some months  
380 after the stress event (Wang *et al.* 2016; Davies *et al.* 2018). In our study, the longer-term  
381 damage was most visible and fruiting most reduced after summer heat stress, particularly in  
382 *S. orbiculatum* (Table 2), suggesting that physiological repair was somewhat suppressed,  
383 possibly due to lack of adequate recovery conditions. Repeat extreme events are likely in  
384 summer and indeed, our summer plants experienced naturally occurring air temperature spikes  
385 in the days before the heat stress treatment. Exposure to repeat heat events could have one of  
386 two likely outcomes: 1) prime plants for higher tolerance thresholds for the next heat event  
387 (Ahrens *et al.* 2021) or 2) cause injury/damage due to accumulated effects (Harris *et al.* 2018).  
388 The second outcome was probably the case for the plants in this study, which experienced  
389 higher heat loads overall. During these desert summers, there is a relative lack of cooler night-  
390 time temperatures (see Fig. C1; Appendix C for example), which coupled with reduced access  
391 to water and/or an increased likelihood of experiencing repeatedly stressfully high temperatures  
392 reduces opportunities for recovery.

### 393 *The effects of nutrient status on protection and downstream costs*

394 We would generally expect that plants with access to more resources will be better able to use  
395 nutrients to protect against heat damage (Heckathorn *et al.* 1996a) or recover (Zhao *et al.* 2008).  
396 Our plants illustrate different responses to summer heat stress, depending on whether this  
397 occurred under high or low nutrient conditions. For example, in *S. oligacanthum*, summer-  
398 stressed plants with access to high nutrients avoided short-term damage to PSII, but incurred  
399 greater membrane (Fig. 3c,d) and visible damage, and had reduced growth rate (Table 2);  
400 whereas under low nutrient conditions, it avoided short-term membrane instability (Table 2).  
401 Interestingly, the long-term outcomes for this species under high and low nutrients appeared to  
402 switch, with high nutrient plants able to survive and reproduce, while low nutrient plants were  
403 not (Table 2; Fig. 6e). Overall, for both species, those with access to high nutrients generally  
404 fared better than plants in low nutrients, even if they incurred damage (Table 2), suggesting  
405 that the cost of heat-related damage and repair on fitness can be mediated if these species have  
406 access to nutrients .

407 *Potential drivers for species-specific responses and fitness*

408 Ultimately, predicting persistence of species under altered occurrences of extreme heat events  
409 requires an understanding of the fitness costs. The resources allocated to reproduction and the  
410 ability to produce fruit are critical components of future survival. In this study, short-term  
411 responses to seasonality of heat stress were generally similar between our two species (Table  
412 2), but there was disparity in longer-term responses of survival and reproductive allocation.  
413 *Solanum orbiculatum* produced more, smaller seeds per fruit than *S. oligacanthum* (Table C1;  
414 Appendix C); however, irrespective of the difference in seed size, overall seed production per  
415 plant was far greater for *S. orbiculatum* than *S. oligacanthum* (Fig. 6h). These species-specific  
416 outcomes may reflect long-term adaptation to different microhabitats which then influence  
417 response to short-term stress.

418 The strategy of die-back and resprouting of ephemeral shoots by *S. oligacanthum* (Fig. A3,  
419 **Appendix A**), a trait used by stress-avoiders (Kassas & Girgis 1970), appears beneficial in its  
420 preferred microhabitat and for withstanding heat stress events. This species showed a strong  
421 tendency for vegetative resprouting, with lower LMA, higher stem:leaf ratio (Fig. 4a,b) and  
422 greatly reduced growth rate of leaves in summer compared with *S. orbiculatum* (Fig. 4e). In a  
423 similar way that fire resprouters allocate more biomass to storage than reseeders (Pate & Bell  
424 1999), the ability to store resources following severe damage may explain why *S. oligacanthum*  
425 had greater survival and maintained flowering after heat stress. Many resprouted  
426 *S. oligacanthum* had developed leaves during the experiment and the high survival rate of this  
427 species infers the potential to reproduce later (Friedman & Rubin 2015). Extended longevity  
428 via resprouting may be an adaptation to stochastic events in arid environments, where  
429 reproductive potential is stored for rare and episodic recruitment events (Nano & Clarke 2011  
430 and references therein). Through vegetative propagation, *S. oligacanthum* avoids the bad and  
431 can exploit the better conditions in the dynamic microhabitat in which it grows.

432 *Solanum orbiculatum*, which typically grows in resource-poor areas such as sandy dunes and  
433 plains, expresses traits reflecting this environment; for example, high LMA leaves. Unlike  
434 *S. oligacanthum*, we did not observe *S. orbiculatum* resprouting following severe heat stress  
435 damage. Instead, *S. orbiculatum* appeared to dedicate meristematic activity to immediate  
436 reproduction, with reduced GR<sub>AG</sub> (Fig. 4e) and increased flower numbers (Fig. 6b). Coupled  
437 with its low survival after heat stress, lack of resprouting in *S. orbiculatum* highlights the  
438 importance of high seed output for its ongoing persistence. Extreme conditions are said to

439 induce more clonal over sexual reproduction (Abeli *et al.* 2012). Given that summer heat has a  
440 marked negative impact on *S. orbiculatum*, and the high energy requirement for reproduction,  
441 there is likely to be strong selection to shift phenological patterns. However, as with many such  
442 species living in extreme regions, the prospects for adaptation outpacing currently poor survival  
443 under heat stress are fair at best.

444 Little work has looked at the timing of heat stress events in natural systems (De Boeck *et al.*  
445 2011; Wang *et al.* 2016) and few heat tolerance studies consider plants from extreme heat and  
446 drought environments. Our findings suggest that, for species that possess high basal thermal  
447 tolerances, like those in deserts, a-seasonal heat stress events occurring in spring pose less risk  
448 than they might for species in more benign environments. However, repeated events during the  
449 height of summer have implications for fitness and survival, especially in low nutrient  
450 conditions. While it would be useful to be able to generalise about how all species will endure  
451 increased summer heat stress, the species-based differences found in this study highlight the  
452 risk in doing so. To best understand and manage productivity and survival in harsh  
453 environments, attention should be directed to plant responses based on ecologically relevant  
454 signatures, such as life history and microhabitat.

#### 455 **Conflicts of Interest**

456 All authors declare no conflict of interest.

#### 457 **Declaration of Funding**

458 This research was undertaken as part of a collaboration agreement between the University of  
459 Technology Sydney and Port Augusta City Council, including support from the Friends of the  
460 Australian Arid Lands Botanic Gardens. This research was supported by an Australian  
461 Government Research Training Program Scholarship to KM.

#### 462 **Acknowledgements**

463 Collection of data was aided by Alicia Cook, Melinda Cook and Neil Berry. Further support  
464 was provided by staff and volunteers at Australian Arid Lands Botanic Gardens.

#### 465 **Author contribution**

466 Together with AL, SV and KF, KM designed the experimental procedure and methods. KM  
467 undertook the field work, analysis and led the writing of the paper. SV supervised lab work,  
468 while AL and KF supported the field work. DK helped with analysis of data. All authors

469 contributed critically to the drafts and gave final approval for the publication. This paper forms  
470 part of the PhD thesis of Milner (2020).

#### 471 **Data availability statement**

472 Upon publication, data will be made available through the Dryad Digital Repository.

#### 473 **References**

- 474 Abeli, T., Rossi, G., Gentili, R., Gandini, M., Mondoni, A. & Cristofanelli, P. (2012) Effect of the  
475 extreme summer heat waves on isolated populations of two orophitic plants in the north  
476 Apennines (Italy). *Nordic Journal of Botany*, **30**, 109-115.
- 477 Ahrens, C. W., Challis, A., Byrne, M., Leigh, A., Nicotra, A. B., Tissue, D., & Rymer, P. (2021).  
478 Repeated extreme heatwaves result in higher leaf thermal tolerances and greater safety  
479 margins. *New Phytologist*, **232**, 1212-1225.
- 480 Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger,  
481 T., Rigling, A., Breshears, D.D. & Hogg, E.T. (2010) A global overview of drought and heat-  
482 induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and*  
483 *Management*, **259**, 660-684.
- 484 Aspinwall, M.J., Vårhammar, A., Blackman, C.J., Tjoelker, M.G., Ahrens, C., Byrne, M., Tissue, D.T.  
485 & Rymer, P.D. (2017) Adaptation and acclimation both influence photosynthetic and  
486 respiratory temperature responses in *Corymbia calophylla*. *Tree Physiology*, **37**, 1095-1112.
- 487 Atkin, O.K., Bruhn, D., Hurry, V.M. & Tjoelker, M.G. (2005) The hot and the cold: unravelling the  
488 variable response of plant respiration to temperature. *Functional Plant Biology*, **32**, 87-105.
- 489 Atkin, O.K., Holly, C. & Ball, M.C. (2000) Acclimation of snow gum (*Eucalyptus pauciflora*) leaf  
490 respiration to seasonal and diurnal variations in temperature: the importance of changes in the  
491 capacity and temperature sensitivity of respiration. *Plant, Cell & Environment*, **23**, 15-26.
- 492 Atkin, O.K. & Tjoelker, M.G. (2003) Thermal acclimation and the dynamic response of plant  
493 respiration to temperature. *Trends in Plant Science*, **8**, 343-351.
- 494 Bauweraerts, I., Ameye, M., Wertin, T.M., McGuire, M.A., Teskey, R.O. & Steppe, K. (2014) Water  
495 availability is the decisive factor for the growth of two tree species in the occurrence of  
496 consecutive heat waves. *Agricultural and Forest Meteorology*, **189**, 19-29.
- 497 Bean, A.R. (2004) The taxonomy and ecology of *Solanum* subg. *Leptostemonum* (Dunal) Bitter  
498 (*Solanaceae*) in Queensland and far north-eastern New South Wales, Australia. *Austrobaileya*,  
499 **6**, 639-816.
- 500 Begcy, K., Weigert, A., Egesa, A.O. & Dresselhaus, T. (2018) Compared to Australian cultivars,  
501 European summer wheat (*Triticum aestivum*) overreacts when moderate heat stress is applied  
502 at the pollen development stage. *Agronomy-Basel*, **8**, 99.



503 Berry, J. & Bjorkman, O. (1980) Photosynthetic response and adaptation to temperature in higher-  
504 plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **31**, 491-543.

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

507 Bureau of Meteorology, (BoM). (2019) Australian Climate and Weather Extremes Monitoring System.  
508 Commonwealth of Australia.

509 Ciais, P., Reichstein, M., Viovy, N., Granier, A., Ogée, J., Allard, V., Aubinet, M., Buchmann, N.,  
510 Bernhofer, C. & Carrara, A. (2005) Europe-wide reduction in primary productivity caused by  
511 the heat and drought in 2003. *Nature*, **437**, 529-533.

512 Cook, A.M., Berry, N., Milner, K.V. & Leigh, A. (2021) Water availability influences thermal safety  
513 margins for leaves. *Functional Ecology*, **35**, 2179-2189.

514 Cowan, T., Purich, A., Perkins, S., Pezza, A., Boschhat, G. & Sadler, K. (2014) More frequent, longer,  
515 and hotter heat waves for Australia in the twenty-first century. *Journal of Climate*, **27**, 5851-  
516 5871.

517 Crawley, M.J. (2013) *The R book*, Second edn. John Wiley & Sons, Ltd, Chichester, West Sussex,  
518 United Kingdom.

519 Crous, K.Y., Drake, J.E., Aspinwall, M.J., Sharwood, R.E., Tjoelker, M.G. & Ghannoum, O. (2018)  
520 Photosynthetic capacity and leaf nitrogen decline along a controlled climate gradient in  
521 provenances of two widely distributed *Eucalyptus* species. *Global Change Biology*, **24**, 4626-  
522 4644.

523 Curtis, E.M., Knight, C.A., Petrou, K. and Leigh, A. (2014). A comparative analysis of photosynthetic  
524 recovery from thermal stress: a desert plant case study. *Oecologia*, **175**, 1051-1061.

525 Daniell, J.W., Chappell, W. & Couch, H. (1969) Effect of sublethal and lethal temperature on plant  
526 cells. *Plant Physiology*, **44**, 1684-1689.

527 Davies, M., Ecroyd, H., Robinson, S.A. & French, K. (2018) Stress in native grasses under ecologically  
528 relevant heat waves. *PLoS One*, **13**, e0204906.

529 De Boeck, H.J., Dreesen, F.E., Janssens, I.A. & Nijs, I. (2011) Whole-system responses of experimental  
530 plant communities to climate extremes imposed in different seasons. *New Phytologist*, **189**,  
531 806-817.

532 De Boeck, H.J., De Groote, T. & Nijs, I. (2012) Leaf temperatures in glasshouses and open-top  
533 chambers. *New Phytologist*, **194**, 1155-1164.

534 Djanaguiraman, M., Boyle, D.L., Welti, R., Jagadish, S.V.K. & Prasad, P.V.V. (2018) Decreased  
535 photosynthetic rate under high temperature in wheat is due to lipid desaturation, oxidation,  
536 acylation, and damage of organelles. *BMC Plant Biology*, **18**, 55.

537 Drake, J.E., Tjoelker, M.G., Vårhammar, A., Medlyn, B.E., Reich, P.B., Leigh, A., Pfautsch, S.,  
538 Blackman, C.J., López, R., Aspinwall, M.J., Crous, K.Y., Duursma, R.A., Kumarathunge, D.,  
539 De Kauwe, M.G., Jiang, M., Nicotra, A.B., Tissue, D.T., Choat, B., Atkin, O.K. & Barton,

540 C.V.M. (2018) Trees tolerate an extreme heatwave via sustained transpirational cooling and  
541 increased leaf thermal tolerance. *Global Change Biology*, **24**, 2390-2402.

542 Erskine, P.D., Stewart, G.R., Schmidt, S., Turnbull, M.H., Unkovich, M. & Pate, J.S. (1996) Water  
543 availability – a physiological constraint on nitrate utilization in plants of Australian semi-arid  
544 mulga woodlands. *Plant, Cell & Environment*, **19**, 1149-1159.

545 FloraNT (2013) FloraNT - Northern Territory flora online. (ed. N.T. Herbarium). Department of Land  
546 Resource Management.

547 Fox, J. & Weisber, S. (2011) *An R Companion to Applied Regression*, 2nd edn. Sage, Thousand Oaks,  
548 CA.

549 French, K., Jansens, I.B., Ashcroft, M.B., Ecroyd, H. & Robinson, S.A. (2019) High tolerance of  
550 repeated heatwaves in Australian native plants. *Austral Ecology*, **44**, 597-608.

551 Friedman, J. & Rubin, M.J. (2015) All in good time: Understanding annual and perennial strategies in  
552 plants. *American Journal of Botany*, **102**, 497-499.

553 Guha, A., Han, J.M., Cummings, C., McLennan, D.A. & Warren, J.M. (2018) Differential  
554 ecophysiological responses and resilience to heat wave events in four co-occurring temperate  
555 tree species. *Environmental Research Letters*, **13**, 065008.

556 Handley, L.L., Austin, A.T., Stewart, G.R., Robinson, D., Scrimgeour, C.M., Raven, J.A., Heaton,  
557 T.H.E. & Schmidt, S. (1999) The  $^{15}\text{N}$  natural abundance ( $\delta^{15}\text{N}$ ) of ecosystem samples reflects  
558 measures of water availability. *Functional Plant Biology*, **26**, 185-199.

559 Harris, R.M.B., Beaumont, L.J., Vance, T.R., Tozer, C.R., Remenyi, T.A., Perkins-Kirkpatrick, S.E.,  
560 Mitchell, P.J., Nicotra, A.B., McGregor, S., Andrew, N.R., Letnic, M., Kearney, M.R.,  
561 Wernberg, T., Hutley, L.B., Chambers, L.E., Fletcher, M.S., Keatley, M.R., Woodward, C.A.,  
562 Williamson, G., Duke, N.C. & Bowman, D.M.J.S. (2018) Biological responses to the press and  
563 pulse of climate trends and extreme events. *Nature Climate Change*, **8**, 579-587.

564 Havaux, M. (1993a) Characterization of thermal-damage to the photosynthetic electron-transport  
565 system in potato leaves. *Plant Science*, **94**, 19-33.

566 Havaux, M. (1993b) Rapid photosynthetic adaptation to heat-stress triggered in potato leaves by  
567 moderately elevated-temperatures. *Plant Cell & Environment*, **16**, 461-467.

568 Heckathorn, S.A., Poeller, G.J., Coleman, J.S. & Hallberg, R.L. (1996a) Nitrogen availability alters  
569 patterns of accumulation of heat stress-induced proteins in plants. *Oecologia*, **105**, 413-418.

570 Heckathorn, S.A., Poeller, G.J., Coleman, J.S. & Hallberg, R.L. (1996b) Nitrogen availability and  
571 vegetative development influence the response of ribulose 1,5-bisphosphate  
572 carboxylase/oxygenase, phosphoenolpyruvate carboxylase, and heat-shock protein content to  
573 heat stress in *Zea mays* L. *International Journal of Plant Sciences*, **157**, 546-553.

574 Hoffmann, A.A., Rymer, P.D., Byrne, M., Ruthrof, K.X., Whinam, J., McGeoch, M., Bergstrom, D.M.,  
575 Guerin, G.R., Sparrow, B., Joseph, L., Hill, S.J., Andrew, N.R., Camac, J., Bell, N., Riegler,

576 M., Gardner, J.L. & Williams, S.E. (2019) Impacts of recent climate change on terrestrial flora  
577 and fauna: Some emerging Australian examples. *Austral Ecology*, **44**, 3-27.

578 Holmgren, M., Stapp, P., Dickman, C.R., Gracia, C., Graham, S., Gutiérrez, J.R., Hice, C., Jaksic, F.,  
579 Kelt, D.A., Letnic, M., Lima, M., López, B.C., Meserve, P.L., Milstead, W.B., Polis, G.A.,  
580 Previtali, M.A., Richter, M., Sabaté, S. & Squeo, F.A. (2006) Extreme climatic events shape  
581 arid and semiarid ecosystems. *Frontiers in Ecology and the Environment*, **4**, 87-95.

582 Hovenden, M.J., Miglietta, F., Zaldei, A., Vander Schoor, J.K., Wills, K.E. & Newton, P.C.D. (2006)  
583 The TasFACE climate-change impacts experiment: Design and performance of combined  
584 elevated CO<sub>2</sub> and temperature enhancement in a native Tasmanian grassland. *Australian  
585 Journal of Botany*, **54**, 1-10.

586 Jentsch, A., Kreyling, J. & Beierkuhnlein, C. (2007) A new generation of climate-change experiments:  
587 Events, not trends. *Frontiers in Ecology and the Environment*, **5**, 365-374.

588 Jump, A.S. & Penuelas, J. (2005) Running to stand still: adaptation and the response of plants to rapid  
589 climate change. *Ecology Letters*, **8**, 1010-1020.

590 Kassas, M. & Girgis, W.A. (1970) Habitat and plant communities in the Egyptian desert: VII.  
591 geographical facies of plant communities. *Journal of Ecology*, **58**, 335-350.

592 Kimball, B.A., Conley, M.M., Wang, S., Lin, X., Luo, C., Morgan, J. & Smith, D. (2008) Infrared heater  
593 arrays for warming ecosystem field plots. *Global Change Biology*, **14**, 309-320.

594 Langsrud, Ø. (2003) ANOVA for unbalanced data: Use Type II instead of Type III sums of squares.  
595 *Statistics and Computing*, **13**, 163-167.

596 Leigh, A., Sevanto, S., Ball, M.C., Close, J.D., Ellsworth, D.S., Knight, C.A., Nicotra, A.B. & Vogel,  
597 S. (2012) Do thick leaves avoid thermal damage in critically low wind speeds? *New Phytologist*,  
598 **194**, 477-487.

599 Lenth, R. (2018) emmeans: Estimated marginal means, aka least-squares means. R package version  
600 1.3.1. Retrieved from <https://cran.r-project.org/web/packages/emmeans/index.html>.

601 Lewis, S.C., King, A.D. & Mitchell, D.M. (2017) Australia's unprecedented future temperature  
602 extremes under Paris limits to warming. *Geophysical Research Letters*, **44**, 9947-9956.

603 Marchand, F.L., Mertens, S., Kockelbergh, F., Beyens, L. & Nijs, I. (2005) Performance of High Arctic  
604 tundra plants improved during but deteriorated after exposure to a simulated extreme  
605 temperature event. *Global Change Biology*, **11**, 2078-2089.

606 Marchin, R.M., Backes, D., Ossola, A., Leishman, M.R., Tjoelker, M.G. & Ellsworth, D.S. (2022)  
607 Extreme heat increases stomatal conductance and drought-induced mortality risk in vulnerable  
608 plant species. *Global Change Biology*, **28**, 1133-1146.

609 Milner, K. (2020) The price of heat stress: functional and resource constraints to thermal tolerance in  
610 arid zone plants. PhD Thesis, University of Technology Sydney.  
611 (<https://opus.lib.uts.edu.au/bitstream/10453/142273/2/02whole.pdf>)

612 Mittler, R., Finka, A. & Goloubinoff, P. (2012) How do plants feel the heat? *Trends in Biochemical*  
613 *Sciences*, **37**, 118-125.

614 Nano, C.E.M. & Clarke, P.J. (2011) How do drought and fire influence the patterns of resprouting in  
615 Australian deserts? *Plant Ecology*, **212**, 2095-2110.

616 Nijs, I., Kockelbergh, F., Teughels, H., Blum, H., Hendrey, G. & Impens, I. (1996) Free Air  
617 Temperature Increase (FATI): A new tool to study global warming effects on plants in the field.  
618 *Plant, Cell & Environment*, **19**, 495-502.

619 O'Sullivan, O.S., Weerasinghe, K.L.K., Evans, J.R., Egerton, J.J., Tjoelker, M.G. & Atkin, O.K. (2013)  
620 High-resolution temperature responses of leaf respiration in snow gum (*Eucalyptus pauciflora*)  
621 reveal high-temperature limits to respiratory function. *Plant, Cell & Environment*, **36**, 1268-  
622 1284.

623 Parsons, P.A. (1990) The metabolic cost of multiple environmental stresses - implications for climatic-  
624 change and conservation. *Trends in Ecology & Evolution*, **5**, 315-317.

625 Pate, J.S. & Bell, T.L. (1999) Application of the ecosystem mimic concept to the species-rich Banksia  
626 woodlands of Western Australia. *Agroforestry Systems*, **45**, 303.

627 Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte,  
628 M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B.,  
629 Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., de Vos, A.C., Buchmann, N., Funes,  
630 G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., ter Steege, H., van der Heijden,  
631 M.G.A., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M.V., Conti, G., Staver, A.C., Aquino,  
632 S. & Cornelissen, J.H.C. (2013) New handbook for standardised measurement of plant  
633 functional traits worldwide. *Australian Journal of Botany*, **61**, 167-234.

634 Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J. & Villar, R. (2009) Causes and consequences of  
635 variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*, **182**, 565-588.

636 R Core Team (2018) R: A language and environment for statistical computing R Foundation for  
637 Statistical Computing, Vienna, Austria

638 Rajametov, S.N., Yang, E.Y., Jeong, H.B., Cho, M.C., Chae, S.Y. & Paudel, N. (2021) Heat treatment  
639 in two tomato cultivars: A study of the effect on physiological and growth recovery.  
640 *Horticulturae*, **7**, 119.

641 Reddy, P.J., Perkins-Kirkpatrick, S.E. & Sharples, J.J. (2021) Intensifying Australian heatwave trends  
642 and their sensitivity to observational data. *Earths Future*, **9**, e2020EF001924.

643 Reyer, C.P.O., Leuzinger, S., Rammig, A., Wolf, A., Bartholomeus, R.P., Bonfante, A., de Lorenzi, F.,  
644 Dury, M., Gloning, P., Abou Jaoudé, R., Klein, T., Kuster, T.M., Martins, M., Niedrist, G.,  
645 Riccardi, M., Wohlfahrt, G., de Angelis, P., de Dato, G., François, L., Menzel, A. & Pereira,  
646 M. (2013) A plant's perspective of extremes: terrestrial plant responses to changing climatic  
647 variability. *Global Change Biology*, **19**, 75-89.

648 Sharkey, T.D. (2005) Effects of moderate heat stress on photosynthesis: importance of thylakoid  
649 reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by  
650 isoprene. *Plant Cell & Environment*, **28**, 269-277.

651 Stafford Smith, D.M. & Morton, S.R. (1990) A framework for the ecology of arid Australia. *Journal of*  
652 *Arid Environments*, **18**, 255-278.

653 Steffen, W., Hughes, L. & Perkins, S.E. (2014) Heatwaves: hotter, longer, more often. Climate Council  
654 of Australia Ltd.

655 Tang, Y., Wen, X., Lu, Q., Yang, Z., Cheng, Z. & Lu, C. (2007) Heat stress induces an aggregation of  
656 the light-harvesting complex of photosystem II in spinach plants. *Plant Physiology*, **143**, 629-  
657 638.

658 Teskey, R., Wertin, T., Bauweraerts, I., Ameye, M., McGuire, M.A. & Steppe, K. (2015) Responses of  
659 tree species to heat waves and extreme heat events. *Plant Cell and Environment*, **38**, 1699-  
660 1712.

661 Valladares, F. & Pearcy, R.W. (1997) Interactions between water stress, sun-shade acclimation, heat  
662 tolerance and photoinhibition in the sclerophyll *Heteromeles arbutifolia*. *Plant Cell and*  
663 *Environment*, **20**, 25-36.

664 Vicente-Serrano, S., M. , Lopez-Moreno, J.I., Beguería, S., Lorenzo-Lacruz, J., Sanchez-Lorenzo, A.,  
665 García-Ruiz, J., M. , Azorin-Molina, C., Morán-Tejeda, E., Revuelto, J., Trigo, R., Coelho, F.  
666 & Espejo, F. (2014) Evidence of increasing drought severity caused by temperature rise in  
667 southern Europe. *Environmental Research Letters*, **9**, 044001.

668 Wang, D., Heckathorn, S.A., Mainali, K. & Tripathee, R. (2016) Timing effects of heat-stress on plant  
669 ecophysiological characteristics and growth. *Frontiers in Plant Science*, **7**, 1629.

670 Wang, Z.L. & Huang, B.R. (2004) Physiological recovery of Kentucky bluegrass from simultaneous  
671 drought and heat stress. *Crop Science*, **44**, 1729-1736.

672 Ward, E.J., Domec, J.-C., Laviner, M.A., Fox, T.R., Sun, G., McNulty, S., King, J. & Noormets, A.  
673 (2015) Fertilization intensifies drought stress: Water use and stomatal conductance of *Pinus*  
674 *taeda* in a midrotation fertilization and throughfall reduction experiment. *Forest Ecology and*  
675 *Management*, **355**, 72-82.

676 Zhao, W.Y., Xu, S., Li, J.L., Cui, L.J., Chen, Y.N. & Wang, J.Z. (2008) Effects of foliar application of  
677 nitrogen on the photosynthetic performance and growth of two fescue cultivars under heat  
678 stress. *Biologia Plantarum*, **52**, 113-116.

679 Zscheischler, J. & Seneviratne, S.I. (2017) Dependence of drivers affects risks associated with  
680 compound events. *Science Advances*, **3**, e1700263.

681

682 Table 1. F-values of four factors in models of physiological and growth traits of *Solanum oligacanthum*  
683 and *S. orbiculatum*; factors were species, season (spring *versus* summer), nutrient treatment (high  
684 *versus* low) and heat stress treatment (ambient *versus* heat stress).

	D <sub>PSII</sub>	MSI	stem: leaf	LMA	GR <sub>AG</sub>	Visibl e dama ge	Survi val	Flowe r no.	Fruit no.	Flower :AG	Fruit: AG
species	0.22	<b>38.22</b> ***	<b>16.91</b> ***	<b>24.29</b> ***	0.01	1.59	<b>10.10</b> **	72.78	1.14	<b>19.45*</b> **	<b>7.57*</b> *
season	<b>8.07</b> **	<b>12.92</b> ***	<b>14.90</b> ***	0.14	<b>58.79</b> ***	0.80	1.42	4.85	3.82	<b>19.21*</b> **	<b>7.83*</b> *
nutrient	1.39	<b>5.23*</b>	<b>14.74</b> ***	0.34	<b>14.25</b> ***	0.10	0.17	<b>129.73</b> ***	<b>39.99</b> ***	1.73	0.21
temperature	<b>8.74</b> **	<b>6.55*</b>	1.26	2.32	<b>4.98*</b>	<b>20.43</b> ***	<b>16.18</b> ***	<b>8.78**</b>	2.77	0.00	0.07
species * season		<b>12.44</b> **	0.96	1.92	<b>4.82*</b>		<b>4.19*</b>	<b>30.57*</b> **	2.14	<b>11.86*</b> *	<b>15.53</b> ***
species * nutrient		2.31	1.53	0.26	0.12		3.03	3.28		0.26	
species * temperature		0.00	1.70	0.26	1.02		0.04	0.37	2.17	0.51	2.12
season * nutrient		<b>14.63</b> ***	0.13	0.47	<b>6.84*</b>	1.01	2.33	<b>5.37*</b>		0.10	3.62
season * temperature		0.24	0.07	1.89	1.59	3.13	<b>6.40*</b>	2.05	1.78	0.21	2.45
nutrient * temperature		0.55	0.00	3.64	0.20	<b>4.33*</b>	1.47	3.60		0.55	
species * season * nutrient			3.07	1.66	1.82			2.76			
species * season * temperature				2.58						<b>4.64*</b>	
species * nutrient * temperature			2.32				<b>5.58*</b>				
season * nutrient * temperature		<b>4.21*</b>								2.86	
<i>degrees of freedom</i>	1,57	1,50	1,51	1,45	1,50	1,54	1,50	1,50	1,54	1,45	1,49
<i>transformation</i>	neg. √	logit	log <sub>10</sub>	√				log <sub>10</sub>	√	√	√

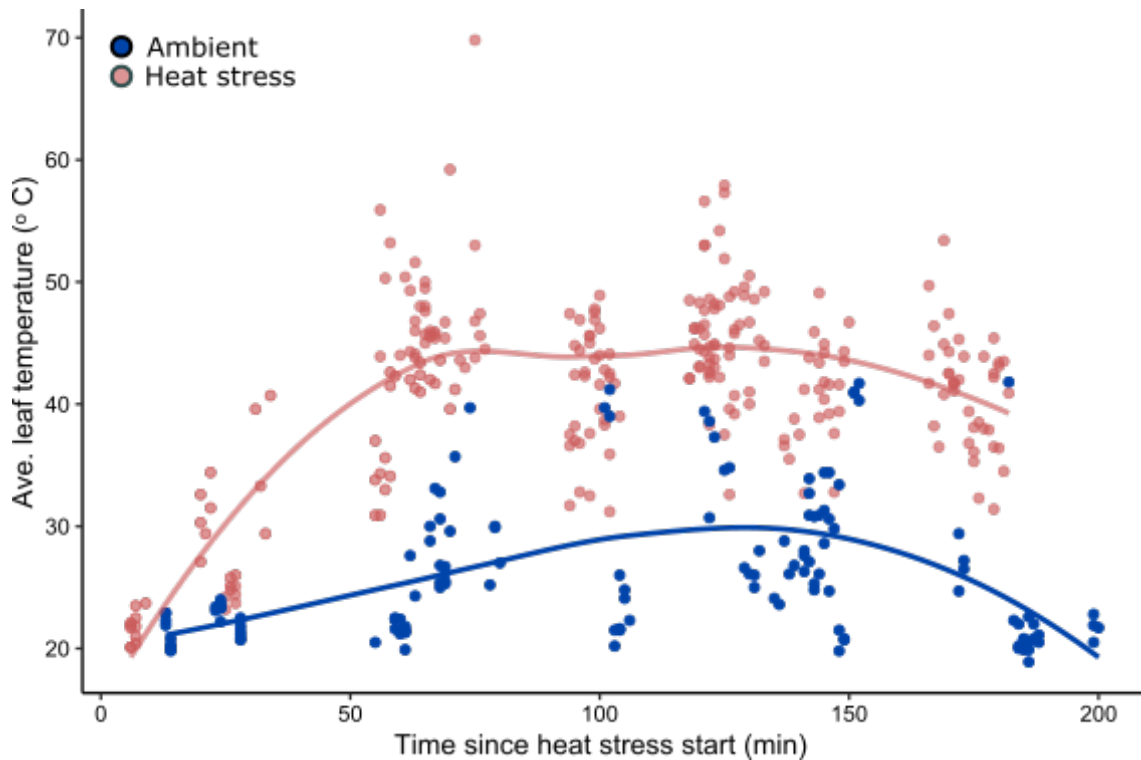
685 Analysis of variance was used for all variables, except damage and survival which were analysed using  
686 general linear models. In both analyses, models were simplified by step-wise removal of non-significant  
687 interactions. Bolded *F*-values are significant at *P*-value < 0.05. Levels of significance denoted as  
688 follows: \*\*\* = *p* < 0.001; \*\* = *p* < 0.01; \* = *p* < 0.05. Variable descriptions: D<sub>PSII</sub>, damage to PSII;  
689 MSI, membrane stability index; stem:leaf, ratio of stem to leaf biomass; LMA, mean leaf mass per area;  
690 GR<sub>AG</sub>, growth rate of aboveground biomass; visible damage, visual damage to plants analysed as a  
691 proportion, where <10% damage = 0, >10% damage = 1; survival, proportion of plants survived;  
692 flower/fruit no, number of flowers/fruits produced per day since heat stress; flower/fruit:AG, ratio of  
693 flower/fruit mass to aboveground biomass. All biomass are dry weights.

694 Table 2. Heat map of *Solanum oligacanthum* and *S. orbiculatum* responses to heat stress normalised  
 695 within a response variable.

Species	Season	Nutrient treat.	Heat stress treat.	D <sub>PSII</sub>	MSI	GR <sub>AG</sub>	Vis. Damage	Survival	Flower /day	Fruit /day	Flower:AG	Fruit:AG	sum total
<i>S. oligacanthum</i>	spring	high	ambient	0.31	0.84	0.93	0.33	0.58	0.01	0.11	0.05	0.08	3
			HS	0.92	0.85	0.94	0.98	0.58	0.01	0.14	0.09	0.25	5
		low	ambient	0.14	0.84	0.95	0.00	0.58	0.02	0.31	0.08	0.20	3
			HS	0.82	0.85	0.96	0.98	0.64	0.09	0.95	0.09	0.14	6
	summer	high	ambient	-0.12	0.91	0.98	1.00	0.66	0.02	0.19	0.40	0.15	4
			HS	0.11	1.00	0.99	1.00	0.58	0.02	0.10	0.12	0.01	4
		low	ambient	-0.04	0.85	1.00	0.00	0.58	0.07	1.00	0.40	0.67	5
			HS	0.54	0.86	0.99	0.85	0.78	0.11	0.69	0.45	0.02	5
<i>S. orbiculatum</i>	spring	high	ambient	0.12	0.83	0.93	0.00	0.58	0.05	0.05	0.32	0.01	3
			HS	1.00	0.83	0.94	0.63	0.78	0.06	0.08	0.29	0.01	5
		low	ambient	0.41	0.83	0.96	0.00	0.58	0.53	0.25	1.00	0.01	5
			HS	0.83	0.84	0.99	0.98	1.00	1.00	0.47	0.22	0.01	6
	summer	high	ambient	0.08	0.83	0.98	0.38	0.70	0.02	0.08	0.38	0.04	3
			HS	-0.01	0.87	0.98	0.63	0.87	0.04	0.21	0.54	0.04	4
		low	ambient	-0.05	0.83	0.97	0.33	0.64	0.12	0.77	0.49	0.29	4
			HS	0.85	0.83	0.99	0.53	0.70	0.15	0.68	0.47	1.00	6

696 Within each variable, the severity of response incurred during the seasonal heat stress experiment was  
 697 normalised to the treatment group with the strongest mean response/damage. Normalised vales were  
 698 coloured to aid in comparison, where 1 = most damage (red) and 0 = no damage (blue). Note: Values  
 699 displayed to 2 decimal places, but colours are derived from more decimal places. Variable  
 700 descriptions: D<sub>PSII</sub>, damage to PSII; MSI, membrane stability index; GR<sub>AG</sub>, growth rate of  
 701 aboveground biomass (GR<sub>AG</sub> included negative values so transformed by adding 1); Vis. damage,  
 702 proportion of plants showing visible damage; Flower/Fruit:AG, flower/fruit mass to above ground  
 703 biomass; Flower/Fruit per day, number of flowers or fruits produced per day since heat stress;  
 704 Survival, proportion of plants that survived the experiment; Sum total, sum of all variables. A sum  
 705 total close to nine indicates that plants did poorly across all response measures.

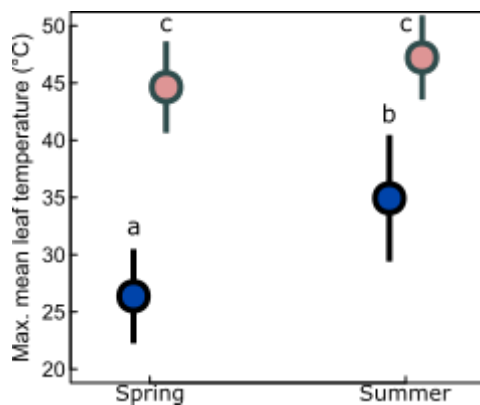
706



707

708 **Fig. 1** Mean leaf temperatures during imposed heat stress (red points) and of ambient plants  
 709 (dark blue points). Temperature was ramped in first 60 min, then maintained at ~45°C for  
 710 remaining 120 min (see Appendix C for detail). Data shown are all measured *Solanum* plants  
 711 from spring and summer. Mean leaf temperature is the average of three leaves per plant. Solid  
 712 lines show the loess smoothing of leaf temperatures.

713

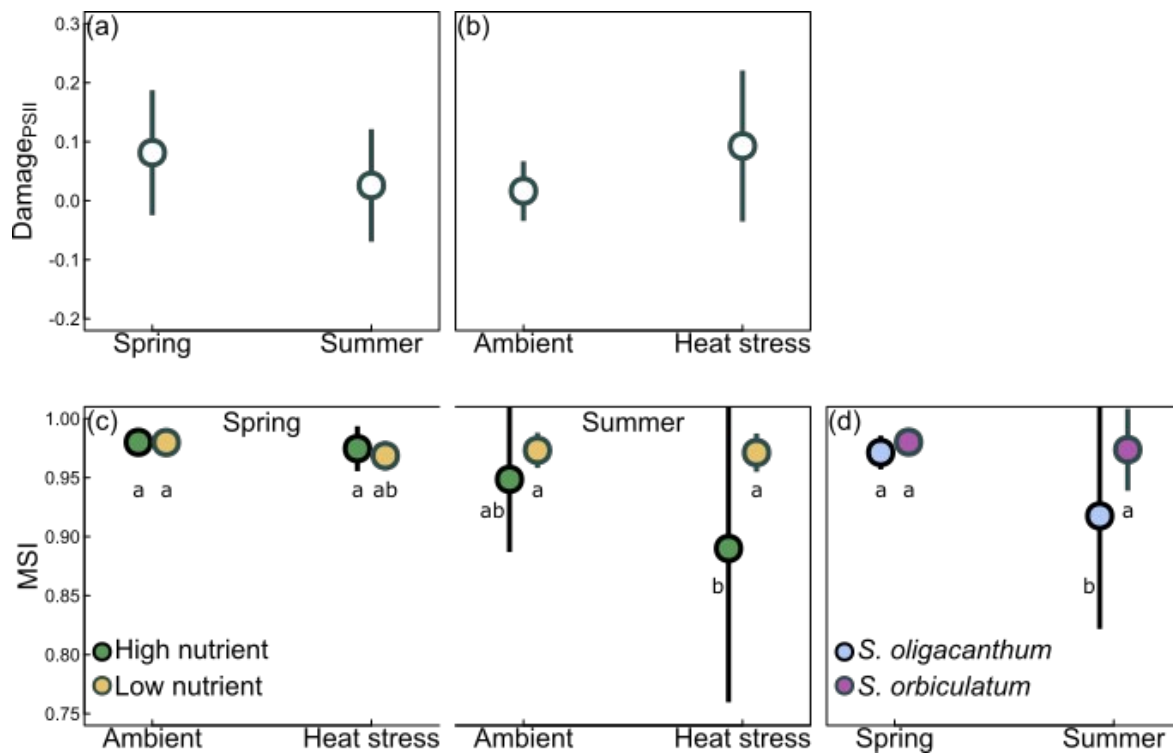


714

715 **Fig. 2** Mean maximum leaf temperatures ( $\pm$  SD,  $n = 4$ ) recorded during a seasonal heat stress  
 716 experiment on *Solanum oligacanthum* and *S. orbiculatum* in southern arid Australia. Plants  
 717 were placed in chambers for imposed heat stress (red) or left in ambient conditions (dark blue).  
 718 Different letters indicate significant ( $p < 0.05$ ) differences between the means of treatment  
 719 responses.

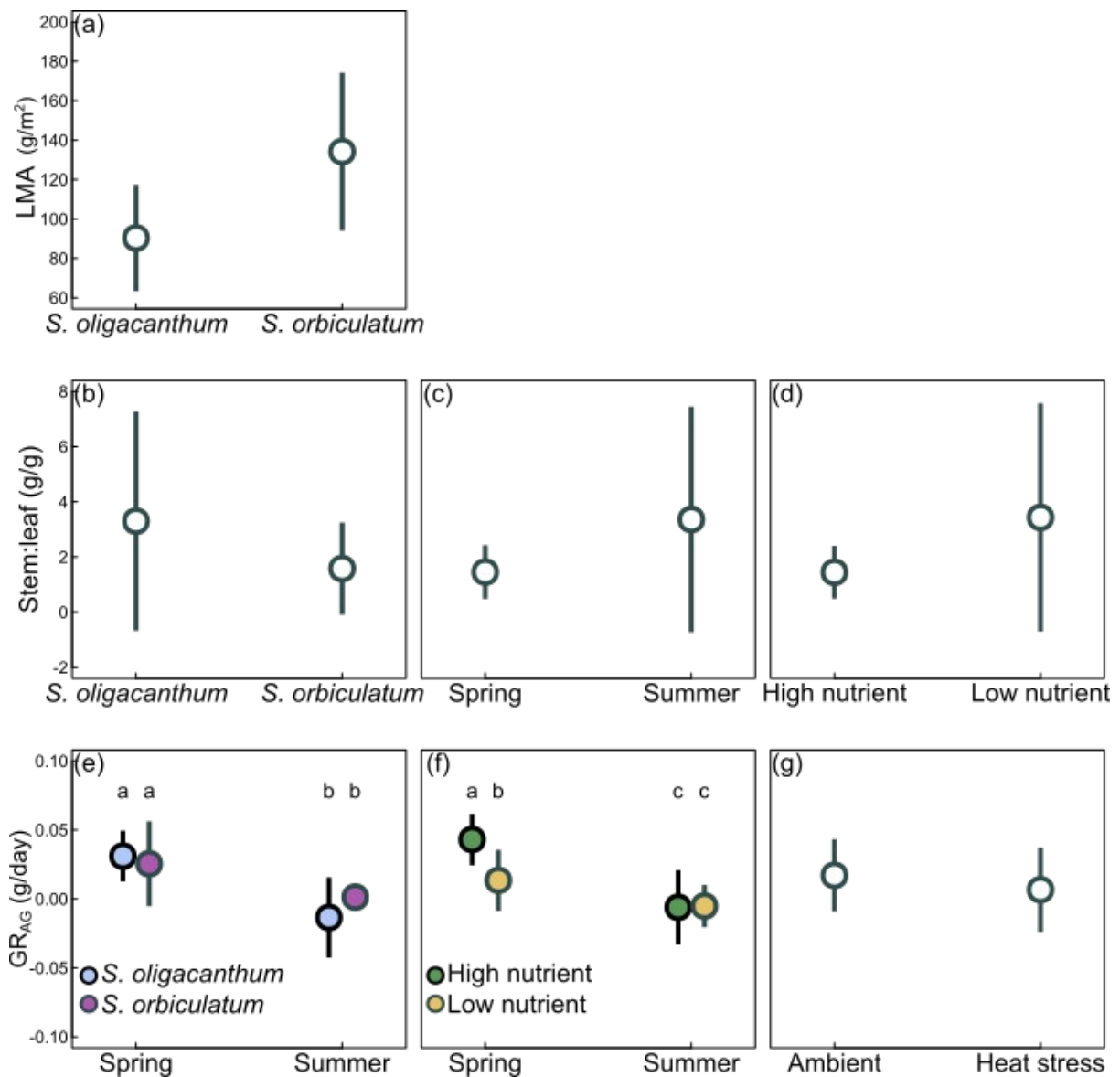


720



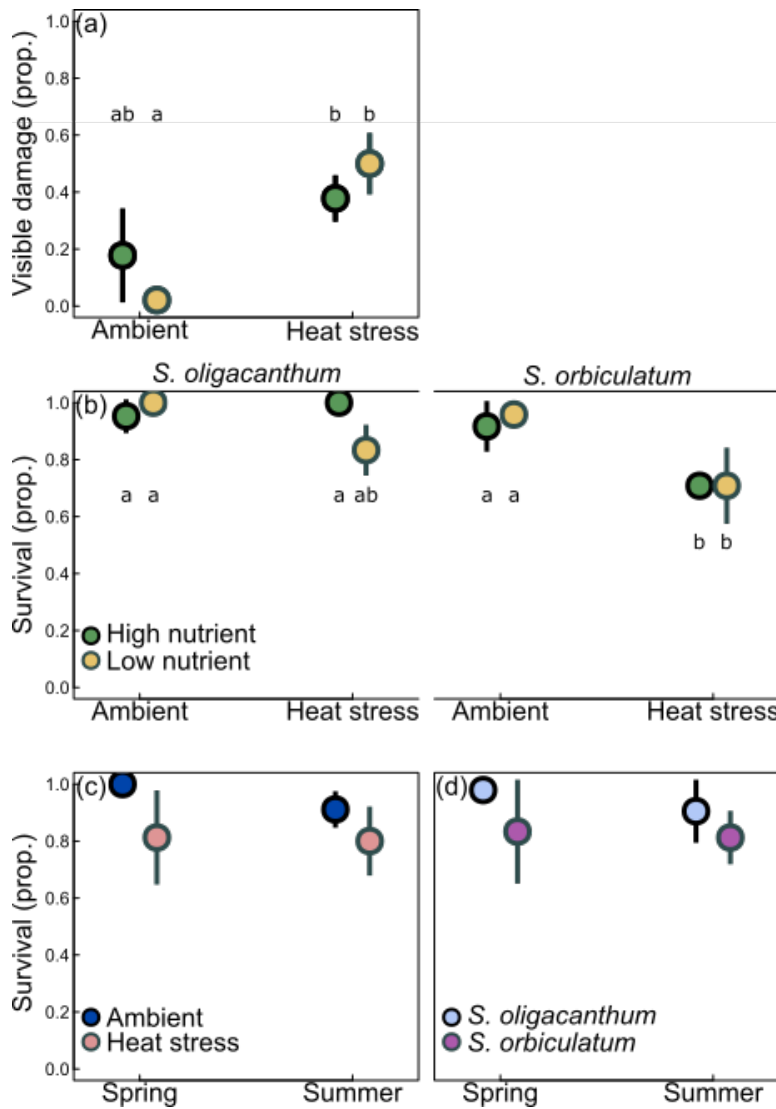
721

722 **Fig. 3** Mean ( $\pm$  SD) short-term physiological responses of *Solanum oligacanthum* and *Solanum*  
723 *orbiculatum* during a heat stress experiment in southern arid Australia. Main factor effects of  
724 damage to PSII (a,b). Factor interactions influencing membrane stability (MSI; c,d): c  
725 represents a three-way interaction with the colours of symbols indicative of whether plants  
726 were grown in high or low nutrients (dark green and yellow, respectively); d shows a two-way  
727 interaction with *S. oligacanthum* (blue) and *S. orbiculatum* (purple). ,n = 4 with exception of  
728 *S. oligacanthum* summer high nutrient C and HS where n = 3. Different lower-case letters  
729 above symbols indicate significant differences ( $p < 0.05$ ) among the means of treatments.



730

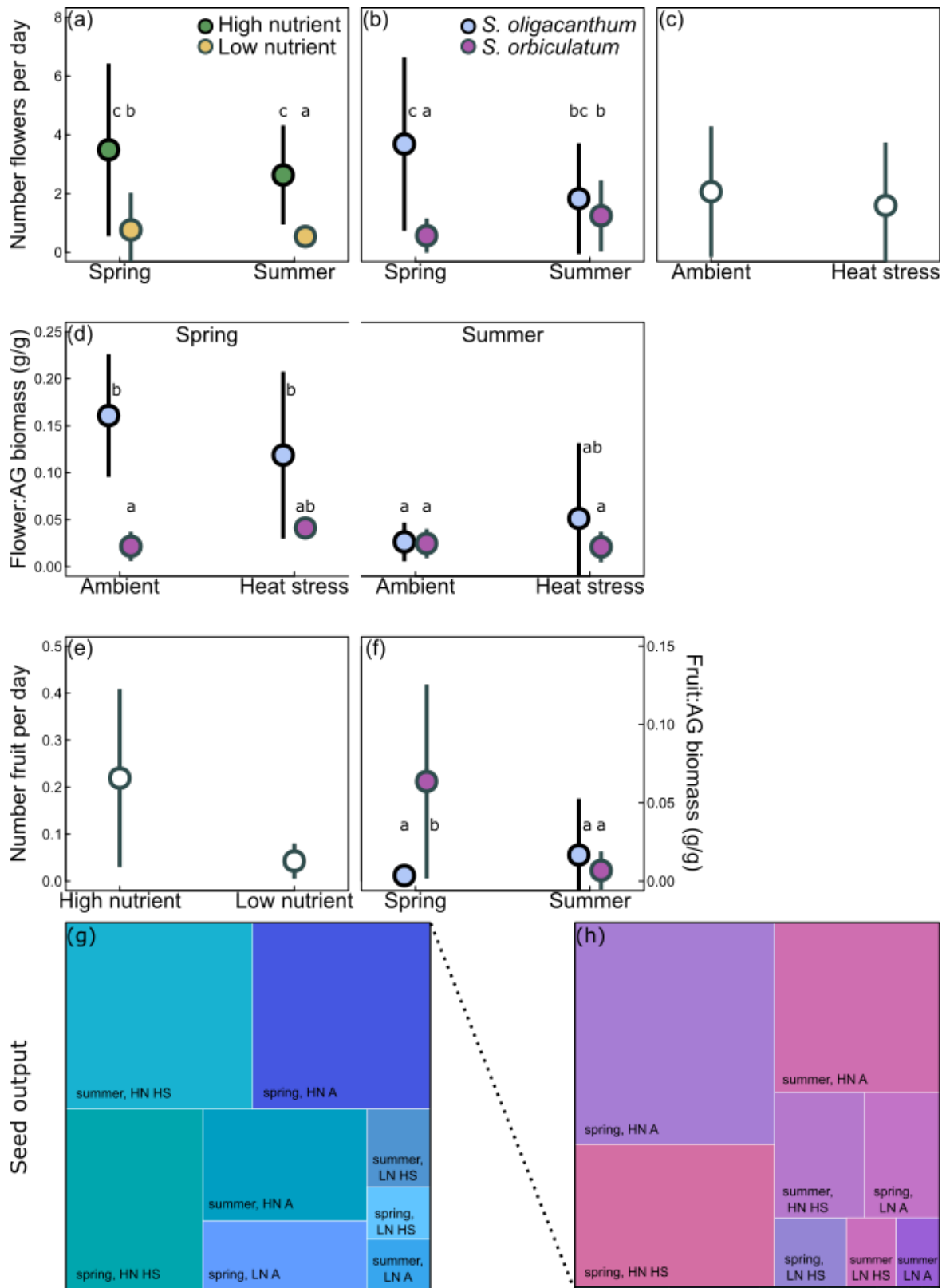
731 **Fig. 4** Responses of growth and allocation of biomass in two species of *Solanum* subject to  
 732 heat stress (mean  $\pm$  SD). *Solanum oligacanthum* and *S. orbiculatum* were grown in high or low  
 733 nutrients and subjected to heat stress or ambient conditions in either spring or summer. Colours  
 734 are described in Fig. 3. Variables are: LMA (a); stem to leaf ratio (b); and growth rate of leaves  
 735 (GR<sub>AG</sub>). All biomass are dry weights. Significant interactions are plotted in e and f. LMA n =  
 736 4, except *S. oligacanthum* summer high nutrient ambient and HS and *S. oligacanthum* spring  
 737 low nutrient HS where n = 3 and *S. orbiculatum* spring low nutrient ambient where n = 2. Stem  
 738 to leaf ratio and GR<sub>AG</sub> n = 4 with exception of *S. oligacanthum* summer high nutrient ambient  
 739 and HS where n = 3. Different lower-case letters above symbols indicate significant ( $p < 0.05$ )  
 740 differences between the means of treatments. Note that panels e and f show two-way  
 741 interactions and a-d,g are main factors. Means of main factors of aboveground biomass can be  
 742 seen in Table A1; Appendix A.



743

744 **Fig. 5** Visible damage and survival of desert *Solanum* species (*Solanum oligacanthum* and  
 745 *S. orbiculatum*) following heat stress treatment (mean  $\pm$  SD). Ambient in dark blue, heat stress  
 746 in red, other colours are explained in Fig. 3. Proportion of plants with visible damage greater  
 747 than 10% (a); proportion of surviving plants (b-d). Significant two-way interactions are shown  
 748 in panels a, c and d and three-way interaction in panel b.  $n = 4$  with exception of  
 749 *S. oligacanthum* summer high nutrient ambient and HS where  $n = 3$ . Different lower-case  
 750 letters above symbols indicate significant ( $p < 0.05$ ) differences between the means of  
 751 treatments. Note that panel b represents a three-way interaction and a, c and d show two-way  
 752 interactions.

753



754

755 **Fig. 6** Fitness and allocation of resources to reproductive structures of desert *Solanum* species  
 756 in response to nutrient availability and seasonal heat stress (mean  $\pm$  SD). Colours are explained  
 757 in Fig. 3. Number of flowers produced per day following heat stress treatment (a-c); flower  
 758 mass to aboveground (AG) biomass (d); Number of fruits produced per day following heat

759 stress treatment (e); flower mass to AG biomass (f). Note, panels c and e show main factors,  
760 two-way interactions are shown in panels a,b,f and a three-way interaction in panel d. Flower  
761 and fruit per day  $n = 4$  with exception of *S. oligacanthum* summer high nutrient ambient and  
762 HS where  $n = 3$ . Flower and fruit to AG  $n = 4$ , except *S. oligacanthum* summer high nutrient  
763 ambient and HS and *S. oligacanthum* spring low nutrient HS where  $n = 3$  and *S. orbiculatum*  
764 spring low nutrient ambient where  $n = 2$ . Different letters indicate significant ( $p < 0.05$ )  
765 differences between the means of treatments. Relative proportional representation of estimated  
766 seed output of *Solanum oligacanthum* (g) and *S. orbiculatum* (h). Fruit were harvested  
767 following heat stress treatment (ambient, A; or heat stress, HS) on plants grown in low (LN)  
768 or high (HN) nutrients in spring or summer. The mean number of seeds  $\text{plant}^{-1}$  was calculated  
769 using the mean number of seeds  $\text{fruit}^{-1} \times \text{number of fruit plant}^{-1} \text{ day}^{-1}$ . Note that the panel on  
770 the right contains both species, with *S. oligacanthum* represented by the very narrow strip at  
771 bottom, which is magnified on the left to show *S. oligacanthum* seed output only. Mean seed  
772 output by each species by factor is shown in Table A1; Appendix A. All biomass are dry  
773 weights.

Effects of seasonal heat events on arid zone plant species Milner et al.

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

K.V. Milner, K. French, D.W Krix, S.M. Valenzuela, A. Leigh

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

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

**Fig. A1** Schematic of experimental design and timeline of seasonal heat stress experiment.

**Fig. A2** Maximum quantum yield ( $F_v/F_m$ ) of *Solanum* plants pre- and post-heat stress.

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

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

**Methods B1** Additional methodological details on leaf protein extraction.

**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.

**Fig. B1** The effect of fertiliser application on nitrogen status of *Solanum oligacanthum* (left) and *Solanum orbiculatum* (right) leaves.

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

**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 ( $^{\circ}$ C) and VPD (kPa) during four replicate heat stress treatments (one replicate per row) imposed in spring (a, c) and summer (b, d).

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

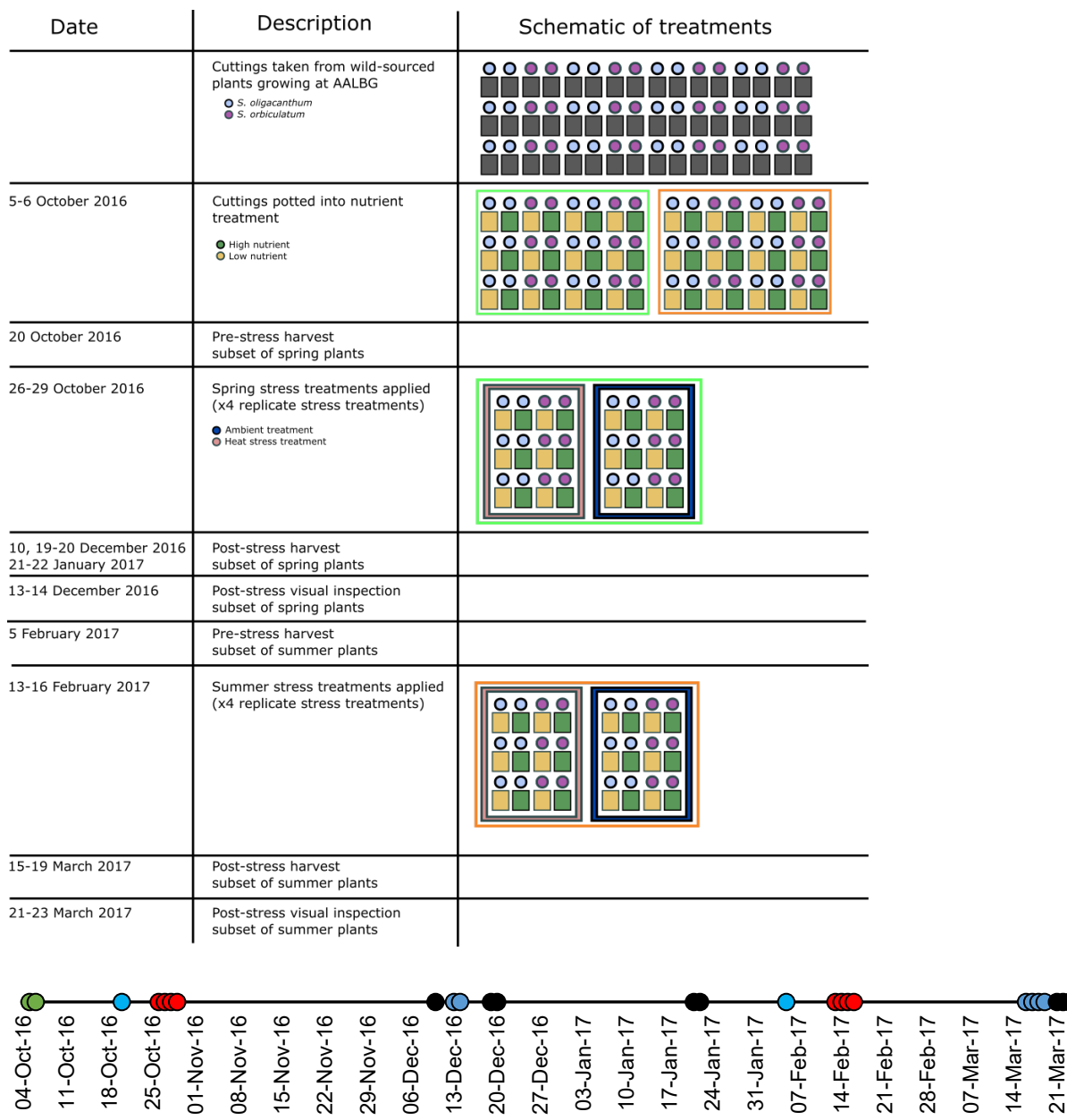
Table A1. Main factor means ( $\pm$  SE) of short- and long-term responses to heat stress experiment during spring *versus* summer.

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

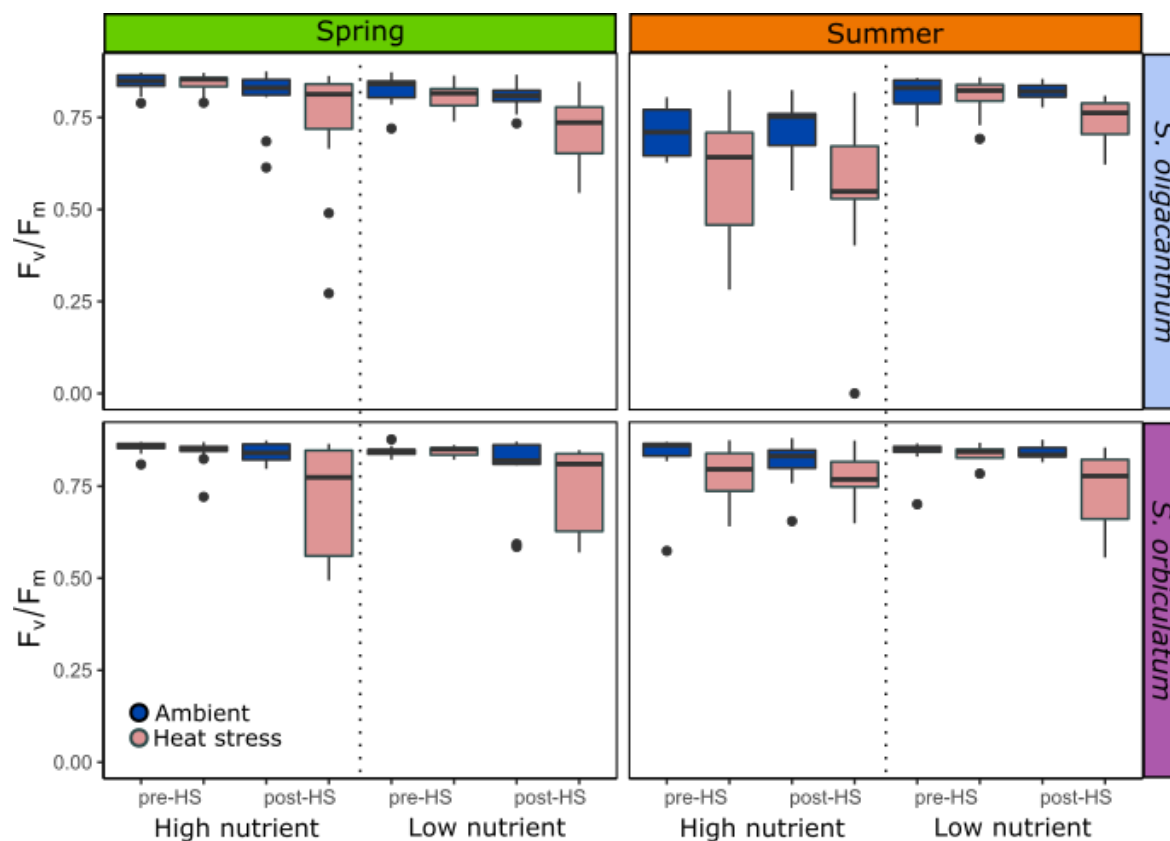
	Species		Season		Nutrient		Heat stress treatment	
	<i>S. oligacanthum</i>	<i>S. orbiculatum</i>	Spring	Summer	High	Low	Ambient	Heat stress
LA (m <sup>2</sup> )	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.01 ± 0	0.05 ± 0.01	0.04 ± 0.01
Pre-Root mass (g)	8.69 ± 3.93	11.28 ± 4.36	0.8 ± 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)	<i>S. oligacanthum</i> (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
	<i>S. orbiculatum</i> (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<sup>2</sup>), total LA of plant; Seed output, the number of seeds fruit<sup>-1</sup> 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  $1.5 \times \text{IQR}$  of lower and upper quartiles respectively).



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

## **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 powder 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.

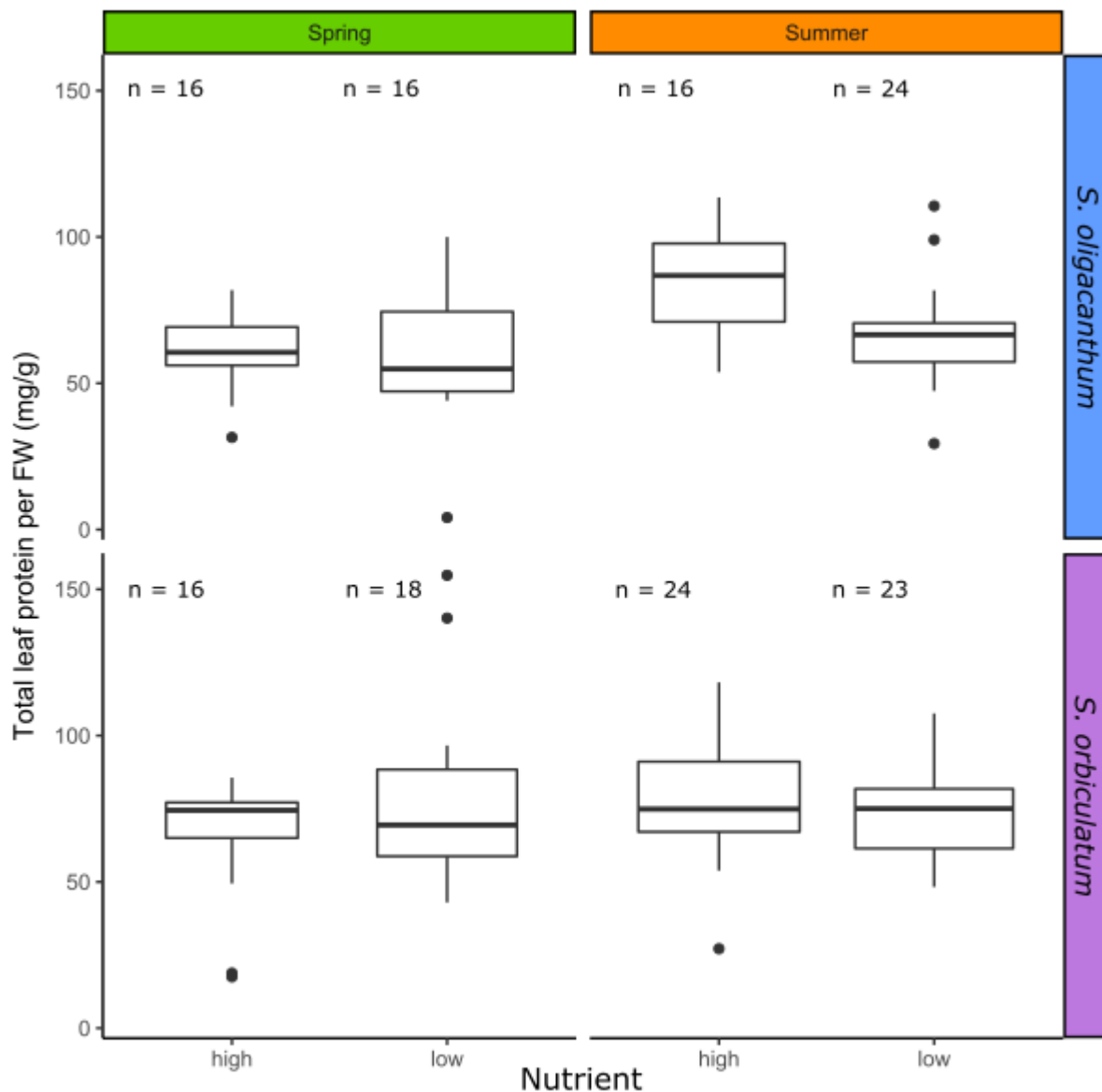
Additional fertiliser increased the leaf nitrogen content of fertilised plants in comparison with plants without fertiliser, although the differences were affected by season and species (Table B1; Fig. B1). The leaf protein content of plants sampled in spring did not differ, regardless of nutrient treatment; however, in summer, leaf protein content was significantly 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.

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



**Fig. B1** The effect of fertiliser 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).

### 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 ( $\Psi_L$ ) was compared with that of well-watered plants grown alongside experimental plants. In spring, although non-significant  $\Psi_L$  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,  $\Psi_L$  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  $1347 \pm 118 \text{ mmol m}^{-2} \text{ s}^{-1}$  was similar to saturating light levels for Australian desert plants (e.g.,  $1200 \text{ mmol m}^{-2} \text{ s}^{-1}$  PAR for *Acacia anuera* in arid Northern Territory; (Wujeska-Klaue *et al.* 2015). Photoperiod in October and February when heat stresses were imposed was approximately 13 h. With an average annual PAR of approximately  $1500 \text{ mmol m}^{-2} \text{ day}^{-1}$  (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  $0.04\text{-}1.14 \text{ ms}^{-1}$  inside chambers and  $0.09\text{-}8.9 \text{ ms}^{-1}$  outside chambers, with greater variance (SD) outside than in chambers ( $1.30$  and  $0.20 \text{ ms}^{-1}$  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  $\pm 2.5\%^\circ\text{C}$ ; IP67; Jaycar, NSW, Australia) and a thermographic camera with emissivity set to 0.95 (accuracy  $\pm 2^\circ\text{C}$  or  $\pm 2\%$  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 ( $T_{\text{air}}$ ) 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:

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

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 90<sup>th</sup> 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, 90<sup>th</sup> 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.



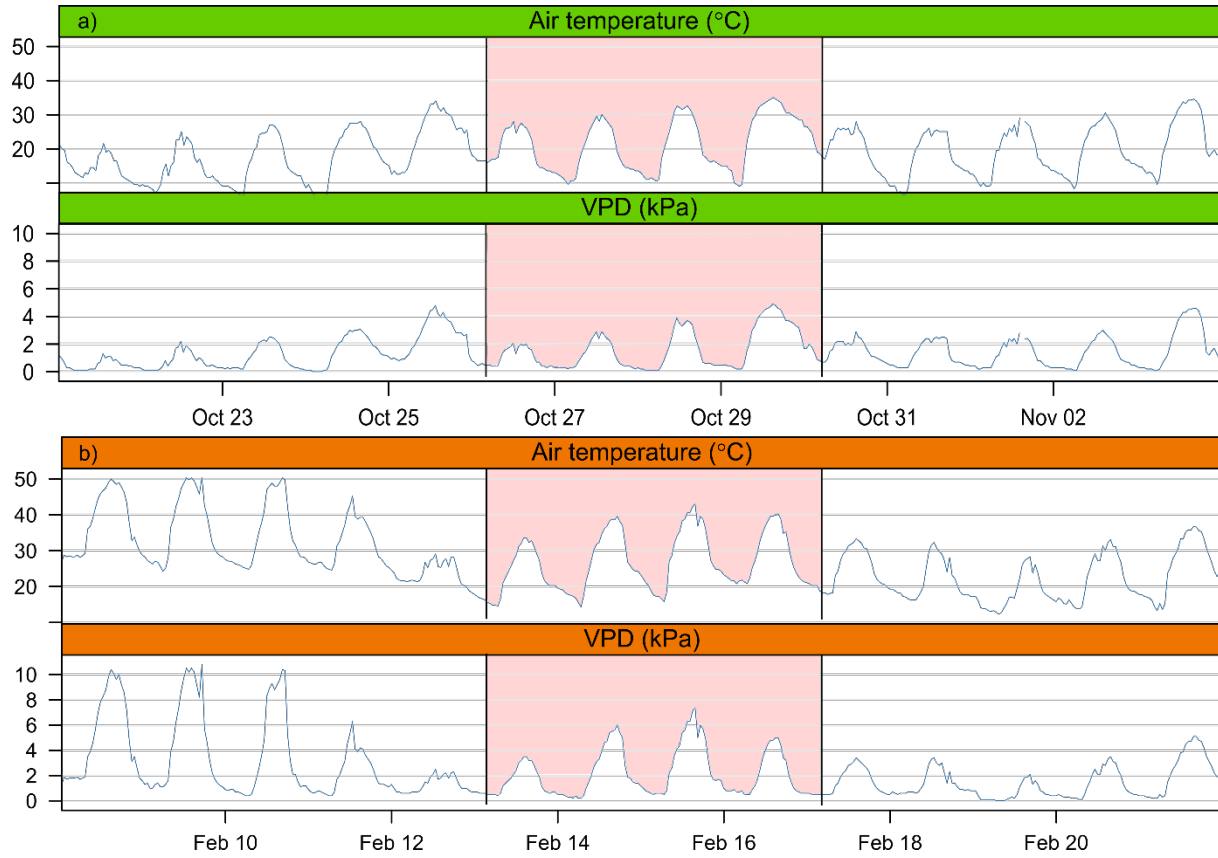
Effects of seasonal heat events on arid zone plant species Milner et al.

Wujeska-Klaue, 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.

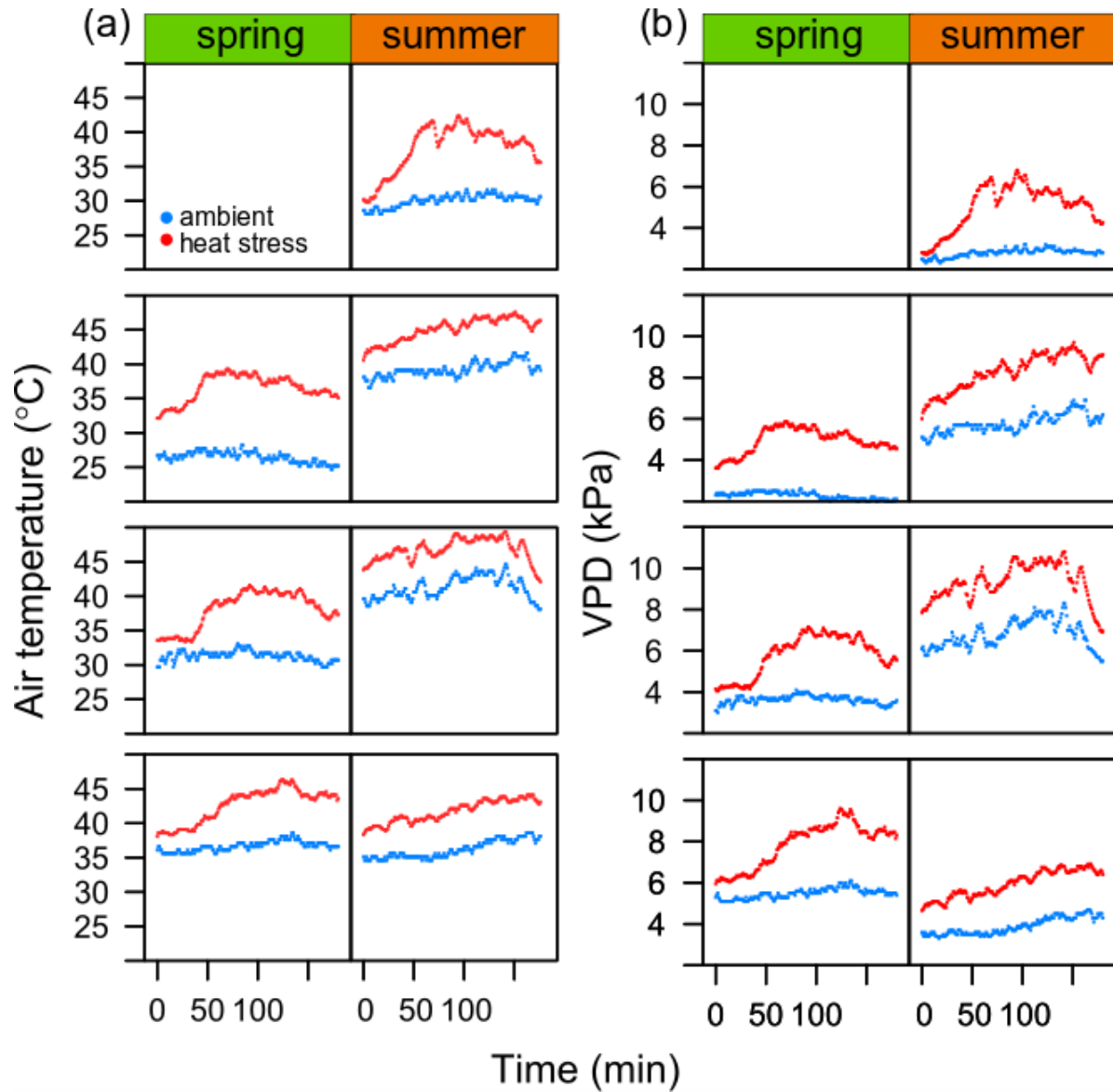
Table C1. Air temperature and VPD during heat stress treatments in spring and summer.

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