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## How does solar ultraviolet-B radiation improve drought tolerance of silver birch (*Betula pendula* Roth.) seedlings?

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1 Title: **By which mechanism does solar ultraviolet-B radiation**  
2 **improve drought tolerance of silver birch (*Betula pendula* Roth.)**  
3 **seedlings?**

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14

## 15 **Abstract**

16 We hypothesized that solar ultraviolet (UV) radiation would protect silver birch seedlings from  
17 the detrimental effects of water stress. To elucidate the mechanism behind this interaction,  
18 plants were grown in an experiment under nine treatment combinations filtering out ultraviolet-A  
19 and ultraviolet-B from solar radiation together with differential watering to create water deficit  
20 conditions. The effects of UV on growth and morphology were evident after the first month of  
21 treatments, and largely persisted, without increasing, over the second month. In seedlings under  
22 water deficit, UV attenuation reduced height growth, leaf production and leaf length compared  
23 with seedlings receiving the full spectrum of solar radiation; whereas the growth and  
24 morphology of well-watered seedlings was largely unaffected by UV attenuation. There was an  
25 interactive effect of the treatment combination on water relations, which was more apparent as a  
26 change in the water potential at which leaves wilted or plants died than through differences in  
27 gas exchange. This suggests changes occur in the cell wall elastic modulus or accumulation of  
28 osmolites in cells under UVB. Overall, the strong negative effects of water deficit are partially  
29 ameliorated by solar UV radiation, whereas well-watered silver birch seedlings are slightly  
30 impaired by the solar UV radiation they receive.

## 31 **Abbreviations**

32 Ultraviolet radiation 280–400 nm (UV). Ultraviolet-B radiation 280–315 nm (UVB). Ultraviolet-  
33 A radiation 315–400 nm (UVA). Attenuated solar UVB treatment (UVB–), Attenuated solar  
34 UVA and UVB treatment (UVA&B–).

35

## 36 **Introduction**

37 During seedling establishment in spring and summer, growth and development are affected by  
38 various environmental constraints that impose selective pressure on plant functional traits  
39 (Bazzaz 2004). Periods of sunny weather associated with high ultraviolet radiation (UV)  
40 frequently occur before and during the onset of drought. In nature, plants perceive these cues  
41 and may be able to pre-emptively acclimate to minimise the detrimental impact of the  
42 “predictable” future water stress. Recent findings that moderate doses of UV act in a regulatory  
43 manner at the cellular level (Heije & Ulm 2012; Hideg *et al.* 2013; Tilbrook *et al.* 2013), should  
44 assist our interpretation of how the physiological processes affected by UV integrate at the whole  
45 plant level. The heightened importance of regulatory- rather than stress responses begs the  
46 question, is UV radiation fulfilling a signaling function that allows plants to prepare for the onset  
47 of seasonal environmental stresses? It would make sense for plants’ response to conditions such  
48 as drought and high radiation to be complementary, and it has even been suggested that detection  
49 of UV radiation may act as a signal to initiate plant defense from photo-oxidative stress (Jansen  
50 *et al.* 2012). In this study, our objective is to test the response of silver birch (*Betula pendula*  
51 Roth.) seedlings to combinations of solar UV radiation and water deficit at realistic values that  
52 might co-occur in nature.

53         Seedlings are at the most critical stage of a trees’ development where selection occurs  
54 through an environmental filter (Clark *et al.* 1999). In Finland, solar UVB radiation is highest in  
55 spring and early-summer when silver birch seedlings come into leaf, and when periods of water  
56 deficit can occur (Atkinson 1992; Kaurola *et al.* 2000; Jylhä *et al.* 2009). Light-demanding  
57 species like silver birch regenerate in open ground and forest gaps where high evaporative  
58 demand and large vapour pressure deficits (VPD) hinder water retention, so escalating any

59 effects of drought (Portsmouth & Niinemets 2006). However, higher physiological activity of  
60 seedlings in sunny than shady conditions also allows for greater flexibility in their mechanisms  
61 to limit water loss, because responses such as tolerance of higher tissue dehydration through a  
62 greater capacity for osmotic adjustment and stomatal regulation of water loss can be employed  
63 while still maintaining a positive carbon balance (Chaves *et al.* 2002; Flexas *et al.* 2006; Robson  
64 *et al.* 2009). Nevertheless, early successional tree species maybe particularly responsive to UVB  
65 with respect to gas exchange and leaf anatomy (Cai *et al.* 2008).

66         Various studies have suggested that UV radiation could confer an advantage on particular  
67 plant species under drought stress, but an understanding of which mechanisms underpin this  
68 benefit is lacking. Common trait responses to UVB radiation, such as smaller leaf and epidermal  
69 cells with thicker walls and thicker cuticles (Wargeant *et al.* 2009; Hectors *et al.* 2010; Robson *et*  
70 *al.* 2013), largely coincide with those responses that help to protect plants from water stress.  
71 However, experiments have typically concentrated only on specific ecophysiological responses,  
72 hence conclusions have been inconsistent and failed to identify which particular physiological  
73 changes combine to produce a coherent response across levels of organisation when scaling up to  
74 the whole plant. The synergistic effects of UV and water stress have sometimes been attributed  
75 to improved stomatal control and changes in root/shoot allocation (e.g. Duan *et al.* 2008 in  
76 poplar), or alternatively to improved hydraulic acclimation expressed through changes in leaf  
77 water potential (e.g. Hofmann *et al.* 2003 in clover; Feng *et al.* 2007 in wheat) and allowing  
78 continued photosynthetic function at reduced leaf water content (Poulson *et al.* 2006 in  
79 *Arabidopsis thaliana*).

80         Common responses of silver birch to drought include reduced biomass and leaf growth,  
81 and reduced shoot:root ratio; which reflect smaller leaf size, increased stomatal control of water

82 loss and decreased assimilation (Ranney *et al.* 1991; Aspelmeyer & Leuschner 2004; Sellin &  
83 Kupper 2005; Possen *et al.* 2011). Silver birch maintains rather isohydric leaves which wilt  
84 easily and are often shed under relatively mild water stress (leaf pre-dawn water potential ( $\Psi_{pd}$ ) <  
85  $-1.3$  MPa: Aspelmeyer & Leuschner 2006). This is considered to be an adaptive mechanism to  
86 reduce transpirational leaf area under mild drought despite the costs incurred constructing new  
87 leaves and the loss of assimilation capacity.

88         As with many species, the most widely-reported response of silver birch leaves to UV  
89 radiation is a change in the production of UV-absorbing compounds (Searles *et al.* 2001). Most  
90 studies report that flavonoids accumulate during leaf expansion (Laitinen *et al.* 2002, Morales *et*  
91 *al.* 2011), suggesting that newly produced leaves are most susceptible to UV penetration.  
92 Although after reaching maturity the flavonoid content of leaves starts to decline with leaf age  
93 (Kotilainen *et al.* 2010). Flavonoids are produced constitutively in the leaves of many species  
94 (Gotz *et al.* 2010), but even small doses of UV can induce sufficient additional UV-screening  
95 phenolics to effectively limit penetration into the mesophyll to 5–12 % of incident UV radiation  
96 in a range of species (Barnes *et al.* 2008), while higher doses of UV often fail to elicit any further  
97 increase in UV-screening phenolics (de la Rosa *et al.* 2001; Tegelberg *et al.* 2001).

98         Possibly because of the effectiveness of UV-protection, most reported detrimental effects  
99 of UV on growth and leaf traits in silver birch have either been subtle (Kostina *et al.* 2001;  
100 Tegelberg *et al.* 2004; Robson *et al.* 2012) or undetectable (Keski-Saari *et al.* 2005; Kotilainen *et*  
101 *al.* 2009; Morales *et al.* 2010). Of course, the response of silver birch to additional  
102 environmental constraints in these and other experiments may be masking, or superseding, the  
103 effect of UV because of functional trade-offs in response to different factors.

104 Our experiment was explicitly designed to test which plant traits were affected by  
105 combinations of UV and water stress, to identify where these two factors were having synergistic  
106 or antagonistic effects on plant function. We grew seedlings of silver birch outside in a  
107 replicated fully-factorial experiment under filters creating near-ambient full solar UV radiation  
108 treatments (near-ambient UV control) and reduced solar UVB (UVB-) and reduced solar UVA  
109 and UVB (UVA&B-), also giving differential watering to create water deficit conditions during  
110 June and July of 2011. We expected near-ambient UV to reduce leaf area and promote greater  
111 investment in protection, e.g. more phenolics, and thicker leaves, similar responses to those  
112 expected under water deficit. Consequently, we hypothesized that UVB- $\times$ -drought responses  
113 should be complementary, with the trait response to one stress partially alleviating the severity of  
114 the other. Hence the comparison of ambient UV control and reduced UV silver birch seedlings  
115 subjected to water deficit should favour the control seedlings, whereas in the well-watered  
116 treatment the reduced UV seedlings should do better. So as to better understand the mechanisms  
117 underlying any treatment effects, we monitored a suite of traits allowing comparison of  
118 adjustments in hydraulic sufficiency, stomatal control, and changes in morphology and whole  
119 plant performance.

120

## 121 **Materials and methods**

### 122 **Preparation of silver birch seedlings**

123 Silver birch seeds (*Betula pendula* Roth.) of central Finnish provenance (Seed orchard 379,  
124 Ey/FIN M29-93-0001), were soaked in water for 24 hours prior to being sown on 2<sup>nd</sup> May and

125 were maintained at 25° C in a greenhouse receiving no UVB during germination until they  
126 produced two true leaves and attained a height of 2–3 cm. On 1<sup>st</sup> June, seedlings were  
127 transplanted, one individual seedling per cell of volume 275 cm<sup>3</sup>, in trays (400 × 300 × 130 cm;  
128 Plantek PL 35F, BCC Oy, Finland). Seedlings were grown in a standard soil mixture of peat,  
129 sand and vermiculite (6:2:1 v/v respectively) to which 2 g of slow release nutrient pellets (15% N;  
130 4.4% P<sub>2</sub>O<sub>5</sub>; 8.3% K<sub>2</sub>O; 1.8% MgO, TE; Scotts International BV, Netherlands) per cell were  
131 added, plus water to field capacity. A total of 455 silver birch seedlings of equal size were  
132 randomly selected and divided between 13 trays, each containing 35 seedlings in five rows of  
133 seven plants. Each of the three middle rows was allocated to a drought treatment, and a  
134 surrounding border of plants was left untreated. This ensured that all plants included in the  
135 experiment were surrounded by eight neighbouring silver birches providing a homogeneous light  
136 environment at the start of the experiment.

137         Prior to their transfer outside under the UV filters, the transplanted silver birch seedlings  
138 were kept in the greenhouse for a further 5 days to become established in the cells. At this stage  
139 any dead seedlings were replaced. To obtain an estimate of the initial state of the seedlings  
140 directly prior to the experiment, the chlorophyll content of the first leaf produced was measured  
141 non-invasively using a SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka, Japan), as  
142 was the leaf length and width of the second leaf using digital callipers, and the soil moisture in  
143 each cell (SM200 Moisture Sensor with HH2 Moisture Meter, Delta-T Devices, Cambridge, UK).  
144 In order to minimise soil-surface disturbance by the probe during subsequent soil moisture  
145 measurements a thin layer of sand was sprinkled on top of the soil.

146



## 147 **Design of the UV filtration experiment**

148 The UV- $\times$ -drought experiment was performed outside in an open area at the greenhouse-and-  
149 field-trials complex of University of Helsinki, Viikki Campus (60°13' N, 25°1' E). Plastic filters  
150 were used to create treatments comprising the full spectrum of solar UV (near-ambient UV  
151 control: 0.05 mm thick polythene, 04 PE-LD; Etola, Jyväskylä, Finland), reduced UVB (UVB- :  
152 0.125 mm thick polyester, Autostat CT5; Thermoplast, Helsinki, Finland) and reduced UVA and  
153 UVB (UVA&B- : Rosco theatrical 'gel' E+# 226; Rosco Labs, Stamford, Connecticut, USA).  
154 Filters attached to frames (80  $\times$  100 cm area) were hung from wooden posts and their height was  
155 adjusted throughout the experiment to keep them suspended *c* 20 cm above the upper leaves of  
156 the silver birch seedlings. The three UV treatment plots were arranged in a random position  
157 within four replicate blocks. On June 8<sup>th</sup>, trays were systematically allocated to the three  
158 different ultraviolet radiation treatments such that trays of similarly sized seedlings were evenly  
159 distributed among the treatments to ensure that there were no initial treatment differences in  
160 seedling size. Trays of seedlings were placed at the centre of each plot to minimize the scattered  
161 and diffuse UV radiation they received around the sides of the filters.

162         Directly prior to the start of the experiment the complete solar spectrum was measured at  
163 the location of the seedlings under each of the 12 filters and in the open under an almost  
164 completely clear sky close to solar noon on 8<sup>th</sup> June (Maya 2000 Pro CCD array  
165 spectroradiometer, Ocean Optics, Florida, USA, D7-H-SMA cosine diffuser, Bentham, Reading,  
166 UK). Each measurement consisted in three consecutive scans, one with the bare cosine diffuser,  
167 one in the dark, and third using a polycarbonate filter blocking UV radiation. The post  
168 processing of the spectra included both a correction for the shape of the slit function and for stray  
169 light (Ylianttila *et al.* 2005; Kreuter & Blumthaller 2009). This protocol was cross-validated at

170 the Finnish Radiation Authority in spring 2011 against a Bentham DM 150 double  
171 monochromator spectroradiometer (Bentham Instruments Ltd., Reading, UK) under natural  
172 sunlight. The average of 20 solar spectra was used for each filter, and the mean calculated from  
173 four plots of each treatment (Table 1; Fig. S1). All three UV treatments reduced the  
174 photosynthetically active radiation (PAR) received by just over 10% but the spectral composition  
175 of radiation under the polyethene (near-ambient UV control) filter was unchanged compared  
176 with unfiltered sunlight. The UVB- and UVA&B- treatments did not completely exclude UV  
177 radiation but provided less than 10% of the control dose to the central area of the plot where the  
178 plants were situated (Table 1).

179

## 180 **Watering treatments**

181 All 15 seedlings ( $5 \times 3$  rows) from every tray, excluding the border plants, were subjected to  
182 three different watering regimes designed to impose differential water stress treatments: (1)  
183 water-stressed, (2) limited-water and (3) well-watered. Every 2–3 days during the experiment  
184 the seedlings were individually given a measured volume of water using a syringe. As the  
185 seedlings grew at different rates they dried the soil to varying degrees, so the soil moisture in  
186 each seedling's cell was measured (HH2 Moisture Meter) prior to watering and the administered  
187 volume of water adjusted to raise the soil to approximately 10% v/v in the water-stressed  
188 treatments ( $c 5 \text{ ml day}^{-1}$  on average), 20% v/v in the limited-water treatment ( $c 10 \text{ ml day}^{-1}$ ), and  
189 30% v/v in the well-watered treatment ( $c 15\text{--}20 \text{ ml day}^{-1}$ ). The rate of depletion of soil moisture  
190 depended on the weather and on the size of the seedlings (Fig. S2), but there was no significant  
191 difference in soil moisture among UV treatments for a given watering treatment over

192 experimental period (Table 2). To maintain the differences between watering treatments, more  
193 water was required by larger seedlings and consequently towards the end of the experiment soil  
194 moisture was similarly depleted prior to watering in the water-stressed treatment and limited-  
195 water treatment where plants had grown larger. Soil moisture in the border seedlings was  
196 depleted quicker than from the rest so they required more water (*c* 15–20 ml day<sup>-1</sup>) to attain  
197 values equivalent to the limited-water treatment.

198

### 199 **Other environmental variables**

200 The air temperature and relative humidity in each plot was monitored at 5 cm above the soil  
201 surface using an *in situ* miniature logger (shielded from direct sunlight) that recorded data every  
202 hour (DS1923 iButton, Maxim Integrated Products, Inc., Sunnyvale, CA, USA) (Fig. 1). A white  
203 plastic sheath was used to shield the sides of the plastic trays from direct sunlight so reducing the  
204 heat load on the soil of the border plants.

205

### 206 **Measurements of seedling growth, leaf traits and water potential**

207 The experiment ran from 8<sup>th</sup> June when silver birch seedlings all had two small leaves, until 2<sup>nd</sup>  
208 August when the seedlings were harvested for both stem and root biomass. The seedling height,  
209 number of leaves, length and width of the largest leaf of each seedling were measured twice,  
210 mid-way through (5<sup>th</sup> July) and at the end of the experiment (27<sup>th</sup> July), and evidence of wilting  
211 was recorded on 11<sup>th</sup> July as well as on these two occasions. At the start of the experiment on  
212 June 3<sup>rd</sup>, the length and width of the 2<sup>nd</sup> proximal leaf were measured on all seedlings. Silver  
213 birch seedlings produce successively larger leaves as the growing season proceeds. Hence, the

214 most recently produced fully-expanded leaf provided the most consistent comparison of traits  
215 among treatments: in practice this was the largest sun leaf. Seedlings grew at different rates in  
216 our treatments meaning that the 4<sup>th</sup> or 5<sup>th</sup> proximal leaf was measured on July 5<sup>th</sup> and again on  
217 July 27<sup>th</sup>. The same leaves were used for all non-destructive measurements and were sampled at  
218 the end of the experiment except when leaves died during the experiment. Additional  
219 measurements of the number of lateral shoots or branches, root collar diameter, and number of  
220 buds were made at the end of the experiment only. Leaf damage, such as herbivory, was  
221 extremely low throughout the experiment. The July stem extension rate in  $\text{mm day}^{-1}$  was  
222 calculated by taking the difference in natural logs of stem extension between the two  
223 measurement dates relative to the final height,  $(\ln H_1 - \ln H_2) / \ln H_2$ .

224 . Leaf pre-dawn water potential ( $\Psi_{pd}$ ) was taken from the 5<sup>th</sup> proximal leaf (04:30–  
225 05:30); plants were shaded overnight to ensure pre-dawn-like conditions in the early morning.  
226 Midday leaf water potential ( $\Psi_{md}$ ) water taken from the 6<sup>th</sup> proximal leaf receiving full sun to the  
227 leaf lamina during the sampling period (12:00–15:00: 2<sup>nd</sup> August). On the same day, the mid-day  
228 water potential of the main stem ( $\Psi_{md-stem}$ ) just above the root collar was also measured using a  
229 pressure chamber (Model 1000, PMS Instrument Co., Albany, Oregon, USA) in all seedlings at  
230 the time of final harvest

231

## 232 **Measurement of leaf optical properties and phenolics**

233 The chlorophyll content of the largest fully-expanded full-sun leaf (4<sup>th</sup> or 5<sup>th</sup> proximal leaf) was  
234 measured non-invasively (SPAD chlorophyll meter) immediately prior to the experiment, then  
235 one week after the start of the experiment (17<sup>th</sup> June), and at the end of the experiment (27<sup>th</sup> July).

236 Leaf chlorophyll contents were calculated from SPAD absorbance units using the equation [Chl]  
237 =  $10^{(SPAD^{0.267})}$  obtained for this particular machine with other species (Randriamanana *et al.*  
238 2012).

239 The adaxial epidermal flavonoid content of these same leaves was assessed prior to the  
240 experiment and on three occasions during the experiment (29<sup>th</sup> June, 11<sup>th</sup> July, and 27<sup>th</sup> July)  
241 using the Dualex FLAV 3.3 (FORCE-A, Orsay, France). This instrument measures, the  
242 absorbance of epidermal flavonoids, particularly flavones and flavonols, at 375 nm (Goulas *et al.*  
243 2004). By the end of the experiment, the 4<sup>th</sup> or 5<sup>th</sup> proximal leaf was no longer receiving full sun  
244 all day in those treatments where seedlings had continued to grow during July so an additional  
245 measurement was made of the youngest fully-expanded leaf, usually the 2<sup>nd</sup> distal leaf on each  
246 seedling. Dualex absorbance units at  $\lambda_{375}$  were converted to flavonoid contents according to the  
247 calibration given by Morales *et al* (2010) for silver birch leaves. SPAD and Dualex  
248 measurements were performed together on sunny days in the early afternoon just after the daily  
249 peak in solar UVB radiation, this also eliminated any possible variability due to diurnal  
250 relocation of chlorophylls (Naus *et al.* 2010) or phenolics (Barnes *et al.* 2008) within the leaf.

251 Methanol-extractable phenolics were also measured in leaves sampled at the end of the  
252 experiment (27<sup>th</sup> July), from mature fully-expanded sun leaves harvested at dawn and midday,  
253 and the youngest fully-expanded leaves harvested at midday. Two leaf disks (6 mm in diameter)  
254 were sampled from one leaf of every plant surviving until harvest, and immediately placed in 3  
255 ml acidified methanol mechanically shaken in the dark at 4° C for 4 hours prior to analysis in a  
256 scanning spectrophotometer (Shimadzu UV-2501 PC UV-VIS, Kyoto, Japan) for the spectral  
257 absorbance over the range  $\lambda_{190}$  to  $\lambda_{1100}$ . If the absorbance was saturating at some  $\lambda$  the extract  
258 was diluted with 1 ml acidified methanol until absorbance < 2.0 to avoid non-linearity and the

259 peaks were subsequently recalculated using a calibration curve produced by diluting other  
260 samples in the same way.

261

## 262 **Measurement of leaf physiological and morphological traits**

263 Gas exchange of fully-expanded sun leaves was measured on two occasions, mid-way through  
264 the experiment (7–8<sup>th</sup> July) and towards the end of the experiment (20<sup>th</sup> July). On each occasion  
265 one leaf from three silver birch seedlings of each water treatment under each filter were  
266 measured: i.e. 3 seedlings  $\times$  3 water-treatments  $\times$  3 UV-treatments  $\times$  4 replicate filters  $\times$  2 dates =  
267 216 leaves measured. Measurements were performed under clear-sky conditions on sunny days  
268 using a LI-COR 6400 infra-red gas analyser (LI-COR Inc., Lincoln, NE, USA) equipped with a  
269 6 cm<sup>2</sup> leaf chamber. The seedlings received light-saturating conditions solar radiation  
270 (photosynthetic photon flux density  $>1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). The environment in the chamber was  
271 maintained close to air temperature 25°C and 23°C on each date, the relative humidity  $35 \pm 2 \%$ ,  
272 vapour pressure deficit  $2.1 \pm 0.2 \text{ K Pa}$ , the CO<sub>2</sub> concentration was 380 ppm, and flow rate of gas  
273 through the chamber was  $600 \mu\text{mol s}^{-1}$ . Once net photosynthesis ( $A_{\text{net}}$ ), stomatal conductance of  
274 water vapour ( $g_s$ ) per m<sup>2</sup> leaf area and intercellular CO<sub>2</sub> concentration ( $C_i$ ) were considered to be  
275 stable for each leaf these parameters were recorded for the next 10 s and the mean value of this  
276 period for each leaf was used in subsequent analyses.

277 To assess how spectral quality was influencing the relationship between photosynthesis  
278 and stomatal conductance, the same gas exchange measurements detailed for light-saturating  
279 conditions were performed on seedlings acclimated to blue and red light (25–26<sup>th</sup> July). This  
280 enabled us to examine whether acclimation to UV radiation selectively reduced the blue-light

281 specific opening response of stomata as a means of controlling water loss (Negash & Björn  
282 1986), or whether this was a more general response. Prior to gas exchange measurements,  
283 seedlings were acclimated for approximately 1 hour to the conditions under the filter boxes,  
284 filters transmitting only blue or red sunlight attached to a cubic frame (Roscolux#80 Primary  
285 Blue and Rosco E-colour#106 Primary Red filters; Rosco Labs, Stamford, Connecticut, USA;  
286 photo: supplemental material). In addition to this, light response curves to red light, and to  
287 saturating red plus a low dose of blue light, were performed on 19<sup>th</sup> July using red and blue light  
288 sources (Norlux NHXRGB090S00S red-green-blue LED array, Norlux, 575 Randy Road, Carol  
289 Stream, IL 60188, USA).

290         At the end of the experiment (27<sup>th</sup> July), two leaves from each water treatment under each  
291 filter were harvested (72 leaves total). The same leaves, the 4<sup>th</sup> proximal leaf of known  
292 dimensions, were used as those harvested for leaf disks used for leaf phenolics extracts. An  
293 additional leaf disk (6 mm diameter) was removed from the lamina of the leaf avoiding the  
294 midrib and weighed immediately after harvest for fresh weight, then oven dried at 65° C for 3  
295 days until a constant weight, then reweighed for dry weight. The leaf relative water content  
296 (RWC) was calculated from these measurements as  $(FW-DW)/FW$ . These data were also used to  
297 calculate leaf mass area (LMA), DW divided by the known area of the leaf disk.

298         Silver birch leaves are hypostomatic, they have stomata only on their abaxial epidermis.  
299 To inspect the number of stomata on the abaxial epidermis , an impression of the leaf surface  
300 was made using nail varnish, which was peeled from the leaf using transparent adhesive tape.  
301 The density of stomata on the leaf impression, and of trichomes on the adaxial epidermis of leaf  
302 itself, were quantified under a microscope with a times 40 objective and a times 10 ocular lens.

303 Between harvesting and taking leaves impressions (later the same day) leaves were kept hydrated  
304 using damp filter paper in sealed zip-lock plastic bags.

305

## 306 **Data analyses**

307 A mixed model ANOVA was performed to test for the main fixed effect of UV treatment and the  
308 split-plot effect of water treatment and their interaction (4 replicate filters  $\times$  3 filter types creating  
309 UV treatments  $\times$  3 drought treatments under each filter) fitted using restricted maximum  
310 likelihood estimation (REML). Block was included as a random effects factor within the model.

311 In addition to this ANOVA, a three-way factorial ANOVA including date was performed for  
312 parameters which were repeatedly measured during the course of the experiment, including  
313 growth, leaf length, wilting, and epidermal flavonoids. The three-way factorial output is given in  
314 the supplemental material, and any differences over time which affect our interpretation of the  
315 results are also mentioned in the text. The correct variance structure to use in the ANOVA  
316 model was determined by comparison of Akaike's Information Criterion and the log-likelihood  
317 ratio (Zuur *et al.* 2009 p83). The best variance structure was produced by fitting different  
318 variances per stratum (VarIdent).

319 Pairs of UV treatments were compared by t-tests adjusted using Holm's (1979) correction for  
320 multiply comparisons, for each date and drought treatment. Significant differences ( $P < 0.05$ ) are  
321 shown by different letters within each treatment combination in the Figures. All statistical  
322 analyses were performed in R version 2.14.1 (R Core Team 2011).

323

324



## 325 **Results**

### 326 **Effects of UV- $\times$ -Drought treatments on silver birch growth**

327 At commencement of the experiment there were no significant differences in leaf length among  
328 the treatments, but at each subsequent measurement there were very clear effects of both UV  
329 treatments and watering treatments. After 4 weeks of treatments, on 5<sup>th</sup> July, the largest leaf (2<sup>nd</sup>  
330 distal leaf) was larger in the well-watered seedlings, smaller in the limited-water treatments and  
331 smallest in the water-stressed treatment, but there was only a difference in leaf size due to UV  
332 treatment in the two water-deficit treatments (Fig. 2), where leaves of the near-ambient UV  
333 seedlings were larger than those of both the UVB- and UVA&B- seedlings. Towards the end of  
334 the experiment (27<sup>th</sup> July), the effect of UV reduction in the water-stressed seedlings persisted,  
335 but in the limited-water seedlings it was no longer evident, and in the well-watered seedlings the  
336 effect was contrary to that in the two water-deficit treatments with slightly larger leaves under  
337 UVA&B- than the rest (Fig. 2). Hence, among the water-deficit seedlings, most of the benefit to  
338 growth in the UVB- and UVA&B- treatments occurred during the first four weeks of the  
339 experiment and not the latter part of the experiment when the difference was maintained or  
340 slightly reversed.

341 The leaf length-to-width ratio (L:W ratio) was neither affected by watering nor UV treatment  
342 except at the final measurement when L:W ratio was smaller in seedlings from both the UVB-  
343 and UVA&B- treatments compared with seedlings in the near-ambient UV treatment under the  
344 two water-deficit treatments but not the well-watered treatment (Table 3).

345 All seedlings had two leaves and were approximately 2–3 cm tall at the start of the  
346 experiment. After 4 weeks of treatments, during June (when the weather in Helsinki was sunny  
347 and solar UVB relatively high), the near-ambient UV seedlings had produced more leaves than  
348 the UVB– and UVA&B– seedlings (Fig. 3). This difference was particularly evident in the  
349 water-stressed treatment. At the end of July, these UV effects on the number of leaves on the  
350 main stem remained in the two water-deficit treatments, but not in the well-watered seedlings  
351 (Fig. 3).

352 The number of new leaves produced on lateral buds at the end of the experiment followed  
353 a similar pattern of response to the treatment combinations as that of the number of leaves on the  
354 main stem; in that the water-stressed seedlings produced more leaves in the near-ambient-UV  
355 treatments and fewer in both the UVB– and UVA&B– treatments, although this effect of  
356 different UV treatments was not so evident in the well-watered seedlings (Table 3).

357 At the first measurement of seedling height after 4 weeks of treatments (5<sup>th</sup> July) the  
358 near-ambient-UV seedlings were taller than the UVB– or UVA&B– seedlings (Fig. 4). This  
359 effect was most evident in the water-stressed seedlings. This difference due to UV treatment  
360 persisted for the two water-deficit treatments after a further 3 weeks (27<sup>th</sup> July), but there was no  
361 longer any statistically significant difference in seedling height in the well-watered treatment  
362 between the UVA&B– seedlings and the near-ambient-UV treatment (Fig. 4). As expected,  
363 there was also strong overall impairment of height growth due to water-deficit.

364 The treatment effects on stem- dry-weight of seedlings harvested at the end of the  
365 experiment followed a similar pattern of response to the treatment combinations as those effects  
366 on height and other growth parameters, except that the tendency for larger UVA&B– seedlings  
367 than the UVB– seedlings was more clearly apparent in the stem biomass data than from the

368 height data (Table 3). The response of root dry-weight was similar to but more variable than the  
369 stem biomass, so no significant treatment differences were detected. This was also the case for  
370 the stem-to-root- biomass ratio (Table 3).

371

### 372 **Effects of UV- $\times$ -Drought treatments on silver birch leaf pigments**

373 The reduced UV treatments did not significantly affect chlorophyll content per unit leaf area (Fig.  
374 5) or per unit leaf mass (not shown), which was higher in the water-stressed than well-watered  
375 seedlings; an effect possibly associated with the smaller leaf size yet equivalent N fertilisation  
376 under water stress (Figs. 3 & 6). The flavonoid content of the upper leaf epidermis, estimated  
377 using Dualex, responded to both factors and was the only trait to respond more to UV-reduction  
378 than watering treatment. Flavonoid content was highest in the near-ambient UV seedlings and  
379 lowest in the UVA&B- seedlings in all watering treatments throughout the experiment (Fig. 6).  
380 Flavonoid content was also higher in the well-watered seedlings than in the water-stressed  
381 seedlings. The flavonoid content of mature leaves was highest in early July and declined over  
382 time, however flavonoid content of young fully-expanded leaves at the end of the experiment  
383 was the highest of any recorded, suggesting that its decline in mature leaves was an effect of  
384 aging rather than of season or light acclimation (Fig. 6).

385 Peaks of absorbance were determined from the results of the spectral spectrophotometry which  
386 mostly gave consistent differences with those measured non-invasively. Since more detailed  
387 studies of phenolic responses to UV in silver birch have already been published elsewhere  
388 (Julkunen-Tiitto *et al.* 2005; Kotilainen *et al.* 2009; Morales *et al.* 2010) we just highlight the  
389 main results here (Table S2). The largest peak at 262 nm also produced the clearest differences

390 between near-ambient UV and the two reduced UV treatments in mature leaves, much more so  
391 than the peak at 279 nm, where in particular UV treatment had no effect on absorbance in the  
392 well-watered treatment. A smaller peak at 335 nm was highest in the near-ambient UV seedlings  
393 under all watering treatments, even in the young leaves, whereas at 414 nm the opposite trend  
394 with UV treatment occurred. As with the non-invasive measurements, the peaks for chlorophyll  
395 *a* did not differ among treatments, and neither did anthocyanins (data not shown).

396

### 397 **Effects of UV- $\times$ -Drought treatments on silver birch mortality and water** 398 **relations**

399 Silver birch seedlings in the two water-deficit treatments exhibited wilting which in some cases  
400 eventually led to seedling mortality, although this was largely restricted to the most water-  
401 stressed treatment (Table 3). The proportion of wilted (and dead) seedlings measured prior to  
402 daily watering increased during the experiment and was highest in the UVA&B- seedlings and  
403 lowest in the near-ambient UV seedlings (Fig. 7).

404 These observations of wilting were corroborated by the results of water potential  
405 measurements. Pre-dawn leaf water potential ( $\Psi_{pd}$ ) was similar across all the treatment  
406 combinations, but midday water potential ( $\Psi_{md}$ ) was more negative in the near-ambient UV  
407 seedlings than in the UVB- seedlings in the two water-deficit treatments (Table 4). This  
408 produced a larger pre-dawn to mid-day difference in water potential ( $\Psi_{diff}$ ) in the near-ambient  
409 UV seedlings than in those receiving UVA&B- and UVB- under the two water-deficit  
410 treatments, and contrasted with the well-watered treatments, where  $\Psi_{md}$  was higher (less-  
411 negative) overall and differences among UV treatments were absent (Table 4).

412           Since we were able to measure the midday stem water potential ( $\Psi_{\text{md-stem}}$ ) of every plant  
413 surviving until harvest (up to 14 plants per treatment combination) this analysis had greater  
414 statistical power than the leaf water potential measurements during the experiment (4 plants per  
415 experimental unit), but nevertheless the two types of midday-water-potential measurement  
416 produced similar results. Stem water potential was most negative in the near-ambient UV  
417 seedlings under the two water-deficit treatments, and least negative in the UVB– seedlings. In  
418 the well-watered seedlings, there was no difference in  $\Psi_{\text{md-stem}}$  attributable to UV treatments  
419 (Table 4). This contrast was responsible for the significant UV-by-drought interaction term for  
420  $\Psi_{\text{md-stem}}$ .

421

### 422 **Effects of UV- $\times$ -Drought treatments on silver birch leaf anatomy**

423 There were no treatment effects on leaf mass ratio (LMA) at the end of the experiment (Table 4).  
424 The relative leaf water content was lower in the water-stressed treatment than the well-watered  
425 treatment but no significant effects of UV treatment on leaf water content were detected (Table  
426 4).

427           No differences in stomatal density on the leaf abaxial epidermis were detected among the  
428 seedlings in any of the UV-treatments, but there was an interactive UV-by-drought treatment  
429 effect on the density of glandular trichomes on the adaxial epidermis (Table 4). In the two  
430 water-deficit treatments there was a higher density of glandular trichomes on leaves of near-  
431 ambient UV seedlings compared with the UVA&B– and UVB– seedlings, whereas in the well-  
432 watered seedlings there were no differences among the UV treatments (Table 4).

433

## 434 **Effects of UV- $\times$ -Drought treatments on silver birch leaf gas exchange**

435 The responses of the seedlings under the treatment combinations were fairly consistent on the  
436 two sampling dates in July, so both are considered together here (Table 5). As would be expected,  
437 water deficit reduced  $A_{\text{net}}$  and  $g_s$ , and increased the instantaneous water use efficiency (IWUE) of  
438 gas exchange (measured as  $A_{\text{net}} / g_s$ ). The IWUE of seedlings in the limited-water treatment was  
439 as high as in the water-stressed treatment, supporting the premise that by mid-July the larger  
440 plants in the limited-water treatment were drying the soil so rapidly that this treatment's severity  
441 was almost equivalent to the water-stressed treatment despite receiving more water. There was  
442 no general effect of UV treatment on  $A_{\text{net}}$  nor on  $g_s$ , although the UV-by-drought interaction  
443 effect on  $A_{\text{net}}$  suggests that there was a difference between the responses of near-ambient UV and  
444 UVB- seedlings under well watered- compared with under water-stressed conditions (Table 5).  
445 In comparison, the IWUE results more-clearly highlighted a difference in the response of near-  
446 ambient UV seedlings compared with the UVB- and UVA&B- seedlings that depended on  
447 watering treatment: that with increasing water-deficit treatment the IWUE of near-ambient UV  
448 seedlings increased relative to the reduced UV treatments (Table 5: IWUE UV-by-drought  
449 interaction:  $P = 0.003$ ).

450 While there was no difference in  $C_i/C_a$  among the three UV treatments for the well-  
451 watered seedlings,  $C_i/C_a$  was lower in the near-ambient UV treatment than in the UVB- and  
452 UVA&B- treatments for seedlings under the two water deficit treatments (Table 5).

453 Under a filter that transmitted only blue light ( $\sim 50 \mu\text{m m}^{-2} \text{s}^{-1}$ ) the patterns of response to  
454 UV and watering treatments were similar to those recorded without the blue filter (Table 5).  
455 The interactive UV-by-drought treatment effect was evident in  $g_s$ , which tended to be lower in  
456 the near-ambient UV seedlings than the UVB- and UVA&B- seedlings under the two water-

457 deficit treatments but not the well-watered treatment. The response of Ci/Ca to the treatments  
458 also followed the same pattern under the blue filter as without the blue filter (Table 5).

459 Under a filter that transmitted only red light ( $\sim 225 \mu\text{m m}^{-2} \text{s}^{-1}$ ) the treatment differences  
460 in gas exchange due to UV treatment were functionally small in terms of their likely biological  
461 significance. The UV-by-drought interaction was largely due to higher  $g_s$  in seedlings under the  
462 near-ambient UV treatment in the well watered treatment (Table 5). There was a tendency for  
463 Ci/Ca to be higher in well-watered treatment for near-ambient UV seedlings, and lower in the  
464 water-deficit treatments for the near-ambient UV seedlings compared with the UVB– and  
465 UVA&B– seedlings (Table 5).

466

## 467 **Discussion**

468 Our water deficit treatments had a greater effect than our UV reduction treatments on most of the  
469 traits we measured, with the exception of leaf flavonoid content which was more responsive to  
470 UV reduction than to watering. Water stress was evident producing smaller plants, fewer leaves,  
471 and leaf traits suggestive of a more conservative strategy under both water deficit treatments.  
472 Whereas, any negative effects of near-ambient UV were either absent (as in leaf production,  
473 seedling height,  $\Psi_{\text{md-stem}}$ , LMA) or only apparent after eight weeks of treatments (e.g. short leaf  
474 length), as consistent with previous studies of silver birch seedlings (de la Rosa *et al.* 2001;  
475 Kotilainen *et al.* 2009; Robson *et al.* 2012). However, when a water deficit was imposed on  
476 seedlings growing under near-ambient UV conditions, their acclimation to UV was consistent  
477 over a suite of traits encompassing leaf traits, growth, and water relations, which conferred an  
478 advantage on them compared with seedlings from the UVB– or UVA&B– treatments. This

479 advantage was particularly evident during the first month of treatments and subsequently  
480 diminished for most traits. Similar benefits of plant acclimation to UV radiation prior to the  
481 imposition of water stress have been reported in experiments with clover (Hofmann *et al.* 2003)  
482 and poplar (Duan *et al.* 2008) but not across such a broad suite of traits.

483

#### 484 **Interactive effects of UV- $\times$ -Drought treatments**

485 Our watering treatment commenced when the seedlings were put under the filters, but the  
486 gradual reduction in soil moisture from initial field capacity in the water-deficit treatments  
487 provided seedlings with a period to acclimate to the differential UV treatments while weather  
488 conditions were mild in mid-June (Fig. 2). Seedlings could establish prior to the warmest driest  
489 period of the experiment when VPD was highest in early July and again in late July, providing  
490 two occasions for us to measure gas exchange following periods of high evaporative demand.  
491 The responses of gas exchange to UV were small but did partially compensate the effects of  
492 drought, as reflected in improved IWUE in seedlings from the near-ambient UV treatment  
493 compared with UVB- and UVA&B- treatments under the most-severe water deficit. Tighter  
494 regulation of the relationship between  $A_{\text{net}}$  and  $g_s$  is implicit in this effect. Improvements in  
495 stomatal control and photosynthetic assimilation have been reported in other species where high  
496 doses of UVB have been combined with water stress (Nogués *et al.* 1998; 1999). More often  
497 reductions in  $A_{\text{net}}$  due to UVB under well-watered conditions are simply no longer evident  
498 between UV treatments under the drought stress (e.g. in *Arabidopsis*, spruce and poplar: Poulson  
499 *et al.* 2006; Cechin *et al.* 2008; Duan *et al.* 2008; 2011). These changes have been attributed to  
500 more conserved  $C_i/C_a$  and net assimilation rates, reduced lipid peroxidation, and increased



501 proline content and reduced stomatal conductance respectively. In our study, the treatment  
502 differences in  $C_i/C_a$  were maintained even at high PPFD, and the differences in  $g_s$  among  
503 drought treatments were large, particularly in darkness (Table 5; Fig. S3). Leaf morphological or  
504 physiological acclimation to UV provide a more-likely explanation for improved IWUE when  
505 combined with water stress than the direct closure of stomata. Although our measurements of  
506 gas exchange under coloured filters confirmed that small doses of blue light do stimulate  
507 stomatal opening beyond that of red light, they did not reveal any differential stomatal sensitivity  
508 to coloured light with the UV treatments, only a consistent tendency for increased IWUE in  
509 seedlings receiving near-ambient UV under water stress compared with those receiving UVB–  
510 and UVA&B–.

511         Among our water-stressed treatments, the pre-dawn-to-midday difference in leaf water  
512 potential ( $\Psi_{diff}$ ) was largest and the midday stem water potential ( $\Psi_{md-stem}$ ) was most negative in  
513 the near-ambient UV seedlings. Water potential did not drop very low ( $< -1.84$  MPa) even  
514 among the most water-stressed seedlings, and this supports the existing evidence that silver  
515 birch's capacity for osmotic adjustment is relatively limited (Ranney *et al.* 1991). Nevertheless,  
516 the treatments effect we report might reflect a greater capacity for osmotic adjustment among  
517 seedlings under near-ambient UV; since, irrespective of their lower  $\Psi$ , these leaves wilted less  
518 readily indicating that their turgor pressure was higher than in the UVB– and UVA&B–  
519 seedlings. This would signify that leaves receiving near-ambient UV were more drought-adapted  
520 which is consistent with the mechanisms of drought response (stomatal and non-stomatal  
521 limitation of water loss) reported for adult silver birch seedlings in a common garden  
522 (Aspelmeier & Leuschner 2004; 2006). Increased leaf and epidermal thickness in silver birch  
523 under near-ambient UV-treatments (Kotilainen *et al.* 2009), could contribute to these treatment

524 effects since both changes would also favour drought tolerance. Although stomatal density was  
525 apparently unchanged by our UV treatments, the density of glandular trichomes was greater  
526 under near-ambient than reduced-UV, another complementary adaptation to water limitation and  
527 UV (Liakoura *et al.* 1997; Kostina *et al.* 2001; Semerdjieva *et al.* 2003; Valkama *et al.* 2004).

528         The positive interaction of near-ambient-UV under the water-deficit-treatment is also  
529 reflected in the larger leaf area (leaf size- $\times$ -number) maintained by these seedlings than those  
530 under UVB- and UVA&B- in the two water deficit treatments (though not in the well watered  
531 treatments). This whole-plant scale effect may also be responsible for the larger leaf  $\Psi_{\text{diff}}$  in the  
532 near-ambient-UV- $\times$ -water-deficit seedlings compared with other treatments. This evidence of  
533 plasticity of leaf functional traits in acclimation to near-ambient UV is likely to contribute to the  
534 lower mortality in this treatment under water-stress as well as greater height growth than the  
535 UVB- and UVA&B- treatments under equivalent watering regimes. Nevertheless, the benefits  
536 of receiving UV during the onset of water stress did not continue to accumulate, in terms of  
537 effects on growth, over time during the second month of treatments. Since silver birch is known  
538 to sacrifice its leaves quite readily under moderately-severe water stress, the production costs  
539 saved by retaining more leaves, thanks to the various improvements in water relations we report  
540 under near-ambient compared with reduced UV could help to promote survival during spring  
541 drought during seedlings establishment.

542         The greatest specific effect of UV on silver birch in this and other experiments (de la  
543 Rosa *et al.* 2001; Kotilainen *et al.* 2009; Morales *et al.* 2010) was the accumulation of certain  
544 UV-absorbing phenolics in the leaves. In this case the response is evident from increases in some,  
545 but not all, peaks of absorbance within the UV spectrum as well as general epidermal absorbance.  
546 Although UV absorbance was greater in well-watered seedlings receiving near-ambient solar UV

547 than the reduced UV treatments, the lack of growth effects among these treatments implies both  
548 that UV-screening provided effective UV-protection for the plant and that the synthesis and  
549 maintenance of a higher concentration of these phenolic compounds did not impose a significant  
550 cost on plants receiving UV radiation (or that the benefit of having these compounds  
551 counterbalanced this cost).

552

### 553 **Differentiating the effects of removing UVA and UVB**

554 Differences have been reported in specific effects of the UVA and UVB portions of the solar  
555 spectrum on plant traits such as leaf growth (Robson & Aphalo 2012), UV-absorbing compounds  
556 (Morales *et al.* 2010), and gas exchange (Johnson & Day 2002), but the modifications of these  
557 differences by drought have not previously been tested using UVA controls in a filtration  
558 experiment. We found that the relationships between the effects of removing UVB radiation and  
559 removing both UVA and UVB radiation were specific to the types of traits considered. For  
560 growth-related traits, leaf length and number, and height (Figs. 3, 4, & 5), the only unique effect  
561 of filtering UVA in addition to UVB– was that well-watered seedlings had grown more by the  
562 end of the experiment under this treatment compared with UVB– seedlings. Removing UVA  
563 and UVB radiation caused a further decrease in total epidermal flavonoids (monitored non-  
564 invasively with a Dualex), and likewise smaller individual peaks of absorbance, beyond that of  
565 the UVB– treatment. A similar additive increase in phenolics due mainly to UVB but further  
566 strengthened by UVA has been reported (Kotilainen *et al.* 2009), but in contrast other reports  
567 suggest that UVA can affect certain silver-birch phenolics differently from UVB radiation  
568 without significantly affecting total phenolics (Morales *et al.* 2010). This selective response of

569 only silver birch phenolics with absorption peaks at particular wavelengths was not apparent  
570 from our results, but might have been detected by using HPLC rather than spectrophotometry of  
571 leaf extracts.

572

## 573 **Conclusions**

574 Near-ambient UV radiation ameliorated some of the detrimental effects of drought stress for  
575 silver birch. By identifying effects over different scales within the same seedlings, we were able  
576 to begin to elucidate the mechanism of response to this treatment combination. Under all three  
577 watering treatments, seedlings grew better, in terms of size and number of leaves, height and  
578 stem biomass production, under the near-ambient UV treatment than in the UVB- and UVA&B-  
579 treatments. In seedlings receiving the well-watered treatment this difference was marginal, but  
580 under the water-stressed treatment the difference was quite pronounced. Lower stem- and leaf-  
581 water potential in the near-ambient UV seedlings under water deficit provided evidence for  
582 acclimation of hydraulic architecture, and when combined with leaf morphological and  
583 anatomical adaptations (see also Kotilainen *et al.* 2009) would explain the reduced turgor  
584 loss/wilting point for these seedlings. Most of these responses were evident when near-ambient  
585 UV was compared with the UVB- treatment and generally the further filtration of UVA  
586 produced equivalent or more pronounced effects of a similar nature. Our results suggest that in  
587 silver birch hydraulic limitation exerts greater influence than stomatal control in reducing the  
588 effect of water stress under near-ambient UV radiation, although further studies of water  
589 movement through the hydraulic system should explicitly test the functional limitations to water  
590 movement imposed by drought with-and-without solar UV. As the pathways of UV perception

591 and response become better known at a molecular level, it should become possible to identify the  
592 underlying physiology behind these hydraulic and morphological changes in the future. The next  
593 step towards a better functional understanding of this response is to identify whether plants are  
594 utilising UV as a pre-emptive signal for forecasting a change in environmental condition, or  
595 whether the complementary responses to UV and drought, and likewise UV and high PAR  
596 radiation, are purely coincidental.

597

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603

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## Tables

Table 1: Solar radiation received by seedlings under each UV treatment. Clear sky irradiance (energy and photon units) under each filter type and in the open close to solar noon on 8th June directly prior to starting the experiment. The effective dose according to various biological spectral weighting functions was calculated using R package UVcalc (Björn *et al.* 2012): GEN(G) and GEN(T) respectively are Green's *et al.* (1974) formulation of the generalised plant action spectrum extending to 313 nm and Thimijan's *et al.* (1978) formulation extending further to 350 nm: PG is the Plant Growth action spectrum (Flint & Caldwell 2003): DNA(N) action spectrum is for damage to naked DNA (Setlow 1974). CIE action spectrum for erythema induced on human skin (McKinlay & Diffey, 1987). Wavelength ranges are, UVB (280–315 nm) UVA (315–400 nm), PAR (400–700 nm), blue (420–490 nm), green (500–570 nm), red (650–670 nm) far-red (720–740 nm). Mean of 4 filters of each type (each of the 4 replicate plots). For full spectra see Fig. S1.

Filter	Energy Irradiance (W m <sup>-2</sup> )			Photon Irradiance (μmol m <sup>-2</sup> s <sup>-1</sup> )			Effective Irradiance (W m <sup>-2</sup> )					Photon ratio (×1000)			Photon ratio		
	PAR(e)	UVA(e)	UVB(e)	PAR(PPFD)	UVA	UVB	GEN(G)	GEN(T)	PG	DNA(N)	CIE	UVB:UVA	UVB:PAR	R:FR	B:G	B:R	
<b>Polyethene (control)</b>	318.4	114.5	2.72	1461.4	37.7	1.05	0.22	0.27	0.92	0.10	0.15	23.71	1.86	1.11	0.80	0.86	
<b>Rosco #226 (UVB–)</b>	310.9	8.4	0.07	1432.1	2.6	0.03	0.00	0.01	0.03	0.00	0.00	7.75	0.05	1.12	0.80	0.86	
<b>Polyester (UVB–)</b>	314.1	101.3	0.13	1440.7	33.1	0.05	0.01	0.03	0.65	0.01	0.03	1.26	0.09	1.12	0.80	0.88	
<b>Open</b>	350.5	134.1	3.18	1604.1	44.2	1.22	0.25	0.32	1.08	0.12	0.17	23.69	1.98	1.13	0.82	0.90	

Table 2: Soil moisture content of the three watering treatments in each of the three UV treatments. For the whole experimental period means and standard deviations are displayed from measurements taken every 2–3 days just before and following watering and for three dates of particular interest, 8<sup>th</sup> & 20<sup>th</sup> July (gas exchange measurements) and 2<sup>nd</sup> August (water potential measurements). Towards the end of the experiment, plants in the water stressed treatment were mostly small, wilted or dying, so they depleted the soil moisture very little, leading producing similar soil moisture values prior to watering in these and the limited-water treatments. Likewise, at the end of the experiment large seedlings with large leaf area depleted the well watered treatments quickly.

Soil Moisture (% v/v)	1. Water Stressed			2. Limited Water			3. Well Watered			P values		
	Control	UVB–	UVA&B–	Control	UVB–	UVA&B–	Control	UVB–	UVA&B–	UV	Water	UV×Water
<b>Whole Experiment</b>	14.2 ± 1.0	14.3 ± 1.5	14.4 ± 1.2	17.7 ± 0.7	17.4 ± 1.3	17.2 ± 0.7	24.2 ± 3.5	23.3 ± 5.0	23.2 ± 3.2			
<b>8th July</b>	8.8 ± 1.2	8.8 ± 1.1	9.0 ± 1.2	15.5 ± 1.2	16.4 ± 0.8	16.7 ± 0.9	18.7 ± 1.1	18.3 ± 1.0	18.6 ± 0.8	0.415	<0.0001	0.576
<b>20th July</b>	10.1 ± 1.0	10.2 ± 1.3	10.9 ± 2.0	11.4 ± 1.6	12.1 ± 2.8	13.5 ± 2.2	30.5 ± 1.9	28.9 ± 1.2	29.3 ± 1.8	0.649	<0.0001	0.076
<b>2nd August</b>	7.4 ± 0.7	7.4 ± 1.3	6.8 ± 0.7	9.1 ± 0.9	9.9 ± 1.5	11.3 ± 1.2	16.4 ± 3.3	16.4 ± 2.5	16.2 ± 3.2	0.629	<0.0001	0.320

Table 3: Effect of UV and water treatments on growth and seedling mortality parameters. Measurements were from 5 seedlings in each of the 4 replicate plots for each treatment combination (n=4; mean  $\pm$  1 SE). Main treatment and interaction effects from mixed model ANOVA are given (details in Table S1).

Parameter	Date	1. Water Stressed			2. Limited Water			3. Well Watered			P values		
		Control	UVB-	UVA&B-	Control	UVB-	UVA&B-	Control	UVB-	UVA&B-	UV	Water	UV×Water
Small Secondary Leaves (#)	-05	1.45 $\pm$	0.55 $\pm$	0.75 $\pm$	2.55 $\pm$	2.05 $\pm$	1.95 $\pm$	3.10 $\pm$	2.95 $\pm$	2.30 $\pm$	<b>0.023</b>	<b>&lt;.0001</b>	0.702
	Jul	0.25	0.25	0.25	0.29	0.32	0.35	0.40	0.39	0.34			
Leaf Length/Width (1)	05 Jul	1.02 $\pm$	1.01 $\pm$	1.01 $\pm$	1.01 $\pm$	0.99 $\pm$	0.98 $\pm$	0.99 $\pm$	1.02 $\pm$	1.02 $\pm$	0.945	0.305	0.469
		0.01	0.02	0.02	0.01	0.01	0.03	0.01	0.01	0.03			
Leaf Length/Width (2)	27 Jul	1.13 $\pm$	1.01 $\pm$	1.06 $\pm$	1.09 $\pm$	1.08 $\pm$	1.10 $\pm$	1.14 $\pm$	1.12 $\pm$	1.14 $\pm$	<b>0.056</b>	<b>0.010</b>	0.148
		0.03	0.02	0.04	0.01	0.02	0.03	0.01	0.01	0.03			
Seedling mortality (proportion)	27 Jul	0.25 $\pm$	0.45 $\pm$	0.65 $\pm$	0.25 $\pm$	0.15 $\pm$	0.05 $\pm$	0.00 $\pm$	0.00 $\pm$	0.00 $\pm$	0.574	<b>&lt;.0001</b>	<b>0.005</b>
		0.10	0.11	0.11	0.10	0.08	0.05	0.00	0.00	0.00			
Stem Extension Rate (mm day <sup>-1</sup> )	July	0.78 $\pm$	0.76 $\pm$	0.83 $\pm$	1.10 $\pm$	1.24 $\pm$	1.20 $\pm$	1.08 $\pm$	1.16 $\pm$	1.22 $\pm$	<b>0.010</b>	<b>&lt;.0001</b>	0.736
		0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08			
Root Collar Diameter (RCD; mm)	27 Jul	2.09 $\pm$	1.41 $\pm$	1.85 $\pm$	3.08 $\pm$	2.65 $\pm$	2.76 $\pm$	3.74 $\pm$	3.50 $\pm$	3.58 $\pm$	<b>0.000</b>	<b>&lt;.0001</b>	0.281
		0.07	0.08	0.10	0.08	0.09	0.10	0.13	0.09	0.10			
Branches (#)	27 Jul	0.61 $\pm$	0.00 $\pm$	0.33 $\pm$	0.60 $\pm$	0.71 $\pm$	0.75 $\pm$	1.80 $\pm$	1.15 $\pm$	1.45 $\pm$	0.218	<b>&lt;.0001</b>	0.578
		0.27	0.00	0.21	0.17	0.19	0.19	0.28	0.24	0.39			
Stem Biomass (g)	02	0.20 $\pm$	0.08 $\pm$	0.17 $\pm$	0.71 $\pm$	0.56 $\pm$	0.58 $\pm$	1.36 $\pm$	1.22 $\pm$	1.35 $\pm$	<b>0.000</b>	<b>&lt;.0001</b>	0.417
	Aug	0.02	0.01	0.03	0.02	0.03	0.03	0.04	0.04	0.05			
Root Biomass (g)	02	0.20 $\pm$	0.11 $\pm$	0.14 $\pm$	0.45 $\pm$	0.44 $\pm$	0.33 $\pm$	1.31 $\pm$	1.28 $\pm$	1.18 $\pm$	0.506	<b>&lt;.0001</b>	0.994
	Aug	0.02	0.01	0.02	0.04	0.05	0.03	0.18	0.09	0.21			
Relative Stem/Root Biomass	02	2.04 $\pm$	2.09 $\pm$	1.83 $\pm$	1.65 $\pm$	1.77 $\pm$	1.59 $\pm$	1.98 $\pm$	2.07 $\pm$	1.86 $\pm$	0.062	<b>&lt;.0001</b>	0.983
	Aug	0.13	0.22	0.09	0.06	0.07	0.05	0.12	0.09	0.13			

Table 4: Effect of UV and water treatment on physiological, morphological, and anatomical, measurements of stem and leaf water relations (sampled 2<sup>nd</sup> August). Measurements were from 5 seedlings in each of the 4 replicate plots for each treatment combination (n=4; mean  $\pm$  1 SE). Main treatment and interaction effects from mixed model ANOVA are given (details in Table S1).

Parameter	1. Water Stressed			2. Limited Water			3. Well Watered			P values		
	Control	UVB-	UVA&B-	Control	UVB-	UVA&B-	Control	UVB-	UVA&B-	UV	Water	UVxWater
Pre-dawn leaf water potential ( $\Psi_{pd}$ ) MPa	-1.23 $\pm$ 0.27	-1.00 $\pm$ 0.07	-1.18 $\pm$ 0.09	-1.15 $\pm$ 0.05	-0.97 $\pm$ 0.03	-1.21 $\pm$ 0.10	-0.50 $\pm$ 0.13	-0.63 $\pm$ 0.16	-0.71 $\pm$ 0.09	0.657	0.388	0.180
Midday leaf water potential ( $\Psi_{md}$ ) MPa	-1.84 $\pm$ 0.14	-1.47 $\pm$ 0.20	-1.62 $\pm$ 0.17	-1.75 $\pm$ 0.09	-1.47 $\pm$ 0.18	-1.70 $\pm$ 0.08	-1.11 $\pm$ 0.27	-1.03 $\pm$ 0.10	-1.13 $\pm$ 0.18	<b>0.022</b>	<b>0.000</b>	0.848
Difference Midday-Predawn ( $\Psi_{diff}$ ) MPa	-0.62 $\pm$ 0.06	-0.47 $\pm$ 0.26	-0.44 $\pm$ 0.11	-0.60 $\pm$ 0.05	-0.50 $\pm$ 0.09	-0.49 $\pm$ 0.07	-0.61 $\pm$ 0.06	-0.40 $\pm$ 0.15	-0.42 $\pm$ 0.14	0.095	0.426	0.411
Midday Relative Water Content (FW-DW)/FW	0.70 $\pm$ 0.01	0.69 $\pm$ 0.01	0.70 $\pm$ 0.01	0.72 $\pm$ 0.01	0.71 $\pm$ 0.01	0.71 $\pm$ 0.01	0.74 $\pm$ 0.01	0.73 $\pm$ 0.01	0.72 $\pm$ 0.01	0.611	<b>0.006</b>	0.850
Midday stem water potential ( $\Psi_{md-stem}$ ) MPa	-1.47 $\pm$ 0.08	-1.23 $\pm$ 0.08	-1.30 $\pm$ 0.01	-1.79 $\pm$ 0.03	-1.63 $\pm$ 0.06	-1.68 $\pm$ 0.04	-1.09 $\pm$ 0.05	-1.17 $\pm$ 0.09	-1.21 $\pm$ 0.06	<b>0.028</b>	<b>&lt;.0001</b>	<b>0.056</b>
Leaf Mass Area (LMA) mg cm <sup>-2</sup>	0.38 $\pm$ 0.01	0.34 $\pm$ 0.04	0.40 $\pm$ 0.02	0.38 $\pm$ 0.02	0.39 $\pm$ 0.02	0.39 $\pm$ 0.02	0.39 $\pm$ 0.02	0.40 $\pm$ 0.01	0.38 $\pm$ 0.01	0.782	0.594	0.630
Stomatal Density (mm <sup>-2</sup> )	206 $\pm$ 19	211 $\pm$ 21	237 $\pm$ 24	201 $\pm$ 22	199 $\pm$ 14	211 $\pm$ 27	201 $\pm$ 13	196 $\pm$ 7	221 $\pm$ 10	0.607	0.478	0.861
Glandular Trichome Density (mm <sup>-2</sup> )	27 $\pm$ 6	14 $\pm$ 5	10 $\pm$ 2	53 $\pm$ 8	40 $\pm$ 7	37 $\pm$ 5	36 $\pm$ 3	43 $\pm$ 5	47 $\pm$ 5	<b>0.0541</b>	<b>&lt;.0001</b>	<b>&lt;.0001</b>



Table 5: Effect of UV and water treatment on leaf gas exchange parameters measured through IRGA. Clear sky measurement from two dates 8<sup>th</sup> & 20<sup>th</sup> July. Blue and red light treatments were treatments under coloured filters on box-frames providing complete filtration of solar radiation apart from blue and red sunlight respectively on 25<sup>th</sup> & 26<sup>th</sup> July. Measurements were from 5 seedlings in each of the 4 replicate plots for each treatment combination (n=4; mean  $\pm$  1 SE). Main treatment and interaction effects from mixed model ANOVA are given (details in Table S1).

Parameter	1. Water Stressed			2. Limited Water			3. Well Watered			P values		
	Control	UVB-	UVA&B-	Control	UVB-	UVA&B-	Control	UVB-	UVA&B-	UV	Water	UV×Water
<b>Clear Sky (ppfd 1200 <math>\mu\text{mol m}^{-2} \text{s}^{-1}</math>)</b>										F <sub>2,20</sub>	F <sub>2,20</sub>	F <sub>4,20</sub>
$A_{\text{net}}$ ( $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ )	6.5 $\pm$ 0.8	4.3 $\pm$ 0.8	4.4 $\pm$ 0.8	7.7 $\pm$ 0.8	7.7 $\pm$ 0.8	8.6 $\pm$ 0.8	11.1 $\pm$ 0.8	12.4 $\pm$ 0.8	11.1 $\pm$ 0.8	0.825	<.0001	<b>0.037</b>
$g_s$ (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	0.07 $\pm$ 0.04	0.07 $\pm$ 0.04	0.08 $\pm$ 0.04	0.12 $\pm$ 0.04	0.13 $\pm$ 0.04	0.13 $\pm$ 0.04	0.34 $\pm$ 0.04	0.31 $\pm$ 0.04	0.31 $\pm$ 0.04	0.315	<.0001	0.849
IWUE <sub>net</sub> ( $A_{\text{net}} / g_s$ )	98 $\pm$ 5	69 $\pm$ 5	68 $\pm$ 5	74 $\pm$ 5	73 $\pm$ 5	71 $\pm$ 5	45 $\pm$ 5	46 $\pm$ 5	48 $\pm$ 5	0.760	<.0001	<b>0.003</b>
$C_i/C_a$ (proportion)	0.57 $\pm$ 0.02	0.68 $\pm$ 0.02	0.68 $\pm$ 0.02	0.62 $\pm$ 0.02	0.62 $\pm$ 0.02	0.65 $\pm$ 0.02	0.73 $\pm$ 0.02	0.74 $\pm$ 0.02	0.73 $\pm$ 0.02	<b>0.031</b>	<.0001	<b>0.036</b>
<b>Blue light (ppfd 50 <math>\mu\text{mol m}^{-2} \text{s}^{-1}</math>)</b>												
$A_{\text{net}}$ ( $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ )	3.6 $\pm$ 0.5	4.6 $\pm$ 0.5	4.0 $\pm$ 0.5	3.2 $\pm$ 0.5	3.9 $\pm$ 0.5	3.4 $\pm$ 0.5	3.3 $\pm$ 0.5	3.5 $\pm$ 0.5	2.8 $\pm$ 0.5	0.137	0.110	0.785
$g_s$ (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	0.03 $\pm$ 0.02	0.08 $\pm$ 0.02	0.04 $\pm$ 0.02	0.03 $\pm$ 0.02	0.03 $\pm$ 0.02	0.03 $\pm$ 0.02	0.08 $\pm$ 0.02	0.10 $\pm$ 0.02	0.11 $\pm$ 0.02	0.553	<.0001	<b>0.022</b>
IWUE <sub>net</sub> ( $A_{\text{net}} / g_s$ )	146 $\pm$ 16	108 $\pm$ 16	115 $\pm$ 16	133 $\pm$ 16	112 $\pm$ 16	99 $\pm$ 16	43 $\pm$ 16	54 $\pm$ 16	45 $\pm$ 16	0.076	<.0001	0.228
$C_i/C_a$ (proportion)	0.95 $\pm$ 0.04	0.84 $\pm$ 0.04	0.83 $\pm$ 0.04	0.99 $\pm$ 0.04	0.84 $\pm$ 0.04	0.93 $\pm$ 0.04	0.95 $\pm$ 0.04	0.95 $\pm$ 0.04	0.99 $\pm$ 0.04	<b>0.009</b>	<b>0.004</b>	0.161
<b>Red light (ppfd 225 <math>\mu\text{mol m}^{-2} \text{s}^{-1}</math>)</b>												
$A_{\text{net}}$ ( $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ )	5.8 $\pm$ 1.0	4.9 $\pm$ 1.0	3.8 $\pm$ 1.0	6.1 $\pm$ 1.0	7.7 $\pm$ 1.0	6.9 $\pm$ 1.0	6.9 $\pm$ 1.0	7.5 $\pm$ 1.0	8.4 $\pm$ 1.0	0.676	<b>0.021</b>	<b>0.048</b>
$g_s$ (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	0.04 $\pm$ 0.07	0.04 $\pm$ 0.07	0.04 $\pm$ 0.07	0.04 $\pm$ 0.07	0.08 $\pm$ 0.07	0.07 $\pm$ 0.07		0.18 $\pm$ 0.07	0.19 $\pm$ 0.07	<b>0.042</b>	<.0001	<b>0.027</b>
IWUE <sub>net</sub> ( $A_{\text{net}} / g_s$ )	93 $\pm$ 6	75 $\pm$ 6	86 $\pm$ 6	118 $\pm$ 6	104 $\pm$ 6	79 $\pm$ 6	31 $\pm$ 6	49 $\pm$ 6	59 $\pm$ 6	0.326	<.0001	0.706
$C_i/C_a$ (proportion)	0.63 $\pm$ 0.03	0.67 $\pm$ 0.03	0.74 $\pm$ 0.03	0.53 $\pm$ 0.03	0.60 $\pm$ 0.03	0.60 $\pm$ 0.03	0.78 $\pm$ 0.03	0.72 $\pm$ 0.03	0.64 $\pm$ 0.03	0.492	<b>0.005</b>	<b>0.020</b>

## Figure Legends

Figure 1: Daily mean air temperature and vapour pressure deficit (VPD) at 5 cm above the soil surface under the filters in each of the three UV filtration treatments throughout the course of the experiment from inception until final harvest on August 2<sup>nd</sup>. The mean of 4 replicate plots for each treatment is displayed ( $n = 4$ ; mean  $\pm$  1 SE plotted). Overall mean temperatures and VPD for the duration of the experiment were “control” 22.5°C & 0.95 KPa; “UVB–“ 22.9°C & 0.96 KPa; “UVA&B–“ 22.5°C & 0.97 KPa. Solid vertical arrows and dashed vertical arrows, respectively, indicate the values on the dates of the two gas exchange measurements and the four Dualex measurements.

Figure 2: Effects of UV- $\times$ -Drought treatments on silver birch growth measured as leaf length (cm). The first date, 3<sup>rd</sup> June, is prior to the start of the experiment, and subsequent measurements were after 28 days of treatments, on 5<sup>th</sup> July, and after 50 days on 27<sup>th</sup> July. The largest leaf was measured on 5 seedlings in each of the 4 replicate plots for each treatment combination ( $n=4$ ; mean  $\pm$  1 SE plotted). Significant differences ( $P<0.05$ ) in pair-wise comparisons of three UV treatments for each date and watering treatment are shown by different letters. Main treatment and interaction effects from mixed model ANOVA with repeated measures are inset (details given in Table S1).

Figure 3: Effects of UV- $\times$ -Drought treatments on silver birch growth measured as the number of leaves produced on the main stem. The first date, 3<sup>rd</sup> June, is prior to the start of the experiment, and subsequent measurements were after 28 days of treatments, on 5<sup>th</sup> July, and after 50 days on 27<sup>th</sup> July. Leaves were counted from 5 seedlings in each of the 4 replicate plots for each treatment combination ( $n=4$ ; mean  $\pm$  1 SE plotted). Significant differences ( $P<0.05$ ) in pair-wise comparisons of three UV treatments for each date and watering treatment are shown by different letters. Main treatment and interaction effects from mixed model ANOVA with repeated measures are inset (details given in Table S1).

Figure 4: Effects of UV- $\times$ -Drought treatments on silver birch growth on the height of birch seedlings from the base to tip of the main stem. Measurements were made after 28 days of treatments, on 5<sup>th</sup> July, and after 50 days on 27<sup>th</sup> July. Height was measured of 5 seedlings in each of the 4 replicate plots for each treatment combination ( $n=4$ ; mean  $\pm$  1 SE plotted).

Significant differences ( $P < 0.05$ ) in pair-wise comparisons of three UV treatments for each date and watering treatment are shown by different letters. Main treatment and interaction effects from mixed model ANOVA with repeated measures are inset (details given in Table S1).

Figure 5: Effects of UV- $\times$ -Drought treatments on silver birch leaf chlorophyll content. Measurements were made immediately prior to the experiment, on 8<sup>th</sup> June, after 28 days of treatments, on 5<sup>th</sup> July, and 50 days on 27<sup>th</sup> July. The SPAD index was measured from the largest fully-expanded full-sun leaf of 5 seedlings in each of the 4 replicate plots for each treatment combination ( $n=4$ ; mean  $\pm$  1 SE plotted). Significant differences ( $P < 0.05$ ) in pair-wise comparisons of three UV treatments for each date and watering treatment are shown by different letters. Main treatment and interaction effects from mixed model ANOVA with repeated measures are inset (details given in Table S1).

Figure 6: Effects of UV- $\times$ -Drought treatments on silver birch leaf adaxial flavonoid contents (from absorbance at  $\lambda = 375$  nm). Measurements were made immediately prior to the experiment, on 8<sup>th</sup> June, and on three occasions (29<sup>th</sup> June, 11<sup>th</sup> July, 2<sup>nd</sup> August) during the experiment. The largest fully-expanded full-sun leaf at the start of the experiment was repeatedly sampled plus the youngest full-expanded sun leaf at the end of the experiment. Measured leaves from 5 seedlings in each of the 4 replicate plots for each treatment combination ( $n=4$ ; mean  $\pm$  1 SE plotted). Significant differences ( $P < 0.05$ ) in pair-wise comparisons of three UV treatments for each date and watering treatment are shown by different letters. Main treatment and interaction effects from mixed model ANOVA with repeated measures are inset, details given in Table S1 and details of the spectrophotometry in Table S2.

Figure 7: Effects of UV- $\times$ -Drought treatments on silver birch leaf wilting. A census of wilting and seedling mortality on 3<sup>rd</sup> June is prior to the start of the experiment, and subsequently after 34 days of treatments, on 11<sup>th</sup> July, and after 50 days on 27<sup>th</sup> July. All 5 seedlings were monitored in each of the 4 replicate plots for each treatment combination ( $n=4$ ; mean  $\pm$  1 SE plotted). Significant differences ( $P < 0.05$ ) in pair-wise comparisons of three UV treatments for each date and watering treatment are shown by different letters. Main treatment and interaction effects from mixed model ANOVA with repeated measures are inset (details given in Table S1).

## Appendix: Supplemental Material.

3 supplemental Figures and 2 supplemental Tables.

### Supplemental Figure Legends

Figure S1: Solar spectra under each UV treatment and in the open on 8<sup>th</sup> June. The width of the line represents 1 SE either side of the mean values for 4 measurements of each UV treatment. See Table 1 for numerical values detailing the spectral composition.

Figure S2: Soil moisture content of the three watering treatments in each of the three UV treatments during the course of the experiment. Means and standard deviations are displayed from measurements taken every 2–3 days just before and following watering. The soil moisture gradually drops between additions of water. In the water-stressed treatment towards the end of the experiment the presence of small plants, wilted and dying plants depleted the soil moisture very little, leading producing similar minimum values in these and the limited-water treatments. Likewise, at the end of the experiment large seedlings with large leaf area depleted the well watered treatments quickly. Overall means and readings on days when the seedlings were measured are given in Table 2.

Figure S3: Light response curves of photosynthesis, stomatal conductance, and  $C_i/C_a$  to saturating red light, plus blue light under the three watering treatments (19<sup>th</sup> July). Responses were fitted to various functions, and the best fit was used: the Mitscherlich equation to photosynthesis, a linear function for conductance, and a power curve for  $C_i/C_a$ . Stable values of gas exchange were obtained by IRGA at red light PPFD 1, 50, 100, 200, 400, 600, 800, 1000, 1200, and 1400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and saturating red plus blue light at PPFD 0.1, 3, 12, 25, 40, 60, 90, and 120  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .







