1 Title: Blue light advances bud burst in branches of three temperate deciduous
2 tree species under short-day conditions.

3 Authors: Craig C. Brelsford^{1*} and T Matthew Robson¹

4

5 1) Viikki Plant Science Centre (ViPS), Department of Biosciences, 00014, University of Helsinki, Finland

6

7 *Author for Correspondence: craig.brelsford@helsinki.fi

8 Phone number: +358452162825

9 CCB ORCID ID: 0000-0001-7084-352X

10 TMR <https://orcid.org/0000-0002-8631-796X> <http://www.researcherid.com/rid/J-3381-2012>

11

12

13

14 **Abstract**

15 During spring, utilising multiple cues allow temperate tree species to coordinate their bud burst and leaf out, at the right
 16 moment to capitalise on favourable conditions for photosynthesis. Whilst the effect of blue light (400-500nm) has been
 17 shown to increase percentage bud burst of axillary shoots of *Rosa* sp, the effects of blue light on spring-time bud burst of
 18 temperate deciduous tree species has not previously been reported. We tested the hypotheses that blue light would advance
 19 spring bud burst in temperate tree species, and that late-successional species would respond more than early-successional
 20 species, who's bud burst is primarily determined by temperature. The bud development of *Alnus glutinosa*, *Betula pendula*,
 21 and *Quercus robur* branches, cut from dormant trees, was monitored under two light treatments of equal photosynthetically
 22 active radiation (PAR, 400-700 nm) and temperature, either with or without blue light, under controlled environmental
 23 conditions. In the presence of blue light, the mean time required to reach 50% bud burst was reduced by 3.3 days in *Betula*
 24 *pendula*, 6 days in *Alnus glutinosa*, and 6.3 days in *Quercus robur*. This result highlights the potential of the blue region of
 25 the solar spectrum to be used as an extra cue that could help plants to regulate their spring phenology, alongside photoperiod
 26 and temperature. Understanding how plants combine photoreceptor-mediated cues with other environmental cues such as
 27 temperature to control phenology is essential if we are to accurately predict how tree species might respond to climate
 28 change.

29

30 Key Words (4-6 words)

31 Phenology; bud break; leaf flush; spectral composition; spectral quality; photoreceptors.

32

33 Key Message

34 An LED spectrum containing blue light advanced bud burst in branches of *Betula pendula*, *Alnus glutinosa* and *Quercus*
 35 *robur* compared with a spectrum without blue light in a controlled environment.

36

37

38

39

40 **Introduction**

41 Trees at northern latitudes must time the bud burst and unfolding of their leaves in spring so that they are able to
 42 capitalise on favourable conditions for photosynthesis as early as possible, whilst avoiding late frosts which can cause foliar
 43 damage (Hänninen 1991, Augspurger 2009, Bennie et al. 2010). Prior to bud-burst, a tree must go through three dormancy
 44 periods over winter which can be separated into 1) paradormancy – the inhibition of growth by distal organs, 2)
 45 endodormancy - the inhibition of growth by internal bud signals, and 3) ecodormancy – the inhibition of growth based on
 46 temporarily-unfavourable light conditions (Samish 1954, Lang et al. 1987,). The dormancy period between endodormancy
 47 and ecodormancy is jointly controlled by a chilling requirement and by photoperiod, where the chilling requirement is
 48 defined as non-freezing temperatures below 10°C (Battey 2000). After or concomitantly with this period of vernalisation,
 49 temperature is the predominant cue affecting bud burst, and to predict bud burst many models calculate the accumulated daily
 50 mean temperature above a given minimum threshold (e.g. >0°C) after sufficient vernalisation has occurred (Hänninen 1995,
 51 Cesaraccio et al. 2004).

52 Typically, early-successional light-demanding species adopt a risky strategy by timing their bud burst primarily based
 53 on temperature (Körner 2007, Caffarra and Donnelly 2010, Körner and Basler 2010), whereas it is more common for later-
 54 successional shade-tolerant species to be photoperiod sensitive (Basler and Körner 2012). Körner and Basler (2010) propose
 55 that in a warmer climate the absence of a photoperiod requirement in opportunistic early-successional species will probably
 56 give them a competitive advantage over later-successional species. However, this may also lead early-successional species to
 57 be at greater risk from late spring frosts (Cannell and Smith 1986), since a day-length threshold provides a fail-safe
 58 mechanism ensuring that a period of unseasonably warm weather during the winter cannot prematurely fulfil the temperature
 59 requirement. The date of bud burst in tree species is considered to have advanced by 2.5 days per decade at temperate
 60 latitudes since 1971 due to climate change (Menzel et al. 2006, Körner and Basler, 2010), however this trend for promotion
 61 of bud burst date with warmer temperatures has recently started to diminish (Fu et al. 2015). Specifically, leaf unfolding was
 62 advanced by fewer days per additional °C of warming in 13 temperate deciduous woody species measured across northern
 63 temperate Europe and the USA from 1980-2012. Fu et al. (2015) partially attributed this decline to fewer chilling days, but
 64 also to a photoperiod requirement that reduces the influence of warmer springs. Additionally, periods of “global dimming”
 65 caused by anthropogenic and volcanic emissions, affect the spectral composition of incident solar radiation reaching the
 66 Earth’s surface (Stanhill and Cohen 2001, Mercado et al. 2009), increasing its diffuse/direct fraction and consequently also

67 the relative proportion of blue light (420-490 nm) (Urban et al. 2007). If we are to forecast the response of bud burst to
68 climate change, we will need to improve existing models to include and account for all the mechanisms involved (Way and
69 Montgomery 2014, Yun et al. 2017, Mayshev et al. 2018).

70 The effect that spectral composition has on the timing of bud burst and leaf out in tree species has been studied much
71 less than that of photoperiod. The spectral composition that trees receive changes predictably with time of year, day length,
72 and latitude; and over shorter time-scales with cloud cover and other factors that alter the ratio of direct to diffuse solar
73 radiation (Johnson et al. 1967, Smith 1982, Hughes et al. 1984, Urban et al. 2007). These compositional changes have
74 implications for tree species undergoing range shifts under rising global temperatures where differences in spectral irradiance
75 at higher latitudes will produce novel combinations of phenological cues (Wareing 1956, Saikkonen et al. 2012). Recently,
76 light pollution from street lamps has also been shown to advance the bud burst date of tree species *Acer pseudoplatanus*,
77 *Fagus sylvatica*, *Fraxinus excelsior* and *Quercus robur* across the whole UK (Somer-Yeates et al. 2016). Although the
78 regions of the spectrum produced by street lamps that were responsible for the earlier bud burst were not specified, this study
79 highlights the potential ecological role for light quality influencing bud burst. What little research has questioned which
80 regions of spectral irradiance are likely to provide cues for bud phenology has largely focussed on the ratio of red (R, 655-
81 665 nm) to far-red light (FR, 725-735 nm, Smith 1982). For instance, FR light is enriched during the periods of twilight from
82 dawn to sunrise and between sunset and dusk, and thus the R:FR ratio varies with day length, latitude and the time of year
83 (Holmes and Smith 1975, Smith 1982, Hughes et al. 1984). In a controlled-environment study, simulating twilight with a
84 treatment of FR light caused bud burst to advance in *B.pendula* (Linkosalo et al. 2006), implicating phytochromes in the
85 coordination this response. However, bud burst mediated by R and FR light has been shown to be both species-specific and
86 dependent on the population studied (Erez et al. 1966, Mølmann et al. 2006, Girault et al. 2008).

87 Numerous studies have investigated how R and FR light affect bud burst and yet the mechanisms behind this response
88 are not well understood, far less still is known about the effects of blue light on bud burst. The blue light region of solar
89 radiation is also enriched during the hours before sunrise and after sunset (Robertson 1966, Johnson et al. 1967, Hughes et al.
90 1984). Since cryptochrome photoreceptors, that detect blue and ultraviolet-A radiation (UV-A, 315-400 nm), are known to
91 influence the circadian rhythm and photoperiodic flowering responses (Guo et al. 1998, Somers et al. 1998). This result
92 implies that it could be worthwhile testing whether blue light can serve as a cue for bud burst in temperate deciduous tree
93 species. Of the few studies on the effects of blue light on bud burst, Girault et al. (2008) report that blue light (Philips TL-D

18W/18 fluorescent tubes) was as effective as white light (Osram L 18W/77 Fluora fluorescent tubes, 31% blue; both spectra given in SI of Girault et al. 2008) in terms of the percentage of axillary buds reaching bud burst in *Rosa* sp. However, the timing of spring bud burst in trees may not be mediated through the same mechanisms as axillary buds of herbaceous species: for instance, monochromatic blue light (460nm) did not induce bud-burst in the gymnosperm *P.abies* after 6 weeks of 24h photoperiod (Mølmann et al. 2006). To our knowledge, there have been no studies investigating the effects of blue light on the bud burst of temperate deciduous tree species.

Given that blue light has been reported to increase the percentage bud burst in the vegetative shoots of angiosperms including *Rosa* sp. and another Rosaceae, *Prunus cerasifera* (Muleo et al. 2000, Girault et al. 2008), we hypothesised that blue light would advance bud burst in temperate deciduous tree species. Additionally, we expected that bud burst of early successional species, which is predominantly affected by temperature (Basler and Körner, 2012), would be less affected by blue light than late successional species. To this end, we tested the effect on bud burst of light treatments that either included or excluded blue light in branches of the three temperate deciduous species *A.glutinosa*, *B.pendula* and *Q.robur*, differing in their functional strategy from early successional to late successional.

Methods

The collection and care of branch material

We collected dormant branches of *A.glutinosa*, *B.pendula* and *Q.robur* from co-occurring populations of local origin in a natural forest stand in southern Finland (60°13'04.2"N 25°00'31.0"E) and kept them in a temperature-controlled environment under different light treatments while monitoring their bud development. Branches were collected from the lower canopy of trees (2-3 m height above the ground) using sterile garden secateurs on 6th February 2017. We selected eight trees per species, taking 10-15 branches from each tree. Only dormant branches, free of pests and symptoms of disease were selected.

The use of this approach, putting cut twigs in controlled conditions for the study of bud burst phenology, and its reliability have recently been exemplified in several studies (Basler and Körner 2012, Laube et al. 2014, Polgar et al. 2014, Vitasse and Basler 2014, Primack et al. 2015). We followed a similar protocol to these studies, placing the cut end of twigs

immediately into test tubes of water and continuously monitoring their bud development, under two light treatments: a broad spectrum containing blue light, and a broad spectrum of equal PAR and far red but with blue light attenuated (Table S1).

All trees received 111 chilling days prior to harvest: this was calculated according to Murray et al. (1989) as the number of days from 1st of November with a mean temperature $\leq 5^{\circ}\text{C}$. All trees were on the east-facing side of a mixed-forest stand, and only branches that were exposed to sunshine for the majority of the day were selected (spectral composition provided in Table S2, monthly precipitation and temperature in Table S3). Branches were selected which presented at least five buds including the terminal bud. Once collected, branches were all immediately taken into a greenhouse kept at ambient outdoor temperature (0°C), and re-cut to a mean length of 28.35 cm (mean $\pm$ 0.34 SE), and then placed into upright sterilised clear-plastic test tubes (10.26cm long – 15ml volume) containing tap water (Table S4, following Basler and Körner 2012 and Polgar et al. 2014). These twigs were directly removed into a temperature-controlled room with six compartments measuring 97- $\times$ -57- $\times$ -57 cm. Branches from individual trees of each species were randomly assigned into each compartment, so that in each compartment there were 16 branches of *Q.robur*, 20 branches of *B.pendula* and 16 branches of *A.glutinosa*. Twigs were rotated inside the compartments every two days to avoid any bias coming from undetected microclimatic variation inside the compartments. Lights were kept at the same distance from the tips of branches in each compartment.

Light treatments under controlled conditions

Three of the six compartments received a broad spectrum of irradiance from an array of LED lamps (2 $\times$ Valoya AP67, 400-750 nm, Table S1: mean PAR among compartments of $161 \pm \pm \leq 10 \mu\text{mol m}^{-2} \text{s}^{-1}$, plus $28 \mu\text{mol m}^{-2} \text{s}^{-1}$ far red) including blue light (BL, $21.8 \mu\text{mol m}^{-2} \text{s}^{-1}$, 13.5% of total PAR), and in the other three compartments BL was attenuated (no BL) by wrapping film Rosco #313 Canary Yellow (Westlighting, Helsinki, Finland) around the LED lamps (3 $\times$ Valoya AP67), limiting the spectrum to a wavelength range 500-750nm (Table S1: PAR $156 \pm \leq 10 \mu\text{mol m}^{-2} \text{s}^{-1}$, plus $31 \mu\text{mol m}^{-2} \text{s}^{-1}$ far red). Spectral irradiance measurements were taken inside all the experimental compartments with an array spectroradiometer (Maya 2000Pro, Ocean Optics Inc., Dunedin, Florida, USA) to ensure equivalent PAR and R:FR ratios between the treatments (Table S1). Lights were kept on a 12 h light-dark cycle to simulate photoperiod conditions experienced at the beginning of spring in southern Finland ($60^{\circ}13'04.2''\text{N}$ $25^{\circ}00'31.0''\text{E}$), to ensure that photoperiod would not be a confounding factor in the experimental test of light quality (Rinne et al. 1994, Welling et al. 2004, Søggaard et al. 2008).

146

147

148 **Temperature measurements under controlled conditions**

149 Day/night temperature was thermostatically adjusted to maintain $12.6/9.5 \pm 0.05$ °C. Temperatures were monitored
 150 with iButton sensors (Maxim Integrated, San Jose, United States) in each compartment, sampling every one hour for the
 151 entire duration of the experiment, with a resolution of 0.065°C. An infra-red thermal thermometer (Optris LS LT, Berlin,
 152 Germany) was used to measure the temperature on individual buds of branches inside the compartments, to ensure there was
 153 no difference in bud temperature between treatments. The temperature of buds measured with the infra-red thermometer had
 154 a strong positive correlation with mean daily air temperature measured with iButton sensors ($R^2=0.83$, Fig. S1, Table S5),.

155

156 **Monitoring bud development**

157 Bud burst was scored until buds reached stage 4 on a scale of 1-7, made by adapting the scales from Teissier du Crois
 158 et al. (1981) and Kramer et al. (2017) whereby, 1 = dormant bud, 2 = bud swelling, 3 = bud scales begin to split, green visible
 159 on the bud, 4 = bud split, with leaf tip protruding. The experiment lasted 57 days at which point all species in all
 160 compartments had reached at least 50% bud burst (stage 4). Degree-days were calculated as the accumulated mean daily
 161 temperature $> 0^\circ\text{C}$ after the date of December 31st 2016, following methods by Heide (1993), including any degree-days
 162 accumulated by the branches from the dates January 1st to February 6th 2017, during the period prior to the experiment.

163

164 **Data analysis**

165 All results are given as the means ± 1 SE of three replicates, whereby each compartment was treated as one replicate.
 166 We followed statistical methods from Guak et al. (1998), Heide (2003), Sogaard et al. (2008), and Olsen et al. (2014), in
 167 comparing the days and degree-days needed to reach 50% bud burst in the BL and no BL treatments for each species. The
 168 effect of BL on the bud burst of each species was analysed using an ANOVA (R version 3.2.2., R Core Team, 2017).
 169 Pearson's correlation was used to test the relationship between bud temperature and recorded air temperature in each
 170 compartment. Figs. 1 and 2 were produced in Microsoft Excel (2013).

171

172

173 Results and Discussion

174 In all three species, BL decreased the mean number of days until 50% bud burst (Fig. 1). The response to blue light
175 was smallest in the early successional species *B.pendula*, which reached 50% bud burst only 3-days earlier in the BL
176 treatment compared to the no BL treatment ($F_{1,4} = 100$, $p < 0.001$). The later successional *A.glutinosa* and *Q.robur* reached
177 50% bud burst 6 days ($F_{1,4} = 16.20$, $p = 0.016$) and 6.3 days ($F_{1,4} = 90.25$, $p < 0.001$) earlier respectively in the BL treatment
178 compared to the no BL treatment. These results are consistent with other studies showing that early-successional species
179 achieve bud burst sooner than late-successional species (Wesołowski and Rowiński 2006, Basler and Körner 2012).

180 [Insert Fig. 1. here]

181 [Insert Fig. 2 here.]

182 In our experiment, the effect of BL expressed as a degree day difference between treatments was consistent with the
183 days until 50% bud burst (Fig. 2). Our BL light treatment reduced the degree-days required for bud burst in *B.pendula* by
184 17.12 degree-days ($F_{1,4} = 12.3$, $p = 0.025$), in *A.glutinosa* by 28.14 degree-days ($F_{1,4} = 35.8$, $p = 0.04$) and in *Q.robur* by 60.04
185 degree-days ($F_{1,4} = 20.9$, $p = 0.010$): this is the equivalent of a 10.54%, 15.48% and 11.03% reduction due to BL in each
186 species respectively (Table 1). The degree-day requirement for bud burst in *B.pendula* was very similar to that of a natural
187 population in southern Finland (Table S6), and was within the range reported in previous studies (Basler and Körner 2012,
188 Heide 1993), as was *A.glutinosa* (Table S6, Heide 1993). The bud burst of *Q.robur* branches had a lower degree-day
189 requirement than a natural population in southern Finland (Table S6): this discrepancy may derive from the photoperiod
190 sensitivity of bud burst in *Quercus* species (Basler and Körner 2012, Basler and Körner 2014) which could have been
191 activated by the 12h photoperiod we employed. Alternatively, this difference could be due to the reduced number of chilling
192 days that treated branches received prior to February compared with those in forest stands throughout spring (Table S6).
193 When expressed as the difference in the total length of time until bud burst, the effect of BL in days or degree-days was
194 smallest in branches of *B.pendula*: however, because of the three species we compared it required the shortest total period
195 before bud burst, the effect of BL on *B.pendula* was greatest in relative terms when measured as a % difference (Table 1).

196 [insert Table 1 here]

197

198

199 **What are the mechanisms by which blue light advances bud burst?**

200 Our results demonstrate for the first time that blue light advances spring bud burst in temperate deciduous tree species,
 201 through a photoreceptor-dependent mechanism which is yet to be elucidated. This result is consistent with studies of plants of
 202 the *Rosacea* family, including *Rosa* sp. and *Prunus cerasifera* , where a higher percentage bud burst and more axillary
 203 vegetative shoots and branches were produced under blue light compared to red light (Muleo et al. 2001, Girault et al. 2008).
 204 The growth rate and number of preformed leaves in the buds of these species were also higher under blue light. The control
 205 of bud burst was exercised in part through the photoregulation of sugar metabolism in this case (Girault et al. 2010), whereby
 206 the allocation of sugars to the growing bud increased in the light compared to darkness. The authors attribute the regulation of
 207 sugar metabolism in the buds to be primarily mediated by the phytochrome photoreceptors, which have an absorption
 208 spectrum in blue light and R:FR, but also possibly by cryptochromes in response to blue light (Reed et al. 1993, Girault et al.
 209 2010). Interestingly, several tree species including *Q.robur*, *Fagus sylvatica*, *Betula pubescens*, *Fraxinus excelsior* and *Acer*
 210 *pseudoplatanus* (Catesson 1964, Barnola et al. 1986, Cottignies 1986, Kelner et al. 1993, Rinne et al. 1994) increase in bud
 211 sink strength close to bud burst, resulting in increased mobilization of stem carbohydrates. It appears from these studies that
 212 sugar metabolism, at least in part, plays a role in the process of bud burst of tree species.

213 Regulation of flowering time in response to photoperiod by the blue/UV-A cryptochrome and the R:FR phytochrome
 214 photoreceptors has been well established in the model plant *Arabidopsis thaliana* (Guo et al. 1998, Somers et al. 1998). In
 215 comparison, much less is known about the role of photoreceptors that regulate bud burst in response to spectral composition.
 216 In the tree species *P.tremuloides*, the phytochrome gene PHYB₂ was found to be coincident with a quantitative trait locus
 217 affecting bud-set and bud burst (Frewen et al. 2000). Similarly, cryptochromes have been shown to facilitate blue-light-
 218 mediated phenological responses in other plant species (Guo et al. 1998, Meng et al. 2013), so it is reasonable to speculate
 219 that cryptochromes and phytochromes could facilitate bud burst in response to blue light. Conversely, although phototropins
 220 also absorb blue light, there is no evidence that they mediate phenological responses (Casal 2000). Considering that UV-B
 221 can affect bud burst and bud set in *P.tremula* (Strømme et al. 2015), and that plants possess a UV-B-detecting photoreceptor
 222 (Rizzini et al. 2011), we suggest that a productive area of future research would be to determine the relative contribution of
 223 different photoreceptors to regulating bud burst in response to spectral composition.

224

225

226 **What is the ecological significance of blue light?**

227 We provide two possible explanations (in italics) for the ecological role of blue light advancing bud burst, and why it could
 228 have a greater effect on early-successional species, measured in absolute terms as days (or degree days) until bud burst, as
 229 opposed to percentage differences. Firstly, it has been demonstrated that blue light enhances photosynthesis in *B.pendula*
 230 plantlets *in vitro* (Sæbø et al. 1995), and among other plant species (Goins et al. 1997, Matsuda et al. 2004, Hogewoning et al.
 231 2010). Blue light is often associated with conditions that are favourable to photosynthesis and normal photosynthetic function
 232 (Matsuda et al. 2004, Sellin et al. 2011), so it is possible that *blue light advances bud burst in order to allow the plant exploit*
 233 *an opportunity for photosynthetic gains whilst conditions are favourable*. Secondly, the ratio of blue:red light is higher during
 234 the twilight hours of sunrise and sunset than the middle of the day (Johnson et al. 1967, Hughes et al. 1984). Robertson et al.
 235 (1966) proposes that at higher latitudes the extended hours of twilight rich in blue light and FR could have effects on plant
 236 growth not found at lower latitudes, such as the delay in bud dormancy exhibited by *P.abies* grown under day extension using
 237 blue light (Mølmann et al. 2006). Given that at high latitudes the day length and thus hours of twilight vary drastically
 238 throughout the year, *it is possible that blue light provides cues related to lengthening twilight and daylight periods in spring*.
 239 Since photoperiod often has a greater effect on the timing of bud burst in late-successional species (Basler and Körner 2012,
 240 Basler and Körner 2014), we might infer that blue light should also have a greater effect on later successional species due to
 241 its ecological relevance, i.e. changes in spectral composition related to day length and the length of twilight. Since we used a
 242 fixed day length in our experiment, we cannot speculate on the possible interactive effects of blue light and day length here.
 243 Similarly, we cannot say whether the bud burst response that we report is related to the proportion of blue light received or
 244 the total irradiance of blue light. As such, we suggest future research should test whether bud burst in response to blue light
 245 is related to diurnal changes in the proportion of blue light.

246 Under controlled temperature and light conditions we found that blue light advances bud burst in the tree species
 247 *B.pendula*, *A.glutinosa*, and *Q.robur*. Further research into bud burst and other phenological processes is needed to: establish
 248 whether or not the effect of blue light on bud burst is related to diurnal changes in blue light as opposed to a seasonal
 249 increase, describe how plants integrate cues from day length and spectral composition (from UV through to far-red) through

250 crosstalk among photoreceptor mediated responses, and combine this information with other environmental cues (e.g.
251 temperature)to produce an environmentally-appropriate response.

252

253 **Author Contributions**

254 CCB conceived, designed and carried out the experiment and statistical analyses. TMR supervised the design, analyses and
255 interpretation of results. Both authors wrote the manuscript.

256

257 **Funding**

258 We thank the Finnish Academy of Science for funding the project through the funding decisions # 266523 and #304519 to
259 TMR, and Valoya Ltd for providing the LED Lamps.

260

261 **Conflict of Interest Statement**

262 None declared.

263

264 **Acknowledgements**

265 Our acknowledgments to Lammi Biological station staff in particular John Loehr, to Mikko Peltoniemi and the EU Life+
266 MONIMET phenology camera network, to Viikki Greenhouses Facility at the University of Helsinki and Viikki Arboretum,
267 Marta Pieristé, Marieke Trasser and Ulla Riihimäki for help sampling, Titta Kotilainen for processing the spectral irradiance
268 measurements. Thanks to Otmar Urban and Line Nybakken for giving constructive comments an early version of the
269 manuscript.

270

271 **References**

272 Augspurger CK (2009) Spring 2007 warmth and frost: phenology, damage and refoliation in a temperate deciduous forest.
273 Funct Eco 23:1031–1039.

274 Banerjee R, Batschauer A (2005) Plant blue-light receptors. *Planta* 220:498-502.

275 Barnola P, Crochet A, Payan E, Gendraud M, Lavarenne S (1986) Modifications of energy-metabolism and permeability in
276 the apical bud and in stem during the temporary rest of the growth of young oak trees. *Physiol veg* 24:307-314.

277 Basler D, Körner C (2012) Photoperiod sensitivity of bud burst in 14 temperate forest tree species. *Agric For*
278 *Meteorol* 165:73-81.

279 Battey NH (2000) Aspects of seasonality. *J Exp Bot* 51:1769-1780.

280 Bennie J, Kubin E, Wiltshire A, Huntley B, Baxter R (2010) Predicting spatial and temporal patterns of bud burst and spring
281 frost risk in north-west Europe: the implications of local adaptation to climate. *Global Change Biol* 16:1503-1514.

282 Briggs WR, Christie JM (2002) Phototropins 1 and 2: versatile plant blue-light receptors. *Trends Plant Sci* 7:204-210.

283 Briggs WR, Huala E (1999) Blue-light photoreceptors in higher plants. *Annu Rev Cell Dev Biol* 15:33-62.

284 Buse A, Good J (1996) Synchronization of larval emergence in winter moth (*Operophtera brumata* L.) and budburst in
285 pedunculate oak (*Quercus robur* L.) under simulated climate change. *Ecol Entomol* 21:335-343.

286 Caffarra A, Donnelly A (2011) The ecological significance of phenology in four different tree species: effects of light and
287 temperature on bud burst. *Int J Biometeorol* 55:711-721.

288 Cannell MGR, Smith RI (1986) Climatic warming, spring budburst, and frost damage on trees. *J Appl Ecol* 23:177-191.

289 Casal JJ (2000) Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. *Photochem Photobiol* 71:1-
290 11.

291 Catesson AM (1964) Modifications cytochimiques saisonnieres des points vegetatifs dans les bourgeons de lacer
292 pseudoplatanus. *C R Acad Sci* 258:5709.

293 Cesaraccio C, Spano D, Snyder RL, Duce P (2004) Chilling and forcing model to predict bud burst of crop and forest
294 species. *Agric For Meteorol* 126:1-13.

295 Cottignies A (1986) The hydrolysis of starch as related to the interruption of dormancy in the ash bud. *J Plant*
296 *Physiol* 123:381-388.

297 Dhuli P, Rohloff J, Strimbeck GR (2014) Metabolite changes in conifer buds and needles during forced bud break in Norway
 298 spruce (*Picea abies*) and European silver fir (*Abies alba*). Front Plant Sci 5:706.

299 Erez A, Samish RM, Lavee S (1966) The role of light in leaf and flower bud break of the peach (*Prunus persica*). Physiol
 300 Plant 19:650-659.

301 Favory JJ, Stec A, Gruber H, Rizzini L, Oravecz A, Funk M, Albert A, Cloix C, Jenkins GI, Oakeley EJ, Seidlitz HK (2009)
 302 Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis and stress acclimation in *Arabidopsis*.
 303 EMBO J, 28:591-601.

304 Fu YH, Piao S, Vitasse Y, Zhao H, De Boeck HJ, Liu Q, Yang H, Weber U, Hänninen H, Janssens IA (2015) Increased heat
 305 requirement for leaf flushing in temperate woody species over 1980–2012: effects of chilling, precipitation and
 306 insolation. Global Change Biol 21:2687-2697.

307 Frewen BE, Chen TH, Howe GT, Davis J, Rohde A, Boerjan W, Bradshaw HD (2000) Quantitative trait loci and candidate
 308 gene mapping of bud set and bud flush in *Populus*. Genetics 154:837-845.

309 Girault T, Bergougnoux V, Combes D, Viemont JD, Leduc N (2008) Light controls shoot meristem organogenic activity and
 310 leaf primordia growth during bud burst in *Rosa* sp. Plant Cell Environ, 31:1534-1544.

311 Girault T, Abidi F, Sigogne M, Pelleschi-Travier SA, Boumaza R, Sakr S, Leduc N (2010) Sugars are under light control
 312 during bud burst in *Rosa* sp. Plant Cell Environ, 33:1339-1350.

313 Goins GD, Yorio NC, Sanwo MM, Brown CS (1997) Photomorphogenesis, photosynthesis, and seed yield of wheat plants
 314 grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. J Exp Bot, 48:1407-1413.

315 Guak S, Olszyk DM, Fuchigami LH, Tingey DT (1998) Effects of elevated CO₂ and temperature on cold hardiness and
 316 spring bud burst and growth in Douglas-fir (*Pseudotsuga menziesii*). Tree Physiol 18:671-680.

317 Guo H, Yang H, Mockler TC, Lin C (1998) Regulation of flowering time by *Arabidopsis* photoreceptors. Science, 279:1360-
 318 1363.

319 Hänninen H (1991) Does climatic warming increase the risk of frost damage in northern trees? Plant Cell Environ 14:449-
 320 454.

321 Hänninen H (1995) Effects of climatic change on trees from cool and temperate regions: an ecophysiological approach to
322 modelling of bud burst phenology. *Can J Bot* 73:183-199.

323 Heide OM, (1993) Daylength and thermal time responses of budburst during dormancy release in some northern deciduous
324 trees. *Physiol Plant*, 88:531-540.

325 Heide OM (2003) High autumn temperature delays spring bud burst in boreal trees, counterbalancing the effect of climatic
326 warming. *Tree Physiol* 23: 931-936.

327 Hogewoning SW, Trouwborst G, Maljaars H, Poorter H, van Ieperen W, Harbinson J (2010) Blue light dose-responses of
328 leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red
329 and blue light. *J E Bot* 61:3107-3117.

330 Holmes MG, Smith H (1975) The function of phytochrome in plants growing in the natural environment. *Nature* 254:512-
331 514.

332 Hughes JE, Morgan DC, Lambton PA, Black CR, Smith H (1984) Photoperiodic time signals during twilight. *Plant Cell*
333 *Environ* 7:269-277.

334 Johnson TB, Salisbury FB, Connor GI (1967) Ratio of blue to red light: a brief increase following sunset. *Science* 155:1663-
335 1665.

336 Kelner JJ, Lachaud S, Bonnemain JL (1993) Seasonal variations in ABA exchange between the apical bud and the underlying
337 stem of beech. Comparison with nutrients. *Plant Physiol Biochem* 31:523-530.

338 Körner C (2007) The use of ‘altitude’ in ecological research. *Trends Ecology Evolut*, 22:569-574.

339 Körner C, Basler D (2010) Phenology under global warming. *Science*, 327:1461-1462.

340 Kramer K, Ducousso A, Gömöry D, Hansen JK, Ionita L, Liesebach M, Lorent A, Schöler S, Sulkowska M, de Vries S, von
341 Wühlisch G (2017) Chilling and forcing requirements for foliage bud burst of European beech (*Fagus sylvatica* L.) differ
342 between provenances and are phenotypically plastic. *Agric For Meteorol*, 234:172-181.

343 Kubasek WL, Shirley BW, McKillop A, Goodman HM, Briggs W, Ausubel FM (1992) Regulation of flavonoid biosynthetic
344 genes in germinating *Arabidopsis* seedlings. *Plant Cell*, 4:1229-1236.

345 Lang GA, Early JD, Martin GC, Darnell RL (1987) Endo-, para-, and ecodormancy: physiological terminology and
346 classification for dormancy research. *Hort Science*, 22:371-377.

347 Laube J, Sparks TH, Estrella N, Höfler J, Ankerst DP, Menzel A (2014) Chilling outweighs photoperiod in preventing
348 precocious spring development. *Global Change Biol* 20:170-182.

349 Linkosalo T, Lechowicz M J (2006). Twilight far-red treatment advances leaf bud burst of silver birch (*Betula pendula*). *Tree*
350 *Physiol* 26:1249-1256.

351 Malyshev AV, Henry HA, Bolte A, Khan MAA, Kreyling J (2018). Temporal photoperiod sensitivity and forcing
352 requirements for budburst in temperate tree seedlings. *Agric For Meteorol* 248: 82-90.

353 Matsuda R, Ohashi-Kaneko K, Fujiwara K, Goto E, Kurata K (2004) Photosynthetic characteristics of rice leaves grown
354 under red light with or without supplemental blue light. *Plant Cell Physiol* 45:1870-1874.

355 Meng Y, Li H, Wang Q, Liu B, Lin C (2013) Blue light-dependent interaction between cryptochrome2 and CIB1 regulates
356 transcription and leaf senescence in soybean. *Plant Cell* 25:4405-4420.

357 Muleo R, Morini S, Casano S (2001) Photoregulation of growth and branching of plum shoots: physiological action of two
358 photosystems. *In Vitro Cell Dev Biol Plant* 37: 609-617

359 Murray MB, Cannell MGR, Smith RI (1989) Date of budburst of fifteen tree species in Britain following climatic warming. *J*
360 *Appl Ecol* 693-700.

361 Menzel A, Sparks TH, Estrella N, Koch E, Aasa A, Ahas R, Alm-Kübler KE, Bissolli P, Braslavská OG, Briede A,
362 Chmielewski FM (2006) European phenological response to climate change matches the warming pattern. *Global Change*
363 *Biol* 12:1969-1976.

364 Mercado LM, Belloui, N, Sitch S, Boucher O, Huntingford C, Wild M, Cox PM (2009) Impact of changes in diffuse radiation
365 on the global land carbon sink. *Nature* 458:1014.

366 Mølmann JA, Junttila O, Johnsen Ø, Olsen JE (2006) Effects of red, far-red and blue light in maintaining growth in
367 latitudinal populations of Norway spruce (*Picea abies*). *Plant Cell Environ* 29:166-172.

Ohgishi M, Saji K, Okada K, Sakai T (2004) Functional analysis of each blue light receptor, cry1, cry2, phot1, and phot2, by using combinatorial multiple mutants in *Arabidopsis*. *Proc Natl Acad Sci U S A* 101:2223-2228.

Olsen JE, Lee Y, Junttila O (2014) Effect of alternating day and night temperature on short day-induced bud set and subsequent bud burst in long days in Norway spruce. *Front Plant Sci* 5.

Polgar C, Gallinat A, Primack RB (2014) Drivers of leaf-out phenology and their implications for species invasions: insights from Thoreau's Concord. *New Phytol* 202:106-115.

Primack RB, Laube J, Gallinat AS, Menzel A (2015) From observations to experiments in phenology research: investigating climate change impacts on trees and shrubs using dormant twigs. *Annal Bot* 116:889-897.

Pudasaini A, Zoltowski BD (2013) Zeitlupe senses blue-light fluence to mediate circadian timing in *Arabidopsis thaliana*. *Biochemistry* 52:7150-7158.

AD Richardson, J O'Keefe (2009) Phenological Differences between Understory and Overstory: A Case Study Using the Long-Term Harvard Forest Records Book, *Phenology of Ecosystem Processes*, Springer, New York, NY. 87–117

Rinne P, Saarelainen A, Junttila O (1994) Growth cessation and bud dormancy in relation to ABA level in seedlings and coppice shoots of *Betula pubescens* as affected by a short photoperiod, water stress and chilling. *Physiol Plant* 90:451-458.

Robertson G (1966) The light composition of solar and sky spectra available to plants. *Ecology* 47:640-643.

Reed JW, Nagpal P, Poole DS, Furuya M, Chory J (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. *Plant Cell* 5:147-157.

Rinne P, Tuominen H, Junttila O (1994) Seasonal changes in bud dormancy in relation to bud morphology, water and starch content, and abscisic acid concentration in adult trees of *Betula pubescens*. *Tree Physiol* 14:549-561.

Rizzini L, Favory JJ, Cloix C, Faggionato D, O'Hara A, Kaiserli E, Baumeister R, Schäfer E, Nagy F, Jenkins GI, Ulm R (2011) Perception of UV-B by the *Arabidopsis* UVR8 protein. *Science* 332:103-106.

Saikkonen K, Taulavuori K, Hyvönen T, Gundel PE, Hamilton CE, VänninenmI, Nissinen A, Helander M (2012) Climate change-driven species' range shifts filtered by photoperiodism. *Nat Clim Change* 2:239.

Samish RM (1954) Dormancy in woody plants. *Ann Rev of Plant Physiol* 5: 183-204.

392 Sæbø A, Krekling T, Appelgren M (1995) Light quality affects photosynthesis and leaf anatomy of birch plantlets in
393 vitro. *Plant Cell Tiss Organ Cult*, 41:177-185.

394 Sellin A, Sack L, Öunapuu E, Karusion A (2011) Impact of light quality on leaf and shoot hydraulic properties: a case study
395 in silver birch (*Betula pendula*). *Plant Cell Environ* 34:1079-1087.

396 Shalitin D, Yang H, Mockler TC, Maymon M, Guo H, Whitelam GC, Lin C (2002) Regulation of *Arabidopsis* cryptochrome
397 2 by blue-light-dependent phosphorylation. *Nature* 417:763-767.

398 Smith H (1982) Light quality, photoperception, and plant strategy. *Ann Rev Plant Physiol* 33:481-518.

399 Stanhill G, Cohen S (2001) Global dimming: a review of the evidence for a widespread and significant reduction in global
400 radiation with discussion of its probable causes and possible agricultural consequences. *Agric For Meteorol* 107:255-278.

401 Strømme CB, Julkunen-Tiitto R, Krishna U, Lavola A, Olsen JE, Nybakken L (2015) UV-B and temperature enhancement
402 affect spring and autumn phenology in *Populus tremula*. *Plant Cell Environ* 38:867-877.

403 Søgaaard G, Johnsen O, Nilsen J, Junttila O (2008) Climatic control of bud burst in young seedlings of nine provenances of
404 Norway spruce. *Tree Physiol* 28:311.

405 Somers DE, Devlin PF, Kay SA (1998) Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* circadian
406 clock. *Science* 282:1488-1490.

407 Somers-Yeates R, Bennie J, Economou T, Hodgson D, Spalding A, McGregor PK (2016) Light pollution is associated with
408 earlier tree budburst across the United Kingdom. In *Proc. R. Soc. B* 283:20160813

409 Teissier du Crois E, Le Tacon F, Nepveu G, Pardé J, Timbal J (1981). Le hêtre. Département des Recherches Forestières,
410 INRA, Paris, 613.

411 Urban O, Janouš D, Acosta M, Czerný R, Markova I, Navratil M, Pavelka M, Pokorný R, Šprtová M, Zhang R, Špunda V
412 (2007) Ecophysiological controls over the net ecosystem exchange of mountain spruce stand. Comparison of the response in
413 direct vs. diffuse solar radiation. *Global Change Biol* 13:157-168.

414 Vitasse Y, Basler D (2014) Is the use of cuttings a good proxy to explore phenological responses of temperate forests in
415 warming and photoperiod experiments? *Tree Physiol* 34:174-183.

416 Way DA, Montgomery RA (2015) Photoperiod constraints on tree phenology, performance and migration in a warming
417 world. *Plant Cell Environ* 38: 1725-1736.

418 Wareing PF (1956) Photoperiodism in woody plants. *Ann Rev Plant Physiol* 7:191-214.

419 Welling A, Rinne P, Viherä Aarnio A, Kontunen Soppela S, Heino P, Palva ET (2004) Photoperiod and temperature
420 differentially regulate the expression of two dehydrin genes during overwintering of birch (*Betula pubescens* Ehrh.). *J Exp*
421 *Bot* 55:507-516.

422 Wesołowski T, Rowiński P (2006) Timing of bud burst and tree-leaf development in a multispecies temperate forest. *For*
423 *Ecol Manag* 237:387-393.

424 Yordanov YS, Ma C, Strauss SH, Busov VB (2014) EARLY BUD-BREAK 1 (EBB1) is a regulator of release from seasonal
425 dormancy in poplar trees. *Proc Natl Acad Sci U S A* 111:10001-10006.

426 Yun K, Hsiao J, Jung MP, Choi IT, Glenn DM, Shim KM, Kim SH (2017) Can a multi-model ensemble improve phenology
427 predictions for climate change studies? *Ecol Model* 362: 54-64.

428

429 Tables

430

431 Table 1. The effect of BL on bud burst expressed both as the difference in the mean number of days and mean degree-days
 432 until 50% bud burst in *A.glutinosa*, *B.pendula* and *Q.robur*, and the percentage differences in the total number of days or
 433 degree-day. Degree-days were calculated as the accumulated daily mean temperature > 0°C starting from 1st January 2017.

434

	BL effect on bud burst expressed in days	
	Number of days earlier with BL	% difference between treatments
<i>A.glutinosa</i>	6.00	15.65
<i>B.pendula</i>	3.33	18.50
<i>Q.robur</i>	6.33	11.30

438

List of Figures

	BL effect bud burst expressed in degree-days	
	Number of degree-days earlier with BL	% difference between treatments
<i>A.glutinosa</i>	43.61	10.54
<i>B.pendula</i>	31.45	15.48
<i>Q.robur</i>	67.03	11.03

439

440

441 **Fig. 1.** The mean number of days until 50% bud burst in *A.glutinosa*, *B.pendula* and *Q.robur*, in a broad spectrum treatment
 442 with blue light (BL) and without blue light (No BL). Error bars represent standard error (n=3) from three replicate
 443 compartments, each containing 16 *A.glutinosa* branches, 20 *B.pendula* branches and 16 *Q.robur* branches. Asterisks represent
 444 statistically significant differences between treatments, where * = $p \leq 0.05$, ** = $p \leq 0.010$, *** = $p \leq 0.001$. *A.glutinosa* ($F_{1,4}$
 445 = 16.20, $p = 0.016$) *B.pendula* ($F_{1,4} = 100$, $p < 0.001$), *Q.robur* ($F_{1,4} = 90.25$, $p < 0.001$).

446
 447 **Fig. 2.** Mean degree-days until 50% bud burst in *A.glutinosa*, *B.pendula* and *Q.robur*, in a broad spectrum treatment with
 448 blue light (BL) and without blue light (No BL). Error bars represent standard error (n=3) from three replicate compartments,
 449 each containing 16 *A.glutinosa* branches, 20 *B.pendula* branches and 16 *Q.robur* branches. Degree-days calculated as the
 450 accumulated daily mean temperature $< 0^{\circ}\text{C}$ after the date of December 31st 2016. Asterisks represent statistically significant
 451 differences between treatments, where * = $p \leq 0.05$, ** = $p \leq 0.010$, *** = $p \leq 0.001$. *A.glutinosa* ($F_{1,4} = 35.8$, $p=0.04$)
 452 *B.pendula* ($F_{1,4}=12.3$, $p = 0.025$), *Q.robur* ($F_{1,4} = 20.9$, $p= 0.010$).

453
 454