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ABSTRACT

There is a need to reappraise effects of UV-B radiation on plant morphology in light of improved
mechanistic understanding of UV-B effects, particularly elucidation of the UVR8-photoreceptor. We
review responses at cell and organismal levels, explore their underlying regulatory mechanisms,
function in UV-protection, and consequences for plant fitness. UV-induced morphological changes
include thicker leaves, shorter petioles, shorter stems, increased axillary branching and altered
root:shoot ratios. At the cellular level, UV-B morphogenesis comprises changes in cell division,
elongation and/or differentiation. However, notwithstanding substantial new knowledge of
molecular, cellular and organismal UV-B responses, there remains a clear gap in our understanding
of the interactions between these organisational levels, and how they control plant architecture.
Furthermore, despite a broad consensus that UV-B induces relatively compact architecture, we note
substantial diversity in reported phenotypes. This may relate to UV-induced morphological changes
being underpinned by different mechanisms at high and low UV-B doses. It remains unproven
whether UV-induced morphological changes have a protective function involving shading and
decreased leaf penetration of UV-B, counterbalancing trade-offs such as decreased photosynthetic
light capture and plant competitive abilities. Future research will need to disentangle seemingly
contradictory interactions occurring at the threshold UV dose where regulation and stress-induced
morphogenesis overlap.

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INTRODUCTION

Plants use solar radiation, not just as a source of energy, but also as a source of information about the environment. Well documented examples of information-harvesting include the capability to sense and respond to the timing, duration, wavelength, dose and direction of light, and this underlies processes such as photoperiodicity, phototropisms and photomorphogenesis (Kendrick & Kronenberg 1994). The term photomorphogenesis is often used to describe light-regulated plant development, i.e. the induction by light of signalling cascades that trigger a broad range of responses at the molecular, cellular and organismal level. Here, we will use the term photomorphogenesis in a stricter sense, to explore the morphological responses of plants grown under different UV-B conditions.

UV-B wavelengths (280 - 315 nm) are biologically active, with low doses inducing changes in gene-expression, physiology, metabolite accumulation and morphology (Heijde & Ulm 2012; Schreiner *et al.* 2012). A specific UV-B photoreceptor, UVR8, has been identified (Rizzini *et al.* 2011). UVR8-mediated processes include antioxidant defence and accumulation of phenolic pigments (Jenkins 2009; Hideg *et al.* 2013). UVR8 is also involved in the regulation of plant morphology under UV-B radiation. UV-induced architectural changes include, amongst others; thicker leaves; shorter petioles; leaf curling, and alterations in leaf shape and width; decreases in stem elongation; increased axillary branching or tillering, and altered root:shoot ratio and structure of the inflorescence (Jansen 2002; Furness *et al.* 2005a; Hectors *et al.* 2007; Yang *et al.* 2008; Wargent *et al.* 2009a, b; Klem *et al.* 2012; Robson & Aphalo 2012). However, the role of UVR8 in these UV-B mediated morphological changes remains poorly understood.

The analysis of published plant UV-responses, and particularly UV-B induced morphological changes, is complex, due to variations in genotype and experimental conditions (Xu & Sullivan, 2010). For example, different *Arabidopsis thaliana* ecotypes display quantitatively different morphological

64 responses when exposed to UV-B (Biswas & Jansen 2012), while in *Silene noctiflora* vegetative and

65 flowering stems display a different dose-response when exposed to UV-B (Qaderi *et al.* 2008). In

66 Arabidopsis, low UV-doses induce rather different effects on plant morphology than higher doses

67 (Figure 1) (Brodführer 1955). In nature, UV-B levels fluctuate depending on temporal, seasonal and

68 meteorological factors, and these fluctuations often correspond with particular plant developmental

69 phases and the position of leaves in the canopy. In contrast, many UV studies are based on the

70 exposure of plants to a constant, chronic level of UV-B under controlled indoor conditions.

71 Notwithstanding the uncertain environmental relevance of many published plant UV-studies, there

72 now seems to be a consensus that UV-B induced stress is a rare event (Ballaré *et al.* 2011). Thus,

73 over the last decade, plant UV-B research has experienced a major paradigm shift, with the focus

74 moving from stress caused by high UV-B doses and stress-induced signalling *to* UV-B-specific

75 regulatory events triggered by very low UV-B doses and mediated through a dedicated UV-B

76 photoreceptor (Jenkins 2009; Jansen & Bornman 2012). Indeed, realistic experimental manipulations

77 of solar UV-B have demonstrated that damaging UV-B-associated stress is the exception rather than

78 the norm in most natural environments (Searles *et al.* 2001; Li *et al.* 2010). This paradigm shift

79 necessitates a re-appraisal of commonly-reported UV-B mediated morphological responses, while

80 simultaneously creating an opportunity to explore the links between established changes in plant

81 morphology and our new understanding of signalling and molecular-level responses. Here, we will

82 review the concept of the UV-B induced phenotype at the cell and plant level, explore underlying

83 regulatory mechanisms, possible functions as well as consequences for plant growth and plant-plant

84 interactions.

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86 **UV-B RESPONSES AT THE MOLECULAR LEVEL**

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87 Major progress has been made in elucidating the molecular pathways underlying many UV-B

88 responses (Rizzini *et al.* 2011; Tilbrook *et al.* 2013). The UV-B photoreceptor UVR8 mediates

89 acclimation responses to chronic UV-B doses (exposure to moderate doses over several days)

90 (Kliebenstein *et al.* 2002), whereas acute (sudden and short-term) exposure to high UV-B induces

91 more generic mitogen-activated protein kinases (MAPK) (Besteiro *et al.* 2011) and/or signalling

92 cascades activated by Reactive Oxygen Species (ROS) (Hideg *et al.* 2013). At present, it is not known

93 whether UV-B mediated morphological changes at cell, organ and organismal level are underpinned

94 by UV-B-specific low dose signalling, by generic stress-mediated pathways, or are associated with

95 the complex metabolic changes that occur during acclimation. Here we will review the evidence that

96 links specific molecular and cellular processes to UV-B mediated morphological changes.

97

98 (I) Regulation of plant morphology via the UVR8 pathway

99 The UV-B specific photoreceptor, UV RESISTANCE LOCUS 8 (UVR8), is a co-regulator of UV-

100 protection, controlling the expression of genes involved in flavonoid biosynthesis, DNA-repair, and

101 anti-oxidative defence (Brown *et al.* 2005; Jenkins 2009; Heijde & Ulm 2012). The UVR8 protein

102 interacts in a strictly UV-B dependent manner with the ubiquitin ligase CONSTITUTIVELY

103 PHOTOMORPHOGENIC (COP1) (Favory *et al.* 2009), while accumulating in the nucleus where it

104 controls gene-expression. Further components of the UVR8-signalling cascade are ELONGATED

105 HYPOCOTYL 5 (HY5) and REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) and RUP2 (Heijde &

106 Ulm 2012). Both *cop1* and *uvr8* mutants show no UV-B induced photomorphogenic response (Favory

107 *et al.* 2009). Thus, 4-day-old uvr8 seedlings raised under narrowband UV-B fail to show any UV-B

108 responsive hypocotyl shortening, although wild-type plants might display as much as 50% inhibition

109 of elongation (Favory *et al.* 2009). In contrast, transgenic UVR8-overexpressing Arabidopsis display

110 particularly strong inhibition of hypocotyl elongation under UV-B (Favory *et al.* 2009). These

111 responses indicate the importance of UVR8 as a photoreceptor that controls seedling architecture

112 (Kliebenstein *et al.* 2002). Yet, when *uvr8* mutant plants are grown for longer periods under UV-B

113 lamps under control conditions (Favory *et al.* 2009; Wargent *et al.* 2009b) or under ambient sunlight

114 outdoors (Morales *et al.* 2013), they are smaller than wild-type plants, a result which appears to be

115 in direct contrast to the hypocotyl response. It has been argued that the strong growth reduction

116 reflects UV-B stress in the poorly UV-protected *uvr8* mutant plants and consequent growth

117 retardation. Thus, it is considered that *uvr8* mutant plants are insensitive to UV-B as an

118 informational signal, but hypersensitive to UV-B stress (Favory *et al.* 2009). This hypothesis

119 effectively reconciles UV-B stress with UVR8-mediated regulation in one model, with the outcome

120 depending on the balance of the two processes. Such a model can easily accommodate complex

121 dose-responses, such as those reported by Brodführer as long ago as 1955 (Figure 1), that lowering

122 the UV-B dose from 100% down to 33% of ambient solar UV-B stimulated inflorescence elongation in

123 Arabidopsis, while very low UV-B doses (2% of ambient solar UV-B) impeded the same process. Bell-

124 shaped and inversely bell-shaped UV-B dose-response curves have also been observed by Qaderi *et*

125 *al.* (2008) and van de Staaij *et al.* (1997). In this context it should be noted that UVR8-mediated

126 photomorphogenic responses are also subject to further regulatory interactions with factors such as

127 HY5, HYH COP1, RUP 1 and RUP2 (Jenkins 2009; Heijde & Ulm 2012). It is these regulatory

128 interactions that may further modify gene expression patterns, and consequently plant architecture.

129 This was reported by Morales *et al.* (2013) who found that not all the UVR8 activated genes that are

130 expressed under controlled conditions are also expressed under natural outdoor conditions. For

131 example, UVR8 apparently interacts with UV-A photoreceptors to regulate transcript accumulation,

132 such as Pyridoxine Biosynthesis 1 (PDX1), in plants grown outdoors (Morales *et al.* 2013). It is likely

133 that UV-induced morphological responses are likewise co-regulated through feedback-interactions

134 at the physiological, cell and organ level.

135

136 <u>(II) Stress Induced Morphogenic Responses</u>

137 The concept of Stress Induced Morphogenic Responses (SIMR) captures similarities in phenotype

138 induced by a broad range of stressors (Potters *et al.* 2007). The concept extends the General

139 Adaptation Syndrome (GAS), which recognises similarities in biochemical responses to distinct

140 stressors (Leshem & Kuiper 1996). Many stressors induce similar alterations in plant morphology,

141 key components of which are the inhibition of cell elongation, localized stimulation of cell division

142 and alterations in cell differentiation status, i.e. growth redistribution rather than simply growth

143 cessation. Indeed, SIMR-phenotypes involve decreased root, shoot or stem elongation, but increased

144 root or shoot branching, and it is this local growth stimulation that distinguishes SIMRs from the

145 severe stress and damage phenotypes. SIMRs are hypothesised to be caused by common stress-

146 related processes such as increased ROS production and altered phytohormone metabolism and

147 transport (Potters *et al.* 2007). There is extensive evidence that UV-B can cause oxidative stress in

148 plants (cf. Hideg *et al.* 2013), while impacts on auxin metabolism have also been reported (Hectors

149 *et al.* 2012). Another proposed factor in SIMRs is nitric-oxide mediated change in microtubuli

150 organisation (Krasylenko *et al.* 2012). The cytoskeleton, and particularly the microtubular system,

151 plays a key role in plant morphogenesis, being involved in control of cell division and elongation as

152 well as initiation of lateral growth (Wasteneys 2004). Microtubuli depolymerisation, which can occur

153 after exposure to high levels of UV-B, is associated with epidermal cell swelling and the inhibition of

154 root elongation (Krasylenko *et al.* 2012). The concentration of NO increases following exposure to

155 numerous biotic and abiotic stressors (Besson-Bard *et al.* 2008), indicating that this is a generic

156 stress-related response. Indeed, alterations in microtubuli organisation are not evident under low

157 chronic UV-B levels (Jacques *et al.* 2011). Thus, factors associated with SIMR, such as ROS and NO,

158 appear to mostly play a role under stress-inducing UV-B conditions.

159 In summary, there is evidence for distinct UV-B-mediated morphogenic responses; an UVR8-

160 mediated response, perhaps fine-tuned through interactions with other environmental and

161 physiological parameters, and a more generic stress response involving changes in cell cycle activity.

162 A future objective for the UV-B field will be to develop tools that can distinguish between these

163 distinct pathways, and indicate their relative importance under environmentally relevant conditions.

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168 **UV-B RESPONSES AT THE CELLULAR LEVEL**

169 The control of cell division and cell expansion is central to regulating organ size (Sugimoto-Shirasu &

170 Roberts 2003). UV-B can affect cell fate through multiple regulatory and/or stress mediated

171 processes. For example, UV-B can impede cell cycle progression, probably through the accumulation

172 of DNA damage, and this particularly slows the G1-to-S step in the cell cycle (Jiang *et al.* 2011). More

173 generic oxidative stress can also affect cell cycle progression, and this has lead to the postulation of

174 the existence of an 'oxidative stress checkpoint' (De Schutter *et al.* 2007). Cell cycle arrest, involving

175 specific checkpoints, can facilitate DNA repair before further replication occurs (Jiang *et al.* 2011),

176 but may also result in decreased cell numbers and/or endoreduplication (Radziejwoski *et al.* 2011).

177 Notably, UV-B also down-regulates the transcription factor E2Fe/DEL1, which represses the onset of

178 endoreduplication (Radziejwoski *et al.* 2011). The elimination of down-regulation can lead to an UV-

179 B mediated increase in ploidy which, in turn, may result in increased cellular expansion, thus

180 potentially compensating for decreases in cell numbers. Consistently, the UV-B-mediated decrease

181 in leaf area is relatively small in an Arabidopsis E2Fe/DEL1 knockout line, and this is associated with a

182 small increase in both ploidy and cell size (Radziejwoski *et al.* 2011).

183   The balancing act between cell division and cell expansion is highlighted by studies of UV effects on

184   cellular growth. Some UV-exposure studies have revealed inhibition of cell division (Dickson &

185   Caldwell 1978; Wargent *et al.* 2009a), while others report inhibition of cell expansion (Hectors *et al.*

186   2010), or a combination of these two processes. Lake *et al.* (2009) report larger cells on the abaxial

187   leaf surface, although the number of cells per mm^2 was unchanged for the more UV-B exposed

188   adaxial surface of Arabidopsis Col-0 wild type. Cell density markedly increased when the UV-

189   sensitive *fah-1* mutant was exposed to UV-B. However, given the nearly 10-fold reduction in leaf

190   area, the 2-fold increase in abaxial cell density still represents a large overall decrease in leaf cell

191   numbers. Wargent *et al.* (2009a) also report inhibition of cell division in Arabidopsis, yet in this case

192   the decrease in cell numbers was partially compensated by increased cell expansion. Cell

193   morphological studies by Jacques *et al.* (2011) and Hectors *et al.* (2010) have reported that elevated

194   UV-B does not affect the shape of adaxial pavement cells in Arabidopsis. Indeed, an interesting but

195   unproven possibility is that UV-B alters the directional expansion of growth, redirecting cellular

196   expansion from the longitudinal plane to the transverse plane of the leaf, a potential means of

197   increasing leaf thickness. There is a clear need for future studies to investigate how UV-B affects

198   both cell orientation and organisation within a leaf, and the effects of UV-B on cellular

199   differentiation require clarification.

200   Jacques *et al.* (2011) reported that UV-B does not affect cell differentiation, visualised as the

201   stomatal index. Yet, Lake *et al.* (2009) report a significant UV-B-mediated decrease in the adaxial

202   stomatal index (the ratio of stomata: epidermal cells) of Arabidopsis leaves which they attribute to a

203   reduction in abscisic acid content. Although, similar decreases in stomatal index, but not stomatal

204   density, have been reported for *Petunia* X hybrid (Staxen & Bornman 1994), there seems to be no

205   consistent UV-B effect on stomatal density, with other studies finding decreases (*Glycine max:* Gitz

206   *et al.* 2005), increases, or no effects (both *Betula pendula,* Kostina *et al.* 2001; Kotilainen *et al.* 2009

207   amongst others). A special scenario of UV-mediated changes in cell development concerns pollen

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208 biology. The pollen wall is relatively poor at attenuating UV-B, with Yellof *et al.* (2008) reporting up

209 to 20% transmission of UV-B into the cytoplasm. Few studies have investigated the fitness

210 consequences of increased UV-B irradiance on pollen, but those that have reported morphological

211 abnormalities (Rozema *et al.* 2001; Koti *et al.* 2004), sometimes associated with reduced plant

212 reproductive success (Torabinejad *et al.* 1998; Murphy & Mitchell 2013). Thus, at the cellular level,

213 there is good evidence for UV-induced changes in cell division, elongation and differentiation. It is

214 tempting to attribute the observed diversity of cellular UV-responses, at least partially, to the similar

215 diversity at the molecular level, where there is a dynamic balance between UVR8 and stress

216 mediated pathways.

217

218 **WHOLE PLANT PHENOTYPE AND UV-B**

219 <u>(I) UV-induced changes in whole leaf morphology</u>

220 The most frequently reported UV-B induced morphological changes are a decrease in leaf area

221 and/or an increase in leaf thickness (Jansen 2002; Furness *et al.* 2005a; Hectors *et al.* 2007; Yang *et*

222 *al.* 2008; Wargent *et al.* 2009a, b; Klem *et al.* 2012; Robson & Aphalo 2012). Various broad-leaved

223 species display increased palisade thickness when grown under UV-B (Nagel *et al.* 1998; Robson &

224 Aphalo 2012), while increased hypodermal thickness has been reported for pine (*Pinus ponderosa*)

225 (Nagel *et al.* 1998). Detailed studies have shown that UV-effects on leaf area are complex. For

226 example, Robson & Aphalo (2012) compared the effects of supplemental UV-B on leaf development

227 of two species of birch. In silver birch (*Betula pendula*), UV-B transiently impeded leaf expansion,

228 whereas in downy birch (*Betula pubescens*) final leaf size was affected, a persistent effect which was

229 linked to an eventual reduction in height growth (Robson & Aphalo 2012). Transient UV-B induced

230 reductions in leaf expansion were also reported for *Rumex patientia* (Sisson & Caldwell 1976,

231 Dickson & Caldwell 1978) and Arabidopsis (Lake *et al.* 2009), possibly related to stress, followed by

232 recovery to match the leaf area of non-UV exposed plants. Similarly, Hectors *et al.* (2010), noted

233 transient changes in the length: width ratio of Arabidopsis leaves. Exposure to UV-B initially caused a

234 relatively large decrease in cell expansion along the longitudinal leaf axis, but when these leaves

235 grew older, the length:width ratio was restored due to stronger inhibition of expansion along the

236 transverse axis (Hectors *et al.* 2010). We hypothesise that these transient UV effects reflect a short

237 term disruption in leaf development, perhaps as a consequence of UV-exposure for leaves of a non-

238 acclimated plant. It appears that once UV-protection is induced in a UV-B exposed leaf, involving

239 such processes as up-regulation of ROS scavenging, UV-screening and DNA repair capacities, any

240 disruption in leaf development is overcome. This may enable leaf development to resume its original

241 pattern, or even produce a compensatory response whereby greater expansion adjusts for reduced

242 division. The capacity to switch between patterns of leaf development appears to be species

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243 specific; downy birch halts leaf development early under supplemental UV, and this is associated

244 with impaired growth (Ren *et al.* 2006; Robson & Aphalo 2012), while silver birch displays

245 compensatory growth. It appears that both increases in cell number and cell size are expressions of

246 the resumption of "normal" leaf development, whereby the plant attempts to adjust its leaf size to

247 environmental conditions. UV-B driven morphogenesis involves stress and/or UVR8-mediated

248 processes, and may be transient, followed by a return to normal organ development. Given this

249 complexity, it is highly unlikely that commonly-used single time-point, and single species analyses of

250 leaf morphogenesis will adequately reveal the full complexity of UV effects.

251

252 II) UV-induced changes in stem and root morphology

253 Decreases in stem elongation have been reported for a variety of different species (Barnes *et al.*

254 1996; Jansen 2002; Furness *et al.* 2005a; Hofmann & Campbell 2011; Germ *et al.* 2013). There is also

255 extensive evidence that UV-B increases axillary branching and/or tillering (cf. Jansen 2002; Furness

256 *et al.* 2005a). It is likely that these aboveground effects will also affect the root:shoot ratio. Indeed,

257 an increased allocation of biomass to roots has been reported to occur under UV-B irradiance

258 (Bussell *et al.* 2012). A similar allocation shift can be observed when plants raised in the shade are

259 compared to those in full sun. Such effects are, however, species specific and also depend on

260 interactions with other biotic components of the ecosystem such as competition from other plant

261 species (Rinnan *et al.* 2006), mycorrhizae (Zaller *et al.* 2002) and endophytes (McLeod *et al.* 2001).

262 Thus, any UV-B effect on plant morphology needs to be assessed in the context of an array of

263 environmental influences.

264

THE BIOLOGICAL FUNCTION OF UV-INDUCED MORPHOGENESIS

266   Several UV-B induced plant responses (e.g. UV-B induced accumulation of flavonoids) contribute to

267   plant UV-B protection. However, the evidence is weak that actively regulated morphological changes

268   substantially contribute to UV-B protection. Moreover, the transient character of some of the UV-

269   induced changes in leaf development (Lake *et al.* 2009; Robson & Aphalo 2012) would suggest at

270   best a temporary role in UV-protection. The effectiveness of leaf morphological alterations that

271   reduce exposure of the mesophyll to UV-B, such as leaf thickening and changes in epidermal cell

272   shape and size (Nagel *et al.* 1998; Fagerberg & Bornman 2005; Hofmann & Campbell 2012), are often

273   viewed as important adaptations increasing UV-protection (Vogelmann *et al*. 1996), but must be

274   considered in the context of a plant's entire physiological acclimation response (Jansen 2002). Most

275   plant species have efficient photorepair mechanisms and antioxidant scavenging, that under natural

276   growth conditions effectively ameliorate much of the damage caused by UV-B (Ballaré *et al.* 2011).

277   Moreover, penetration of UV photons into the mesophyll is typically less than 10% of incident

278   radiation (Day 1993; Day *et al.* 1994; Barnes *et al.* 2008), while any UV-B that does penetrate the leaf

279   follows a heterogeneous pattern of transmission via stomatal pores and anticlinal cell wall regions

280   (Day *et al.* 1993). These findings raise the question of whether leaf thickening is an acclimative

281   response conferring UV-B protection that is proportionate to the requisite loss of photosynthetic

282   light use efficiency.

283   Adjustments of plant morphology and architecture might be interpreted as providing a flexible and

284   transitory mean for plants to reduce exposure of leaves to UV-B. For example, leaves of short, bushy

285   plants are more likely to be shaded and less exposed to UV-B within a mixed canopy. However, once

286   more this is a rather over-simplified perspective. Radiation measurements show that the diffuse

287   fraction of the global UV-B irradiance varies from 0.57 to 0.91 while that of photosynthetically active

288   radiation (PAR) varies from 0.25 to 0.70 (Webb & Steven 1984). The relatively large diffuse

289   component of UV-B may result in relatively high levels of UV-B radiation within plant canopies. The

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290   foremost example of a canopy is a forest system. Forests produce complex and heterogeneous

291   radiation environments where a very low proportion, down to 1-2% of solar radiation penetrates to

292   ground level. Yet, the UV:PAR ratio deep in the canopy varies enormously, between sunflecks that

293   are relatively depleted in UV-B (UV:PAR<1) to shaded understorey where UV-B is strongly enriched

294   (UV:PAR up to 5) at midday (Yang *et al*. 1993; Brown *et al.* 1994). Changes in the spectral

295   composition are also modified by the optical properties of canopy phyto-elements, particularly the

296   spectral reflectance and transmittance of leaves. Percentage leaf reflectance for many crop and tree

297   species is 0.05 to 0.10% for UV-wavelengths, typically 0.15% for PAR, and approximately 0.4 - 0.6%

298   for near infra-red (Walter-Shea & Norman 1991; Grant 1997; Holmes & Keiller 2002; Sims & Gamon

299   2002). Leaf transmittance for the UV-B and UV-A wavebands is negligible. Canopy UV-B optical

300   properties thus create complex feedback loops whereby the UV-absorbing properties of upper sun

301   leaves modify the UV environment for shade leaves in a comparable manner to the well known

302   changes in red: far-red ratio. This is a species specific process; white clover (*Trifolium repens*)

303   depletes UV-B:PAR with depth into the canopy, a characteristic attributed to the combination to a

304   planophile leaf angle and high UV-B absorbance (Deckmyn *et al.* 2001). In contrast, grasses such as

305   *Dactylis glomerata,* with erectophile leaves facilitate much deeper penetration of both UV-B and

306   PAR into the canopy (Deckmyn *et al.* 2001). Thus, UV-B penetration depends on canopy structure

307   and species-composition, penetration of diffuse light and leaf reflectance and absorbance.

308   Consequently, a more dwarfed phenotype does not necessarily imply exposure to a relatively

309   depleted UV-B to PAR ratio. Furthermore, a shade-utilisation acclimation response would be a costly

310   UV-defence for the plant given the decrease in PAR exposure incurred (Yang *et al.* 1993), enough to

311   affect plant competition for light in the canopy (Barnes *et al.* 1996). Finally, shade utilisation would

312   only feasible to a restricted selection of plant functional types but might induce contradictory

313   developmental changes in other species. For example, Arabidopsis, a plant displaying strong

314   dwarfing under UV-B, responds to shading by flowering and bolting (Callaghan & Pigliucci 2002). This

315 would potentially result in exposure of flowers to full solar UV-B, the acclimative significance of

316 which is at best equivocal.

317 Based on the understanding of morphological responses of plants subject to experimentally-elevated

318 UV-B doses, and given the relatively high UV:PAR ratio in canopy shade compared with the low

319 UV:PAR ratio in clearings and sunflecks (Flint and Caldwell 1998), it might be anticipated that shade

320 leaves are modified by this proportionately large UV-B dose under-canopies beyond those

321 characteristics exhibited in response to PAR. Nevertheless, the functional relevance of such

322 morphological responses remains to be proven. The possibility that UV-B driven morphogenesis has

323 a function other than UV-protection should also be considered. This supposition relies on the

324 premise that UV-B exposure pre-empts or accompanies high PAR, or more tenuously drought stress

325 and other seasonal changes. UV-B exposed leaves tend to be smaller and thicker, typical

326 characteristics of sun-leaves (Lichtenthaler *et al.* 2007); while in the absence of UV-B leaves tend to

327 be larger and thinner, i.e. more similar to shade leaves (Krizek 2004; Niinemets 2010). While it is

328 attractive to hypothesise UV-B-mediated responses as part of a general environmental response to

329 variable light levels, this is neither proven, nor takes in to consideration the enormous variability in

330 plant UV-B morphogenesis.

331

332 **PLANT MORPHOLOGY AND FLAVONOID ACCUMULATION; A TWO WAY INTERACTION?**

333 Although UV-induced morphological changes may not have a direct functional role in UV-protection,

334 morphological changes are associated with regulation of protective flavonoid pigments. Analysis of

335 UV-induced morphogenesis in barley revealed a strong inverse relationship between leaf flavonol

336 content and specific leaf area, i.e. leaves with high flavonoid content were relatively thick (Klem *et*

337 *al.* 2012). Also in silver birch saplings the concentration of flavonoid glycosides correlates negatively

338 with plant height in some studies (Mutikainen *et al.* 2002), but not in others (Lavola *et al.* 2000;

339 Robson & Aphalo 2012). A key question is whether UV-B mediated increases in flavonoid content

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340 and decreases in leaf area or stem length are co-occurring phenomena, or rather are mechanistically

341 related. Flavonoids play a key role in plant UV-B protection, having both anti-oxidant and UV-

342 screening properties (Agati & Tattini 2010), and therefore contribute to the prevention of UV-B

343 stress, and stress-mediated morphogenesis. Flavonoids also regulate multiple developmental

344 processes. Flavonoid aglycones impact on auxin homeostasis by impeding polar transport through

345 efflux carriers (Peer & Murphy 2007), and/or by altering auxin catabolism (Zenk & Müller 1963;

346 Mathesius 2001). The effectiveness of flavonoid-regulated auxin transport has been demonstrated

347 using Arabidopsis tt4 and ugt78d2 flavonoid mutants, which exhibit alterations in both auxin

348 distribution and plant morphology (Peer *et al.* 2004; Besseau *et al.* 2007; Ringli *et al.* 2008; Yin *et al*.

349 2013). Similarly, Arabidopsis plants grown on agar plates containing the flavonoid precursor

350 naringenin possess altered root elongation, similar to plants grown on synthetic auxin transport

351 inhibitors (Brown *et al.* 2001). A link was made between UV-hypersensitivity and auxin transport

352 and/or distribution in the mutant, *ROOT UVB SENSITIVE2* (*RUS2*) (Ge *et al.* 2010). Although

353 understanding of the physiological interactions in UV-B exposed plants is still limited, these data

354 appear to imply a role for flavonoids and/or auxin homeostasis in "fine-tuning" UV-induced

355 morphological responses.

356 Classically, the synthesis of energetically-costly phenolic compounds was expected to incur a cost to

357 growth, however in practice this relationship has rarely been evident (Lavola *et al.* 2000; Kotilainen

358 *et al.* 2009; Robson & Aphalo 2012). Nevertheless, Hofmann *et al.* (2000) reported a trade-off

359 between plant dry matter production and quercetin glycoside accumulation in white clover

360 (*Trifolium repens*). Conversely, higher quercetin glycoside accumulation under UV-B was correlated

361 with tolerance against UV-B-induced growth reduction (Hofmann *et al.* 2003; Hofmann & Campbell

362 2011). Thus, the role of flavonoids in UV-induced morphogenesis is multifaceted, and consequences

363 for growth may depend upon the strength of UV and extent of flavonoid induction. Elucidating this

364 role will require the development of novel tools. Morales *et al.* (2010) reported a linear dose

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365 response of some flavonoids to solar UV-A compared with a quadratic dose response of the same

366 compounds to UV-B. Using differential doses of UV in experiments in this way may help elucidate the

367 role of flavonoids in UV-mediated morphogenesis. Indeed, although co-induction of flavonoid

368 accumulation and morphological changes has been extensively detailed, it remains unknown

369 whether these phenomena are mechanistically related.

370

371 UV-B INDUCED MORPHOGENESIS AND PLANT-PLANT INTERACTIONS

372 UV-induced morphological effects, measured at cell, organ or organismal level cannot easily be

373 scaled up to the vegetation level. Under natural conditions, the greater complexity of biotic

374 interactions as well as interactions with multiple environmental factors mean that UV-B effects on

375 morphology can be masked, or unexpectedly be amplified, through for example altered competitive

376 relationships. Plant-plant interactions include the competition for resources such as light, nutrients

377 and water. Competitive success is directly influenced by the morphology of interacting species.

378 There is strong evidence that light quality, and especially the red: far-red (R:FR) light ratio, through

379 its effects on morphology, plays a pivotal role in influencing interactions among neighbouring plants

380 (Ballaré & Casal 2000; Rajcan & Swanton 2001). Thus far, only a few studies have analysed the

381 impact of UV-B radiation on the interactions between competing plants (e.g. Beyschlag *et al.* 1988;

382 Barnes *et al.* 1996; Furness *et al.* 2005b). However, these studies show that the influence of UV-B

383 radiation on competitive interactions can be substantial, and this derives from the effect of UV-B

384 radiation on shoot morphology and light interception (Barnes *et al.* 1990). For instance, in *Sphagnum*

385 peatlands, solar UV-B caused a decrease of growth of the *Sphagnum magellanicum* carpet, but not

386 of the co-occurring Ericaceae-species *Empetrum rubrum,* which is well defended from UV by its very

387 high needle flavonoid content (Searles *et al.* 2002). However, *Empetrum* stems displayed increased

388 lateral branching under near-ambient solar UV-B radiation, and as a consequence emergent stems

 17

389   tended to be enveloped by the growing *Sphagnum* carpet (Robson *et al.* 2003). The differences in

390   the morphological response of individual species to UV-B radiation can affect competitive abilities,

391   and hence shift competitive balances (Barnes et al. 1995). For example, UV-B induced shifts in

392   competitive interactions were found in a greenhouse study with broccoli (*Brassica oleracea*) and

393   *Chenopodium album* (Furness *et al.* 2005a). Broccoli gained in competitiveness relative to *C. album*

394   in response to above-ambient UV-B exposure conditions. Intraspecific competition was less

395   influenced by UV-B radiation than interspecific competition, emphasizing the importance of

396   differential UV-responses between species. Fox & Caldwell (1978) examined the effects of an

397   increase in UV-B radiation on the competitive interactions of several pairs of species. Statistically

398   significant shifts in the competitive balance were found in *Amaranthus – Medicago* and *Poa – Geum*

399   interactions. In both cases, UV-B caused a shift in favour of the crop (*Medicago* and *Poa*). These data

400   suggest that UV-dependent changes in competitive balance may well be common, perhaps reflecting

401   the variation in morphological responses of plant species, ecotypes and cultivars to UV-B radiation.

402   Generally, monocots appear to be more responsive than dicots (Barnes *et al.* 1990). Yet, genotypic

403   variation in morphological responsiveness to UV-B was also apparent between closely related

404   cultivars of crop species (Yuan *et al.* 2000; Klem *et al.* 2012) and between Arabidopsis ecotypes

405   (Biswas & Jansen 2012).

406   Changes in canopy structure caused by UV-B-induced morphogenesis have been computed to be

407   sufficient to alter light interception and canopy photosynthesis for competing species (Ryel *et al.*

408   1990). Effects of UV-B on shoot elongation can potentially amplify competition for light and

409   therefore be an important mechanism through which changes in the solar UV-B spectrum alter the

410   composition and character of terrestrial vegetation. There are two specific factors that can amplify

411   the importance of even small changes in stem elongation and the relative distribution of foliage

412   within the upper parts of canopies. Firstly, light extinction is exponential within plant canopies (Yang

413   *et al.* 1993). Secondly, the photosynthetic capacity of foliage in the upper leaves is usually much

414   greater than that of leaves located lower in the canopy (Urban *et al.* 2012). The importance of light

    18

415 capture and UV-B-induced morphogenesis is demonstrated by work of Barnes *et al.* (1988) who

416 showed that UV-B induced shifts in the competitive balance between wheat (*Triticum aestivum*) and

417 wild oat (*Avena fatua*) were associated with changes in the relative positioning of leaf area within

418 mixed canopies. Both leaf insertion heights and leaf blade lengths were reduced to a greater extent

419 in UV-B-exposed wild oat than in UV-B-exposed wheat, thus potentially decreasing wild oat

420 photosynthetic light capture. However, alternative mechanisms for UV-B-induced changes in plant-

421 plant competitive balance have also been proposed. UV-B exposure can alter root:shoot ratios and

422 therefore root competition, which can be more critical than above-ground competition (Zaller *et al.*

423 2002; 2004). Additionally, there is some evidence that UV-B may affect the production and release

424 of flavonoid-based allelochemicals (Einhellig 1995; Li *et al.* 2009; Furness *et al.* 2008), and thus

425 indirectly alter the morphology of target species. Finally, it has been reported that UV-B can also

426 alter the flower morphology, with reports both of smaller flowers with shorter petals and stamens

427 and of increased flower diameters, size and number of inflorescences (Helsper *et al.* 2003; Kravets et

428 *al.* 2008). To summarise, there is clear evidence that UV-B-mediated changes in plant morphology

429 can affect plant-plant competition. However, at the moment the relative importance of such

430 competitive changes, within the context of a complex environment, remains to be established.

431

432 **IN CONCLUSION**

433 Many published papers refer to UV-B-induced morphological changes (Figure 2). These

434 morphological changes include, amongst others; thicker leaves; shorter petioles; leaf curling, and

435 alterations in leaf shape and width; decreases in stem elongation; increased axillary branching or

436 tillering, and altered root:shoot ratio and structure of the inflorescence. However, the UV-B induced

437 phenotype is diverse, with many apparently contradictory reports of UV-B effects on plant

438 architecture. The diversity of effects at the organismal level is echoed by similar diversity at the

19

439 cellular level, with reports of increased or decreased cell numbers, increased or decreased cell size,

440 and changes or no changes in cellular differentiation! Variations in experimental conditions,

441 genotypes, and developmental stage have been shown to play a role in causing such a diversity of

442 response. However, UV-B-induced phenotypic diversity probably also reflects diversity in underlying

443 molecular mechanisms. It is likely that UV-induced morphological changes are underpinned by more

444 than one molecular mechanism; at low UV-B doses through an UVR8 mediated response, perhaps

445 fine-tuned through interactions with flavonoids and/or phytohormones; and at high UV-B doses

446 through a more generic (SIMR) stress response possibly involving changes in cell cycle activity (Figure

447 3). Several studies have demonstrated complex UV-B dose-responses, consistent with multiple

448 mechanisms and/or regulatory feedback loops. However, despite a substantially improved

449 understanding of molecular, cellular and organismal UV-B responses, there remains a clear gap in

450 our knowledge of the interactions between these organisational levels, and how they control plant

451 architecture. Furthermore, there is insufficient understanding of the UV-B dose-response

452 underpinning morphogenesis. Future research will especially need to disentangle the complex

453 interactions that occur at the threshold UV-B dose where regulation and stress-induced damage

454 overlap and where plant responses from different experiments can seem confusing and seemingly

455 contradictory. The use of UVR8 and related mutants, as well as generic stress-protected and stress-

456 sensitive mutants, under different UV-B radiation combinations might facilitate this quest. The

457 capability to distinguish UVR8 and generic stress-induced morphogenesis will, in turn, result in

458 meaningful tools to analyse the functional role and/or fitness cost of these processes. Understanding

459 of the functional importance of commonly-reported UV-B-induced morphological changes will be a

460 challenge that requires careful consideration of a suite of plant and environmental factors that

461 combine to produce a particular plant architecture.

462

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Figure legends

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773 Figure 1: An example of a bell-shaped UV dose response curve redrawn from Brodführer (1955). The

774 number of Arabidopsis branches (top panel), height in cm (middle) and number of seedpods (lower),

775 per individual. Data are means ± 1 SE of 20 individuals per UV treatment, and the fitted line is a loess

776 smoother.

777

778 Figure 2: The focus of publications on the effect of UV radiation on plant morphology from 1988 to

779 date. Of 276 articles in Web of Knowledge obtained using the search string, (UV OR ultraviolet) AND

780 morphology AND plant*, 110 actually focussed on the effects of UV-B radiation on the morphology

781 of terrestrial plants. Each experiment from the 110 articles was classified according to the level of

782 organisation studied (where experiments considered multiple levels of organisation publications

783 were put into more than one category). The number of review articles among these 110 was also

784 counted. The number of publications in each category is given in parentheses within each bar. See

785 *Supporting information* for details of the publications censured.

786

787 Figure 3: Schematic overview indicating that different strengths of UV dose produce a response

788 mediated by interacting regulatory pathways and stress induced pathways. The relative importance

789 of these responses is UV-dose dependent and is further modulated through an environmental filter.

Cellular/Pollen (38)

Leaf/Root (75)

Whole Plant (41)

Canopy (18)

Review (14)

Level of organisation

Percentage of published articles (%)

0   20   40   60   80