Part-night exposure to artificial light at night has more detrimental effects on aphid colonies than fully lit nights

Robin Heinen[1], Oriana Sánchez-Mahecha[1], T. Martijn Bezemer[2], Davide M. Dominoni[3], Claudia Knappe[8], Johannes Kollmann[4], Anton Kopatsch[7], Zoë A. Pfeiffer[1], Michael Schloter[5,6], Sarah Sturm[1], Jörg-Peter Schnitzler[7], A. Corina Vlot[8,9], Wolfgang W. Weisser[1]

1 Terrestrial Ecology Research Group, Department for Life Science Systems, TUM School of Life Sciences, Technical University of Munich, Freising, Germany

2 Institute of Biology, Section Plant Ecology and Phytochemistry, Leiden University, Leiden, The Netherlands

3 School of Biodiversity, One Health & Veterinary Medicine, University of Glasgow, Glasgow, Scotland

4 Chair of Restoration Ecology, Department for Life Science Systems, TUM School of Life Sciences, Technical University of Munich, Freising, Germany

5 Research Unit Comparative Microbiome Analysis, Helmholtz Munich, Neuherberg, Germany

6 Chair of Soil Science, Department for Life Science Systems, TUM School of Life Sciences, Technical University of Munich, Freising, Germany

7 Research Unit Environmental Simulation, Helmholtz Munich, Neuherberg, Germany

8 Institute of Biochemical Plant Pathology, Helmholtz Munich, Neuherberg, Germany

9 Chair of Crop Plant Genetics, Faculty of Life Sciences: Food, Nutrition and Health, University of Bayreuth, Kulmbach, Germany

Author for correspondence: Robin Heinen, robin.heinen@tum.de

Author contributions: RH conceived the original ideas for the study, which were further developed in close discussion with DD, TMB, JK, MS, JPS, ACV and WWW. RH designed the experiment. RH, OSM, ZAP and SS conducted the experiment and collected data. CK analyzed gene expression in plant samples. AK measured and visualized spectral composition in the climate chambers. RH analyzed the data. RH led the writing of the manuscript and all authors commented and approved the final version of the manuscript.

Abstract

Artificial light at night (ALAN) threatens natural ecosystems globally. While ALAN research is increasing, little is known about how ALAN affects plants and interactions with other organisms. We explored the effects of ALAN on plant defence and plant–insect interactions using barley (*Hordeum vulgare*) and the English grain aphid (*Sitobion avenae*). Plants received 'full' or 'part' nights of 15–20 lux ALAN, or no ALAN 'control' nights, to test the effects of ALAN on plant growth and defence. Although plant growth was only minimally affected by ALAN, aphid colony growth and aphid maturation were reduced significantly by ALAN treatments. Importantly, we found strong differences between full night and part night ALAN treatments. Contrary to our expectations, part night ALAN – limiting the duration of ALAN – had stronger negative effects on aphid colony growth than full ALAN. Defence-associated gene expression was affected in some cases by ALAN, but also positively correlated with aphid colony size, suggesting that the effects of ALAN on plant defences are indirect, regulated via direct disruption of aphid colonies, rather than via ALAN-induced upregulation of defences. Mitigating ecological side effects of ALAN is a complex problem, as reducing exposure to ALAN increased its negative impact on insect herbivores.

Introduction

Artificial light at night (ALAN) is becoming increasingly common in urban and rural areas of the world (Gaston, Visser & Hölker, 2015; Kyba et al. 2017). ALAN is emitted by various man-made sources including street lights, building lights, and advertising lights. Current levels of ALAN are unprecedented in the ecological history of life on Earth, as light levels followed a predictable periodicity of diurnal and annual rhythms, with little light at night except of stars and the moon. These rhythms are increasingly disrupted by ALAN in many areas of the world, and ALAN therefore is considered an important aspect of global change (Gaston, Visser & Hölker, 2015; Senzaki et al. 2020; Falcón et al. 2020). For instance, ALAN can interfere with natural behaviour and physiology in many taxa (Sanders et al. 2021), including freshwater and marine invertebrates (Duarte et al. 2019; Perkin et al. 2011; Ayalon et al. 2019), terrestrial insects and several vertebrates, such as bats and birds (Desouhant et al., 2019; Stone, Harris & Jones, 2015; Dominoni, Quetting & Partecke, 2013). Surprisingly, given their dependence on the light environment for acquisition of energy, and as signal for photomorphogenesis, phenological and defence processes, much less is known about how ALAN affects plants (Briggs 2006; Bennie et al. 2016). ALAN may for instance interfere with phenology, starch turnover and defence regulation, which may affect interactions with plant-associated organisms (Heinen 2021). As nights will most likely become significantly brighter, it is of utmost importance to better understand the impacts on natural ecosystems (Rich & Longcore, 2013). Therefore, understanding how ALAN affects interactions between plants and associated organisms warrants further studies (Heinen 2021).

Levels of ALAN will increase in the future because of the decreasing production costs, and the development of more energy- and cost-efficient light-emitting diode (LED) technology making light widely available and affordable, which may lead to wider implementation of ALAN (Hölker et al., 2010; Kyba et al. 2017). This will likely also increase the ecological consequences of ALAN. Although many forms of ALAN exist and are still in use, a clear trend towards LED technology has been observed in recent years. The most commonly used LEDs today are cool-white LEDs (with a colour temperature of

67 >4200 K) and these are characterized by a narrow and high peak in the blue part of the irradiation
68 spectrum, and a broad and lower peak in the red part of the spectrum. Cool-white LEDs can have
69 devastating effects on many nocturnal animal species, and to mitigate these detrimental effects,
70 several solutions have been suggested, including the use of different light colours (Van Geffen et al.,
71 2014; Spoelstra et al., 2015), and to reduce light hours to periods that people are generally more active
72 (International Darksky Association, www.darksky.org; Gaston et al. 2012; Day et al. 2015). A plausible
73 scenario that could be implemented would be to strictly limit ALAN to the first part of the night, when
74 human activity is still relatively high, and switching off the lights during the second half of the night.
75 This would provide plants and animals with at least several hours of true darkness to recover.
76 However, how ALAN, and specific light schemes that reduce the number of light hours will affect plant
77 defences and plant-herbivore interactions, is not known.

78 One key plant parameter that is affected by the quality, quantity and duration of light that a
79 plant is exposed to is plant defence. Most of the available literature on the effects of light on plant
80 defences can be divided into two categories. First, a large body of knowledge describes how additional
81 light, i.e., supplementing plants with artificial light *beyond* the daylight hours, can help us understand
82 how extending daylength – as ALAN may be perceived by plants – influences plant defences. Light
83 supplementation studies are generally carried out on plant health and yield in agri- or horticultural
84 settings. Generally, light supplementation into the dark hours increases plant defences. For example,
85 tomato plants supplemented with low-density light at night have significantly higher immunity to
86 pathogens compared to plants that grow in a dark night (Yang et al., 2015a). In another study, chickpea
87 plants supplemented with light at night showed increased levels of the antioxidant ß-carotene (Wu et
88 al., 2007), which may serve as a defence against oxidative stress (Mittler, 2002). One might conclude
89 from this that a longer day leads to a more defended plant. A second body of scientific literature that
90 is helpful to understand effects of ALAN on plants, but that does not directly addresses ALAN,
91 examines the effects of light reduction on plants. This literature examines the consequences of e.g.
92 shading and light competition for plant physiology, including defences, and that are mediated by

93 changes in light intensity and spectral composition (e.g., Bennie et al. 2016; Smith et al. 2017; Grubisic
94 et al. 2018). This literature may also be helpful to understand effects of ALAN, as it represents reduced
95 light levels. Light competition and shading can alter key processes in the jasmonic (JA) and salicylic
96 acid (SA) signalling pathways, i.e., the backbone of plant defences (reviewed in Ballaré, 2009; Ballaré,
97 2014; Ballaré & Pierik, 2017). Generally, reductions in light exposure, through competition and
98 shading, result in decreased sensitivity to and suppressed JA and SA expression (de Wit et al., 2013;
99 Ballaré & Pierik, 2017), although the SA-related processes are less well-understood (Ballaré & Pierik,
100 2017). Light competition and shade may also lead to reduced accumulation of plant secondary
101 metabolites, and structural and indirect defences (Izaguirre et al., 2006; 2013; Engelen-Eigles et al.,
102 2006; Moreno et al., 2009; Agrawal et al., 2013; Cargnel et al., 2014). Relevant to understanding the
103 effects of ALAN on plants, a result of defence suppression under low-light or dark conditions is that
104 plants are more susceptible to pathogens and herbivory at night (Roden & Ingle, 2009; Kraiselburd et
105 al., 2017). This literature shows that ALAN has the potential to affect plant defence but that this
106 depends on the specifics of radiation at night.

107 ALAN may also affect interactions between plants and herbivores (Bennie et al., 2015; Yang et al.,
108 2015b; Sanders et al., 2018; Heinen 2021). Different typical ALAN sources with different spectral
109 characteristics have been found to have differential bottom-up effects on plant–aphid interactions
110 (Bennie et al., 2015). For example, supplementing *Lotus pedunculatus* plants with amber – but not
111 white – ALAN, caused a decrease in densities of the aphid *Acyrthosiphon pisum* compared to no ALAN
112 in the later stages of seasonal colony dynamics (Bennie et al., 2015). Importantly, even low light
113 intensities at night can have significant effects, as ALAN can already suppress aphids under densities
114 as low as 0.1 lux, which is far below commonly observed levels (Sanders et al., 2018). Although the
115 mechanisms are not fully understood yet, a possible mechanism could be that ALAN results in systemic
116 activation of defence mechanisms in the plant. For instance, in one study where tomatoes were
117 supplemented with different spectral irradiance at night, particularly the plants exposed to red light
118 (and to a lesser extent to other light colours) showed strong induced defence responses against the

root-knot nematode *Meloidogyne incognita* (Yang et al., 2015b). These defence responses were accompanied by high levels of SA in root tissues, and upregulated transcript levels of SA-associated genes in the roots (Yang et al., 2015b). How irradiance levels associated with ALAN affects plant defences, however, is not known.

In this study, we used a well-characterized plant–aphid model system, barley (*Hordeum vulgare* L.) and the English grain aphid (*Sitobion avenae* Fabricius), and exposed them to full nights of ca. 20 lux ALAN ('full night'), half nights of ca. 20 lux ALAN ('part-night') or no ALAN ('control') to improve our understanding about the effects of ALAN on plant-herbivore interactions and plant defence. We hypothesized that: (i) Exposure to ALAN reduces aphid growth compared to dark nights, mediated by an upregulation of defence responses. We predict that aphid colonization on plants will be negatively impacted by plants, through an upregulation of plant defences. (ii) Exposure to ALAN affects plant growth and health status, compared to dark nights. We predict that effects of ALAN will be positive, but likely minimal, as the levels of ALAN are expected to be too low to increase photosynthesis. (iii) A partial reduction in ALAN via a midnight elimination of light sources will reduce the hypothesized effects of ALAN, serving as a dark recovery time that benefits both plants and insects.

Materials and Methods

Soil

Soils for this experiment were derived from the Experimental Station Roggenstein (48.17989, 11.32030; altitude 524 m a.s.l.) at the Technical University of Munich. Top soil was excavated from a former flower strip bordering an agricultural field in June 2020 and stored in open-top Big Bags outdoors under a roof shelter at the experimental facility until used in the experiment, which started in late September 2020. The soil was classified as sandy loam (50% sand, 33% silt, 14% clay, 2.4% SOM, pH 6.8, 1130 mg total-N/kg, 31.5 mg available-S/kg, 1.2 mg available-P/kg, 126 mg available-K/kg). The

143 soil was sieved through a 2-cm mesh sieve before use in the experiment, to remove large stones, loam
144 aggregates, and root material.

145

146 *Plant–insect model system*

147 Barley plants (*Hordeum vulgare*, cv. 'Scarlett') were used as model plants. This cultivar was selected
148 as it was used in previous studies in our research group and because it was severely colonized by
149 aphids (Sanchez-Mahecha et al. 2022). As model aphids, we used the English grain aphid (*Sitobion*
150 *avenae* 'Fescue', Hemiptera: Aphididae), a species that is specialized on grasses and is a common pest
151 in cereal cropping systems. We have observed that this barley cultivar and aphid clone respond in
152 terms of growth and population growth, respectively, to brief pulses of light disruption during the
153 night in a pilot study. Aphid colonies have been maintained in a climate chamber at the institute at 15
154 °C (night) to 20 °C (day), under 16h:8h L:D conditions, on barley cultivar 'Chanson' for several years.

155

156 *Climate chamber conditions*

157 This study was conducted in the TUM Model EcoSystem Analyzer (TUM*mesa*; Roy et al. 2021) in
158 Freising, Germany. In this facility, eight separate high-performance climatic chambers allow for a very
159 high level of between-chamber standardization of abiotic parameters and irradiation conditions
160 (Teixeira et al., in prep.). Plants were grown under 16h:8h (L:D) daylength regimes, reflecting their
161 summer growth period, with 21 °C and 16 °C temperatures during day and night, respectively.
162 Daylights turned on at 8am, and were shut off at 12pm, which enabled us to sample under night
163 conditions between 6 and 8am. Relative air humidity was kept constant at 60%. During the day, plants
164 received a full-spectrum light (Suppl. Fig. S1c), and the Photosynthetic Photon Flux Density (PPFD) was
165 set to 500 µmol $m^{-2}s^{-1}$.

166

167 *Experimental design*

168 We performed a full-factorial experiment involving three ALAN treatments, two aphid treatments and
169 destructive (independent) samplings across four sampling points. Seven climate chambers were used
170 from October to December 2020, and were assigned to one of three ALAN treatments. As the number
171 of available chambers limited the replication of our ALAN treatments, the experiment was repeated
172 in two independent runs. In the first run, two chambers were exposed to ALAN for the full night (‘*full*
173 *night*’), three chambers were exposed to ALAN that was shut off after the first four hours (‘*part-night*’),
174 and the final two chambers were not exposed to ALAN (‘*control*’; Fig. S2). In the second run, three
175 chambers received full night ALAN, two chambers received part-night ALAN and two served as no
176 ALAN controls (Fig. S2). Each treatment was switched to another chamber between the two runs, to
177 account for chamber-specific effects. Effectively, this approach resulted in a total of four replicated
178 ‘control’ chambers, and five replicated chambers of each of the two ALAN treatments (Fig. S2).

179 *ALAN treatments* – Each chamber was equipped with four linked LED modules each consisting of six
180 white diodes per module (D-15-01-001, 2W, 12V, www.leds24.com), coupled to a dimmer to control
181 illuminance. Illuminance was measured using a HR4000 spectrometer (using OceanView v1.6.7) paired
182 to an Ocean Optics Custom Fiber Configuration OCF-102313 equipped with a cosine corrector with
183 spectral diffusing material (Ocean Insight, Orlando, Florida, US) at pot level and illuminance was set to
184 levels between 15–20 lux, which are levels commonly observed underneath LED street lights (Bennie
185 et al. 2016). Measurements with the same spectral meter underneath local LED street lights showed
186 noisy signals with high temporal variation during measuring intervals of 5– 00 lux. Note that under the
187 experimental nocturnal light conditions, PPFD levels were below the measurable range of the fixed
188 TUM*mesa* light sensors, indicating fewer than 0.5 µmol photons m^{-2} s^{-1}. The spectral irradiance of the
189 climate chambers during the day and spectral irradiance of the LEDs under maximum output capacity
190 and under the dimmed ALAN treatment levels were measured and visualized after the experiment
191 using an array-radiometer (Newport OSM400 200-800 nm with Sony CCD-Sensor 2046px and 50 µm

192 gap, 1 nm spectral resolution, Newport Spectra Physics GmbH, Darmstadt, Germany). Spectral
193 irradiance for the LED modules under maximum capacity and under set ALAN treatments, and a
194 visualization of a typical daylight spectral irradiance of the used climate chambers can be found in
195 Suppl. Fig S1.

196 *Plant and aphid treatments* – Barley plants were grown with and without aphids in single round plastic
197 pots (400 mL, 10 cm, Meyer, Göttingen, Germany). Pots were filled with soil, and watered with 50 mL
198 of tap water and then left to acclimatize at room temperature for four days, then brought into the
199 climate chambers, where they were left for another three days. Pots were checked daily and watered
200 (20 mL) when the soil surface was dry. After this week of acclimatization, two surface-sterilized barley
201 seeds (5% hypochlorite solution for 2 min, followed by a 1-min rinse with tap water) were planted in
202 1–2 cm deep holes made in the middle of the pot. Four days after sowing, most seedlings had
203 germinated, and the smallest seedling was removed from each pot, so that only one plant per pot
204 remained. Plants were checked daily, and watered to need, three times a week. One week after
205 sowing, the height of each plant was recorded, and two pre-final (N4) instar individuals of *S. avenae*
206 'Fescue' were added to all pots assigned to the aphid treatment. The plants from both the aphid and
207 no-aphid treatments were subsequently bagged around the pot rim with a permeable cellophane bag
208 (Kopp Verpackungen, Höheischweiler, Germany), which was secured with elastic band to contain the
209 aphids. For each of the two experimental runs, an independent set of plants was established.

210 *Temporal sampling* – Plants were sampled 7-, 10-, 14- and 17-days post-aphid infestation, which
211 enabled us to investigate how ALAN treatments affected aphid colony growth as a function of harvest
212 day. At each sampling time point, an *a priori* assigned (independent) subset of plants was sampled. All
213 eight treatment combinations, i.e., plant-aphid combinations (2 aphid treatments x 4 sampling dates),
214 were replicated four times within each ALAN climate chamber (hereafter: within-chamber replicates).
215 This design resulted in a total of 14 (4 control + 5 full night + 5 part-night) ALAN chambers x 2 aphid

216 treatments x 4 time points x 4 within-chamber replicates = 448 pots, which were *a priori* assigned to
217 treatments before sowing.

218 *Sampling and response variables* – On the sampling days, total numbers of aphids were recorded.
219 Among adult aphids, winged and unwinged aphids were distinguished. We measured plant height by
220 measuring the distance between soil surface and the tip of the longest leaf. Chlorophyll levels were
221 measured on the youngest fully expanded leaf at three points and recorded the average, using a
222 chlorophyll meter (Konica Minolta SPAD-502Plus, Tokyo, Japan). Plants were placed on a cart in the
223 evening, and the next morning, destructive sampling occurred between 6am and 7am, before the
224 daylights turned on. This ensured that we assessed the effects of the ALAN treatments – which by
225 design occurred at night – on gene expression. Chambers were opened to remove plants for about 5-
226 10 s. The harvest itself occurred in a dark corner of the corridor, with the only light coming from a dim
227 emergency light above a door. Shoots were harvested within 5 min of removing plants from their
228 chamber. To this end, the fresh shoots were clipped with sterilized scissors, folded in labelled
229 aluminium foil, and flash-frozen in liquid nitrogen. This was done for the first three within-chamber
230 replicates. We clipped and dried shoots of the remaining fourth within-chamber replicate to
231 determine shoot biomass. Roots for all four within-chamber replicates were subsequently washed and
232 dried to determine root biomass. Roots and shoots were oven-dried at 60 °C for at least three days
233 before weighing.

234 *Gene expression assay* – In order to assess the effects of ALAN on plant-aphid interactions, we
235 performed a reverse transcriptase-quantitative PCR on mRNA extracted from the frozen plant leaf
236 material. For the gene expression assay we selected the plants harvested at day 10 post-infestation,
237 as these plants did not yet show signs of aphid stress or yellowing, and hence provided high-quality
238 samples for our gene expression assay. Before RNA extraction, the sets of three within-chamber
239 replicates in liquid nitrogen were pooled for each treatment. Shoots of the three samples were ground
240 and homogenized in liquid nitrogen. Total RNA was extracted from the homogenized sample using a

241 QIAGEN RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol.
242 RNA was converted to cDNA using SuperScriptII reverse transcriptase (Invitrogen, Thermo Fisher,
243 Waltham, Massachusetts, US).

244 We selected four markers that are associated with defence-related proteins. Two markers amplify *PR1*
245 and *PR17b*, two genes that code for pathogenesis-related proteins, and are generally involved in
246 responses to biotrophic pathogens (Christensen et al. 2002; van Loon et al. 2006; Zhang et al. 2012),
247 and are known to be upregulated in response to aphids (Grönberg 2006; Delp et al. 2009). Two other
248 markers, *HvERF-like* and *HvWRKY22,* are associated with systemic defence against bacterial and fungal
249 pathogens in barley (Dey et al. 2014; Lenk et al. 2019). cDNA preparation was followed by qPCR on a
250 7500 Fast real-time qPCR system (Applied Biosystems, Thermo Fisher) with three technical replicates
251 to obtain average Ct values per gene and sample. qPCR was performed with the SensiMix SYBR low
252 ROX kit (Bioline, Meridian Bioscience) using primers from Dey et al. (2014) and Shrestha et al. (2019).

253 For further statistical analysis, we determined delta Ct (dCt) values by Ct_{target} - $Ct_{reference}$. Under the
254 assumption that the endogenous reference gene has stable and high expression in all samples,
255 indicated by a low $Ct_{reference}$, this generally results in a positive dCt value for a sample, where a higher
256 dCt indicates a lower expression. As we were interested in the relative expression of genes across
257 treatments, the dCt values were used for further statistical analysis. However, since the experiment
258 was replicated in two temporally separated runs, and this was an expected source of variation, we z-
259 transformed the dCt values per run by subtracting the *run mean* dCt from each *sample* dCt, and divided
260 this by the *standard deviation of the run mean* dCt, effectively taking out the run effect. The resulting
261 z-score represents an effect size as the distance between run mean dCt and the sample dCt in units of
262 standard deviation. As a positive dCt z-score indicates downregulation, which is visually not intuitive,
263 we inverted the z-score signs, so that higher and lower relative gene expression match with the sign
264 of the plotted values.

265

266 *Statistical analysis*

267 All analyses were performed in R version 3.6.3 (R Development Core Team, 2020). Linear mixed
268 models were performed using the package 'lme4' (Bates et al. 2015), and p-values were estimated
269 using type II Wald Chi-Square tests using car::Anova from the 'car' package (Fox & Weissberg 2019).
270 Data visualization was done using 'ggplot2' (Wickham 2016).

271 *Plant variables* – Plant variables were analysed using linear mixed models; as fixed factors we included
272 'ALAN treatment', 'aphid treatment' and 'harvesting day'. We included 'run' as a covariate in the
273 model, and 'climate chamber' as a random intercept, to correct for potential climate chamber effects.

274 *Aphid variables* – Aphid variables were analysed using generalized linear mixed models, with a Poisson
275 error distribution; as fixed factors we included 'ALAN treatment' and 'harvesting day'. We included
276 'run' as a covariate in the model, and 'climate chamber' as a random intercept, to correct for potential
277 climate chamber effects. Only pots allocated to the aphid treatment were included in the analyses.

278 *RT-qPCR* - Gene expression data were analysed by means of z-transformed dCt values using linear
279 mixed models. As fixed factors we included 'ALAN treatment' and 'aphid treatment', and 'climate
280 chamber' as a random intercept, to correct for potential climate chamber effects.

281 We assessed relationships between gene expression and aphid numbers by averaging the aphid
282 numbers on the samples that were pooled for the gene expression assays. The relationship between
283 aphid numbers and inverted z-transformed dCt values was assessed by linear regression.

284

285 **Results**

286 *ALAN and temporal effects on aphid growth*

287 Aphid numbers increased with time, i.e., harvest day (Table 1, Fig. 1a). The total aphid number per
288 plant was significantly affected by ALAN (Table 1, Fig. 1a). On average, aphid numbers on plants

289 exposed to full night ALAN were similar to those exposed to dark nights (control), whereas plants
290 exposed to part-night ALAN had about 14% lower aphid numbers (Fig. 1a). We also observed a
291 significant interaction between ALAN and harvest day (Table 1, Fig. 1a), indicating that ALAN affected
292 aphid numbers differently over time. Specifically, both full night and part-night ALAN treatments
293 suppressed aphid numbers during the first ten days of infestation, after which the aphids exposed to
294 full ALAN reached numbers similar to aphids exposed to dark night controls, whereas aphids exposed
295 to part-night ALAN remained substantially lower throughout the experimental duration (Fig. 1a).
296 When only adult aphids were considered, their numbers were significantly lower on plants exposed
297 to an ALAN treatment than in the dark night control (Table 1), with adult aphid numbers being 12 and
298 17% lower on plants exposed to full night and part-night ALAN than on control plants exposed to dark
299 nights, respectively (Fig. 1b). Harvest day also affected the number of adult aphids in the population
300 (Table 1), as the number of adult aphids in the population increased over time (Fig. 1b). We observed
301 no significant interaction between harvest day and ALAN treatment in their effects on adult aphid
302 numbers (Table 1).

303

304 *ALAN, aphid and temporal effects on plant performance*

305 Plant height and shoot dry biomass were only marginally (positively) affected by ALAN and plants
306 tended to be higher under full night ALAN treatment (Table 1, Fig. 2a,b), although post-hoc testing
307 revealed no significant differences between ALAN treatments. Root dry biomass was significantly
308 affected by ALAN (Table 1, Fig. 2c), with post-hoc testing revealing that part-night ALAN significantly
309 decreased root size compared to full night ALAN, but not to controls. Plant height and shoot biomass
310 were not significantly affected by aphid treatment, but aphids marginally reduced root biomass (Table
311 1, Fig. 2c). Harvest day positively affected plant height, shoot and root dry biomass (Table 1), as plants
312 increased in size during the experiment (Fig. 2). We observed no significant interactive effects
313 between ALAN and herbivory on plant height, shoot or root biomass (Table 1). ALAN significantly

314 elevated chlorophyll levels in the plants, with plants exposed to full night ALAN exhibiting on average
315 9%, and plants exposed to part-night ALAN 4% higher chlorophyll levels than control plants exposed
316 to dark nights (Table 1, Fig. 2b). Aphid presence did not significantly affect chlorophyll levels (Table 1),
317 but these were significantly affected by harvest day, as levels decreased when plants grew older (Table
318 1, Fig. 2b).

319

320 *ALAN and aphid effects on plant gene expression*

321 The expression of some defence genes was marginally or clearly affected by the ALAN treatments
322 while the presence of aphids increased expression of most of defence genes (Table 2). The expression
323 of *HvWRKY22* was marginally affected by ALAN treatments (Fig. 3a), with expression being
324 downregulated when plants were exposed to full night or part-night ALAN treatments (Fig. 3a). The
325 expression of *HvWRKY22* was strongly upregulated in aphid treatments (Fig. 3a). No interactive effects
326 were observed on the expression of *HvWRKY22* (Table 2). The expression of *PR1* was marginally
327 upregulated by aphid treatments (Fig. 3b), but effects of ALAN treatments or interactive effects on
328 the expression of *PR1* were not observed (Table 2). The expression of *PR17b* differed significantly
329 between ALAN treatments (Fig. 3c) and was marginally upregulated by aphid treatments (Fig. 3c), and
330 expression of *PR17b* was significantly lower when plants were exposed to full night or part-night ALAN
331 (Fig. 3c). The expression of the *HvERF-like* gene was not significantly affected by any of the treatments
332 (Fig. 3d).

333 The total number of aphids on those plants used for qPCR analyses positively correlated with the
334 expression of *HvWRKY22* ($R^2 = 0.31$, $F_{1,12} = 5.5$, $p = 0.037$, Fig. 4a), *PR1* ($R^2 = 0.55$, $F_{1,12} = 14.8$, $p = 0.002$,
335 Fig. 4b), and *PR17b* ($R^2 = 0.71$; $F_{1,12} = 29.4$, $p < 0.001$, Fig. 4c), but not with the *HvERF-like* gene ($R^2 =$
336 0.002, $F_{1,12} = 0.02$, $p = 0.891$, Fig. 4d).

337

338 **Discussion**

339 In this study, we exposed plants and associated aphids to either full nights of ALAN, nights with a part-
340 night ALAN treatment, where illuminance was switched off halfway through the night, or controls with
341 completely dark nights. We found that plant growth was only minimally affected by ALAN, whereas
342 aphid colony size and the number of matured aphids were reduced significantly by ALAN treatments.
343 Importantly, we found strong differences between full and part-night ALAN. Contrary to our
344 hypotheses, ALAN mitigation – by limiting the duration of light exposure at night – had stronger
345 negative effects on aphid colony growth than nights with full exposure to ALAN. Gene expression
346 assays revealed that defence-associated gene expression was in some cases affected by ALAN, but
347 also strongly positively correlated with aphid colony size, suggesting that the effects of ALAN on plant
348 defences are likely regulated through indirect effects of ALAN on plants via direct disruptive effects
349 on aphid colonies, rather than indirectly via upregulation of plant defences in response to ALAN.

350 In agreement with our first hypothesis, exposure to ALAN suppressed aphid colony growth. However,
351 aphid colonization patterns differed with the time since infestation, ALAN treatment, and between
352 the aphid parameters. Both forms of ALAN (i.e., full and shut-off ALAN) tended to suppress aphid
353 colony growth in the early stages of plant colonization. However, in the nights that received full ALAN,
354 this effect disappeared towards the later stages post-infestation at the end of the experiment. Under
355 the part-night ALAN, this was not the case, and aphid numbers 17 days after infestation were 14%
356 lower than in the control or full night ALAN. The number of adults recorded at the end of the
357 experiment was suppressed on plants at both ALAN levels, with a reduction of 12 and 17%,
358 respectively, in full night or part-night ALAN compared to aphid colonies on control plants exposed to
359 dark nights. This is important, as the capacity of an aphid population to grow depends on the number
360 of mature adults present at a given time, as well as their fertility. It is likely that slowed maturation,
361 higher mortality and disturbances leading to increased settling times will lead to suppressed colony

362 formation during the exponential growth phase, although the impact of these individual processes
363 could not be estimated from our data.

364 Our results are in line with previous work showing suppressive ALAN effects on plant–aphid
365 interactions in other model systems, where aphid suppression was associated with changes in plant
366 phenology, and increases in aphid parasitism by parasitoids (Bennie et al. 2015; Sanders et al. 2015;
367 Bennie et al. 2018; Sanders et al. 2018). Importantly, our study shows that aphid reduction also takes
368 place without such external confounding factors. Contrary to the present study, a previous field
369 experiment with *H. vulgare* and *S. avenae* did not detect effects of ALAN on final aphid densities at
370 nine weeks (Sanders et al. 2018). However, it should be noted that the study included parasitoids,
371 which as aphid antagonists, may have affected aphid colony sizes over time. Furthermore, Sanders et
372 al. (2018) did not investigate temporal aspects, and therefore, any temporal effects of ALAN may have
373 been masked, or compensated for, as our own data suggest. Other studies that have assessed ALAN
374 effects on aphid colony size over time have generally included repeated measures of the same colony
375 (Bennie et al. 2015; Sanders et al. 2015), which allows to assess individual colony responses, but also
376 elevates the risk of including colony-specific effects that persist over time. In our study, aphid colony
377 growth was assessed over time on truly independent samples – not by temporal pseudo-replication –
378 which provides a higher temporal resolution, and also reinforces the generality of ALAN impacts on
379 aphid colony formation over time.

380 An important question is what drives the ALAN-induced reduction in total and adult aphid numbers?
381 Two answers are plausible: On the one hand, ALAN could influence aphids indirectly, via changes in
382 plant nutritional quality or quantity (plant defence compound and nutritional quality or plant size),
383 which commonly affects insect performance through bottom-up effects (Will & Van Bell 2006). On the
384 other hand, ALAN may directly impact aphids, through disturbance of natural behaviour such as
385 settling times that may limit feeding, and ultimately, fitness. Our data suggest that plant size was only
386 marginally affected by ALAN. Although various studies have suggested that resource availability could

387 be the main driver through which ALAN impacts insect responses (Sanders et al. 2015, Bennie et al.
388 2015), plant size under levels of infestation observed in our study are unlikely to be limiting to aphid
389 population growth, as plants were large, and aphid numbers relatively low. It thus seems more
390 plausible that potential bottom-up effects are mediated via plant quality, not quantity. We observed
391 a significant increase in chlorophyll levels – a proxy for plant health and quality – when plants were
392 exposed to ALAN, but as our data suggested only a weak and positive relationship with aphid numbers,
393 it is highly unlikely that chlorophyll levels alone are responsible for aphid suppression under ALAN.
394 Future research should focus on how ALAN may affect plant quality for aphids, and especially the
395 diurnal variation in the phloem composition (Douglas 2006), in order to better understand the possible
396 links between ALAN, the plant and ALAN-induced aphid suppression.

397 As plant size and health status seemed to have only minimal influence in the ALAN-driven reduction
398 in aphid colony size, we investigated impacts of ALAN on plant-aphid defence pathways. We assessed
399 the expression of four defence-associated genes in barley corresponding with different defence
400 pathways, to test whether exposure of plants to ALAN would result in a regulation of specific
401 pathways. To this end, we assessed the ALAN-associated transcript accumulation of two *PR* genes,
402 which are commonly associated with innate immunity in plants (van Loon et al. 2006; Wang et al.
403 2018). In particular, *HvPR1* and *HvPR17b* are induced upon infection of barley with pathogens and are
404 presumed to positively correlate with defence (Christensen et al. 2002; Grönberg 2006; Delp et al.
405 2009; Zhang et al. 2012). Further, *HvWRKY22* and *HvERF-like* have previously been positively
406 associated with SAR-like systemic defence in barley (Dey et al. 2014).

407 We observed marginal effects of ALAN on the expression of *HvWRKY22*, and a strongly significant
408 effect of ALAN on the expression of *PR17b*. We did not find any effect of ALAN or aphid treatments on
409 the expression of *HvERF-like* gene expression, suggesting that this gene, which is associated with
410 systemic pathways (Dey et al. 2014), is unlikely to respond to variation in light at night and is not
411 involved in plant defences against aphids. Contrary to our expectation that ALAN would upregulate

412 plant defences, ALAN decreased the expression of *HvWRKY22* and *PR17b*, and we observed similar
413 but non-significant patterns in *PR1*. Only in the case of *PR17b*, the levels of expression were
414 significantly lower under ALAN treatments, compared to levels in control plants exposed to dark
415 nights. Gene expression did not differ significantly between ALAN level treatments and controls in the
416 plants that were not exposed to aphids, suggesting that constitutive levels of defences were not
417 affected by ALAN. In fact, for *HvWRKY22*, *PR1* and *PR17b*, but not for *HvERF-like* gene, the gene
418 expression correlated tightly and positively with the aphid numbers present on the plant.

419 This suggests that patterns of expression differing between ALAN treatments were likely not the result
420 of direct ALAN effects on plant defence regulation, but instead more likely a result of direct disruptive
421 effects of ALAN on aphid feeding or settling behaviour and resultant suppression of colony growth,
422 and that this, in turn, is reflected by a reduced transcript accumulation of certain defence-associated
423 genes. Importantly, from these results, we cannot conclusively state that ALAN does not affect plant
424 physiology. Our analyses focused on a limited number of genes that are involved in constitutive and
425 induced plant defence responses against aphids. A more holistic approach, for instance using meta-
426 transcriptomics of plants under ALAN or dark night treatments could reveal how ALAN may affect
427 transcriptional regulation of phytohormonal defence mediation, as well as other pathways involved,
428 for instance, in secondary metabolism.

429 There are various alternative plant physiological pathways through which ALAN might affect the
430 phytobiome (Heinen 2021). For instance, exposure to ALAN can disrupt stomatal activity, above-
431 belowground allocation, and the nocturnal starch turnover from above- to belowground
432 compartments (Stitt & Zeeman, 2012; Kwak et al. 2017; 2018). Disruptions in starch metabolism may
433 result in changes in concentration and composition of sugars in phloem tissues, and as aphids are
434 directly affected by phloem sugar concentrations via the 'sugar barrier', which requires strong
435 osmoregulation in the aphid gut to excrete it (Douglas 2006), it is entirely plausible that such a
436 pathway might affect probing, feeding and reproductive success, leading to altered aphid colony

437 growth. As such, future studies should investigate ALAN effects on sugars, amino acids, micronutrients
438 and secondary metabolites in the phloem that are important for aphid performance. For instance,
439 changes in carbon metabolism over diurnal cycles can greatly impact interactions between plants and
440 their microbiome (Baraniya et al. 2018), and knowing how ALAN may impact on carbon metabolism
441 and affect interactions between plants and other organisms will be an exciting direction for future
442 research (Heinen 2021).

443 The most likely explanation for the suppressive effects of ALAN on aphid colony growth is via direct
444 effects on aphid behaviour. Aphids perceive light intensity and can respond to low light intensities by
445 producing winged adults (Alkhedir, Karlovsky & Vidal 2010). Aphids also express diurnal rhythmicity in
446 locomotor and metabolic activity (Beer et al. 2017), even independent of their plant hosts, which are
447 strongly rhythmic (Joschinski et al. 2016). Despite strong cyclic circadian clock gene expression, aphids
448 continue the various stages of their feeding cycles throughout the night, which is hypothesized to be
449 an important element for osmoregulation of high phloem sugar content (Douglas 2006; Nalam et al.
450 2021). Light has been shown to disrupt aphid behaviour, and more strongly so during the night
451 (Narayandas & Alyokhin 2006). We thus speculate that ALAN led to disruptions of settling and feeding
452 behaviour, lowering the uptake of resources, which in turn explain smaller aphid colony sizes and
453 number of matured adults, and the tightly linked defence gene expression, as observed under our
454 treatments. Further tests to examine how ALAN impacts aphid feeding behaviour, should make use of
455 electrical penetration graph analyses (Tjallingii 1978), which record the probing behaviour of aphids
456 over time, to compare aphid behaviour under dark and lit nights.

457 An important finding is that contrary to our hypothesis, a mitigation of ALAN levels at night through a
458 midnight shutoff of the light source enhanced the adverse ecological effects of ALAN on insects. A
459 popular belief is that the ecological side effects of light pollution could be mitigated by optimizing
460 lighting schemes to match human activity patterns, and turning off lights after peak activity. However,
461 our data suggest that after experiencing a short duration of ALAN, turning off the lights has more

462 detrimental effects than keeping them on. These findings are difficult to explain. One possibility is that
463 a midnight shutoff strategy may be experienced as a double ecological stressor. First, the organisms
464 experience a lit night, which may be seen as the first stressor, which is then followed by a severely
465 reduced dark recovery period, i.e., further stress, although the mechanism is unclear. However, we
466 argue that the ecological consequences of specific mitigation strategies should be understood before
467 implementation, as despite good intentions, they may in certain conditions do more harm than good.

468 Our study also highlights the physical limitations of ALAN research. One example is the typical choice
469 to use illuminance – expressed in lux, which is a photometric unit – in most ALAN research. This means,
470 that the radiometric quantity will be weighted with a visual function describing the wavelength
471 dependent response of the human eye. The photopic function of the human eye have the maximum
472 at 550 nm and reach from ca. 380 nm to 780 nm, this wavelength range can be named in a strict
473 meaning as light (Aphalo et al. 2012). However, light is in the physical sense better described by the
474 spectral irradiance with the unit $Wm^{-2}nm^{-1}$, radiant energy per time per effective receiver surface and
475 per wavelength interval. This is where ALAN research becomes more problematic, as its spectral
476 irradiance generally falls below thresholds measurable by standard equipment. Another problematic
477 aspect is that in the lower ranges of spectral irradiance, the ratio of noise to signal is very high (cf.
478 Suppl. Fig. S1b). Measurements conducted under LED street lights in the field confirm low signals of
479 irradiance, with comparatively high levels of noise. A better physical characterization of ALAN
480 irradiance spectra, and its reach in nature is essential to understand the breadth of ecological impacts,
481 especially on sessile organisms such as plants.

482 Despite significant advances in the field, the effects of ALAN on plants and plant-associated organisms
483 are understudied (Heinen 2021). We aimed to generate a better understanding of how ALAN impacts
484 on aphid colony formation over time, as mediated via plant quality and quantity. In general, ALAN
485 impacts on barley plants appear to be minor, but regardless exposure to ALAN leads to substantial
486 reductions in aphid colony size and maturation, which appear to be direct disruptive effects of ALAN,

rather than plant-mediated effects. Our study especially highlights the difficulties associated with mitigation of the ecological side effects of ALAN, as a reduction of the light intervals increased the negative impact of ALAN on aphids. Rather little is known about how ALAN affects insect communities on crops and wild plant species. These findings highlight the need to study the impacts of ALAN in natural ecosystems, and we show that the solution to light pollution may be more complex than the flip of a switch.

Acknowledgements

We thank Roman Meier for advice and engineering support in the TUMmesa phytochamber facilities. We thank Hans Hausladen and Stefan Kimmelmann for advice and help in obtaining soil for the pot experiment. We thank Sharon Zytynska for supporting the project. The experiment was supported by DFG grant 397565003 WE 3081/36-1 awarded to Sharon Zytynska and WWW.

500 **Figure legends:**

501 Figure 1: Box plots depicting effects of ALAN treatments and sampling time on a) aphid colony size
502 (n=16-20), and b) number of adult aphids within the population (n=16-20). Box colours depict ALAN
503 treatments, with grey being control, yellow part-night ALAN, and green full-night ALAN. Boxes
504 represent median values with upper and lower quartiles, and whiskers represent 1.5x the
505 interquartile range; individual dots represent outliers. Simplified mixed model output is indicated in
506 the graphs, and detailed model output can be found in Table 1.

507 Figure 2: Box plots depicting effects of ALAN treatments and sampling time on a) plant height (n =
508 16–20), b) chlorophyll content (SPAD units) (n = 16–20), c) root (n = 16–20) and d) shoot dry biomass
509 (n = 4–5). Box colours depict ALAN treatments, with grey being control, yellow part-night ALAN, and
510 green full-night ALAN. Boxes represent median values with upper and lower quartiles, and whiskers
511 1.5x the interquartile range; individual dots are outliers. Simplified mixed model output is indicated
512 in the graphs, and detailed model output can be found in Table 1.

513 Figure 3: Box plots depicting effects of ALAN treatments on standardized gene expression of a)
514 *HvWRKY22,* b) *PR-1*, c) *PR-17b* and d) *HvERF-like gene* markers (each n = 4 (control) or 5
515 (treatment)). Box colours depict ALAN treatments, with grey being control, yellow part-night ALAN,
516 and green full-night ALAN. Boxes represent median values with upper and lower quartiles, and
517 whiskers represent 1.5x the interquartile range; individual dots represent outliers. Simplified mixed
518 model output is indicated in the graphs, and detailed model output can be found in Table 2.

519 Figure 4: Relationships between the average number of aphids on barley plants assigned to a pooled
520 sample, and the standardized gene expression of a) *HvWRKY22,* b) *PR-1*, c) *PR-17b* and d) *HvERF-like*
521 *gene* markers across ALAN treatments. Linear correlations with 95% confidence intervals were fit if
522 the linear relationship was significant.

523

Tables:

Table 1: Output of general linear models testing the effects of ALAN (A), herbivory (H) and time (T) on the number of total aphids, the number of adult aphids, plant height (cm), shoot biomass (g), root biomass (g), chlorophyll levels (SPAD units). Presented are degrees of freedom, Chi-square statistics and p-values. Significant effects are marked in bold, and marginally significant effects in italics.

	Total aphids		Adult aphids		Plant height		Shoot dry mass		Root dry mass		Chlorophyll level	
	d.f.	χ^2 (p)	d.f.	χ^2 (p)	d.f.	χ^2 (p)	d.f.	χ^2 (p)	d.f.	χ^2 (p)	d.f.	χ^2 (p)
Run	1	**51.2 (<0.001)**	1	*2.9 (0.089)*	1	**63.3 (<0.001)**	1	**13.6 (<0.001)**	1	0.8 (0.383)	1	**36.0 (<0.001)**
ALAN (A)	2	**103.7 (<0.001)**	2	**10.4 (0.005)**	2	*4.7 (0.094)*	2	*5.1 (0.078)*	2	**6.9 (0.032)**	2	**8.1 (0.017)**
Herbivory (H)	-	-	-	-	1	0.6 (0.456)	1	2.1 (0.146)	1	*3.7 (0.055)*	1	2.6 (0.109)
Time (T)	3	**3166.4 (<0.001)**	3	**406.8 (<0.001)**	3	**123.8 (<0.001)**	3	**21.0 (<0.001)**	3	**10.7 (0.013)**	3	**73.7 (<0.001)**
A x H	-	-	-	-	2	1.8 (0.412)	2	0.2 (0.911)	2	2.4 (0.301)	2	1.4 (0.504)
A x T	6	**31.2 (<0.001)**	6	7.8 (0.254)	6	1.2 (0.974)	6	8.4 (0.210)	6	5.5 (0.487)	6	4.8 (0.565)
H x T	-	-	-	-	3	0.1 (0.995)	3	3.3 (0.351)	3	2.2 (0.525)	3	0.3 (0.957)
A x H x T	-	-	-	-	6	1.8 (0.934)	6	3.9 (0.697)	6	0.3 (0.999)	6	6.8 (0.338)

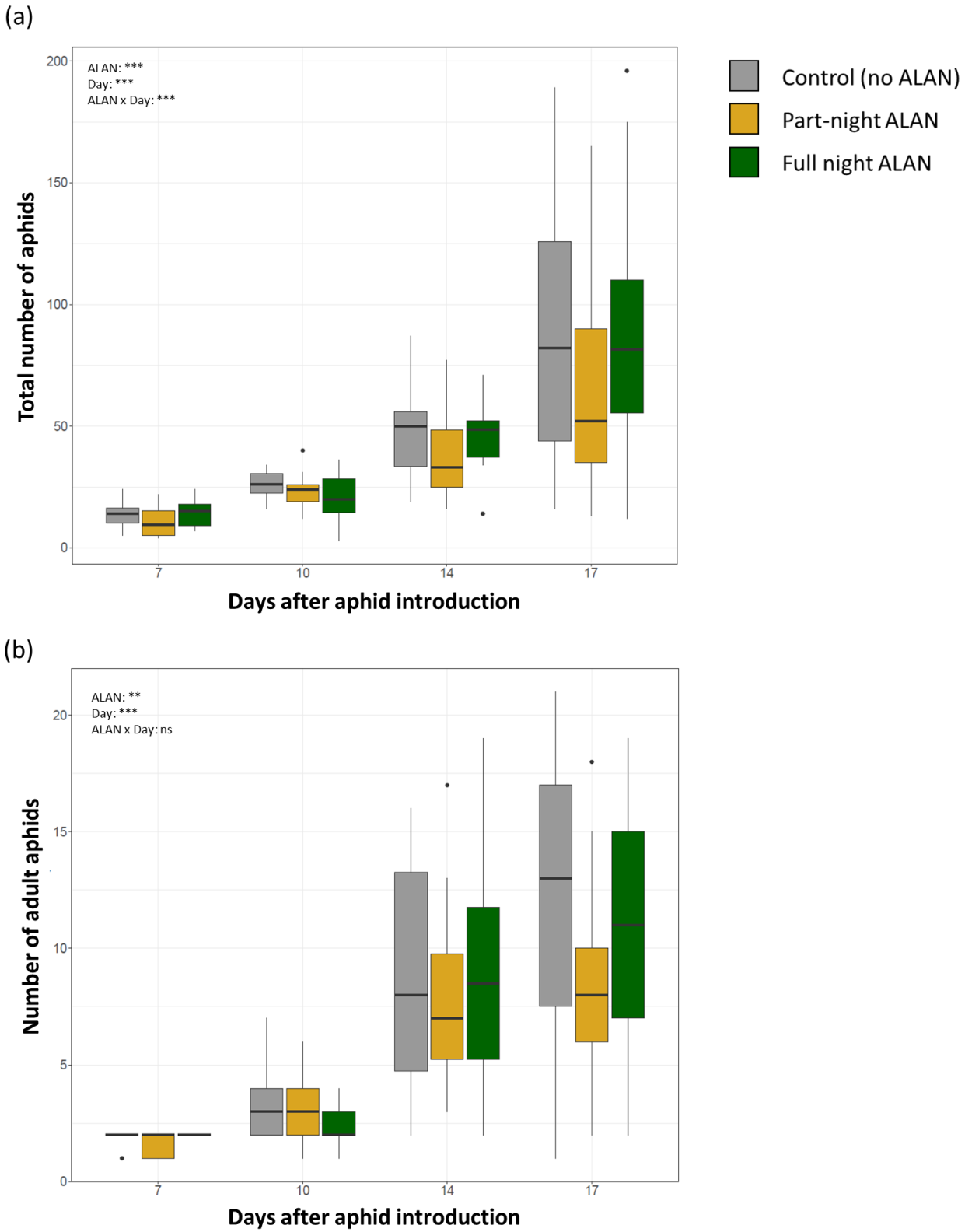
529 Table 2: Output of general linear models testing the effects of ALAN (A), herbivory (H) and time (T) on the standardized expression of *HvWRKY22, PR-1, PR-*
530 *17b,* and *HvERF-like* marker genes. Presented are degrees of freedom, Chi-square statistics and p-values. Significant effects are marked in bold, and
531 marginally significant effects in italics.

	HvWRKY22		*PR-1*		*PR-17b*		*HvERF-like*	
	d.f.	χ^2 (p)	d.f.	χ^2 (p)	d.f.	χ^2 (p)	d.f.	χ^2 (p)
ALAN (A)	2	*5.9 (0.053)*	2	3.3 (0.192)	2	**10.1 (0.006)**	2	0.5 (0.779)
Herbivory (H)	1	**8.0 (0.005)**	1	*3.6 (0.057)*	1	*3.1 (0.077)*	1	0.0 (0.998)
A x H	2	3.5 (0.170)	2	2.4 (0.302)	2	1.9 (0.381)	2	1.3 (0.531)

532

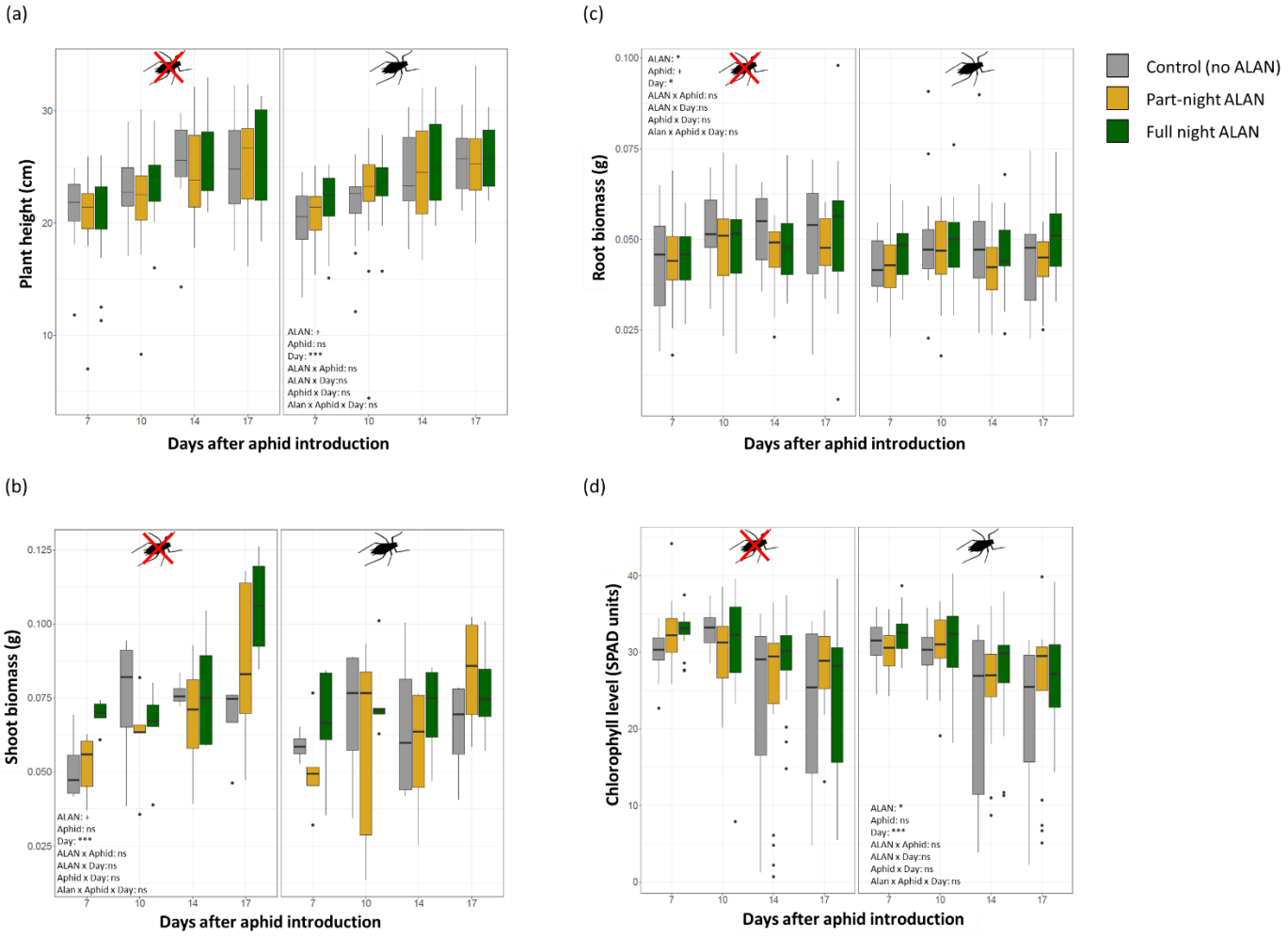
533 **Figures:**

534 Figure 2:



535

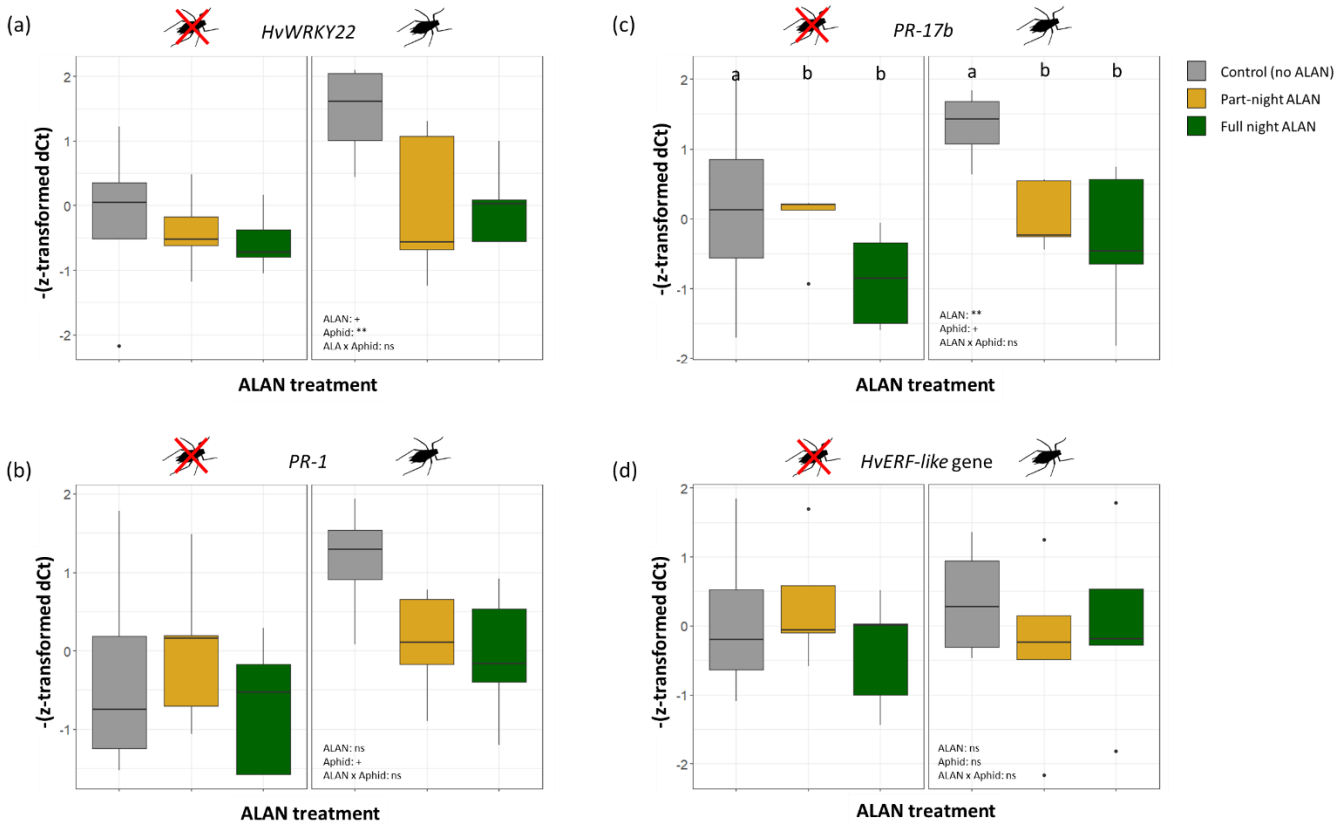
536 Figure 3:



537

538

539 Figure 4:

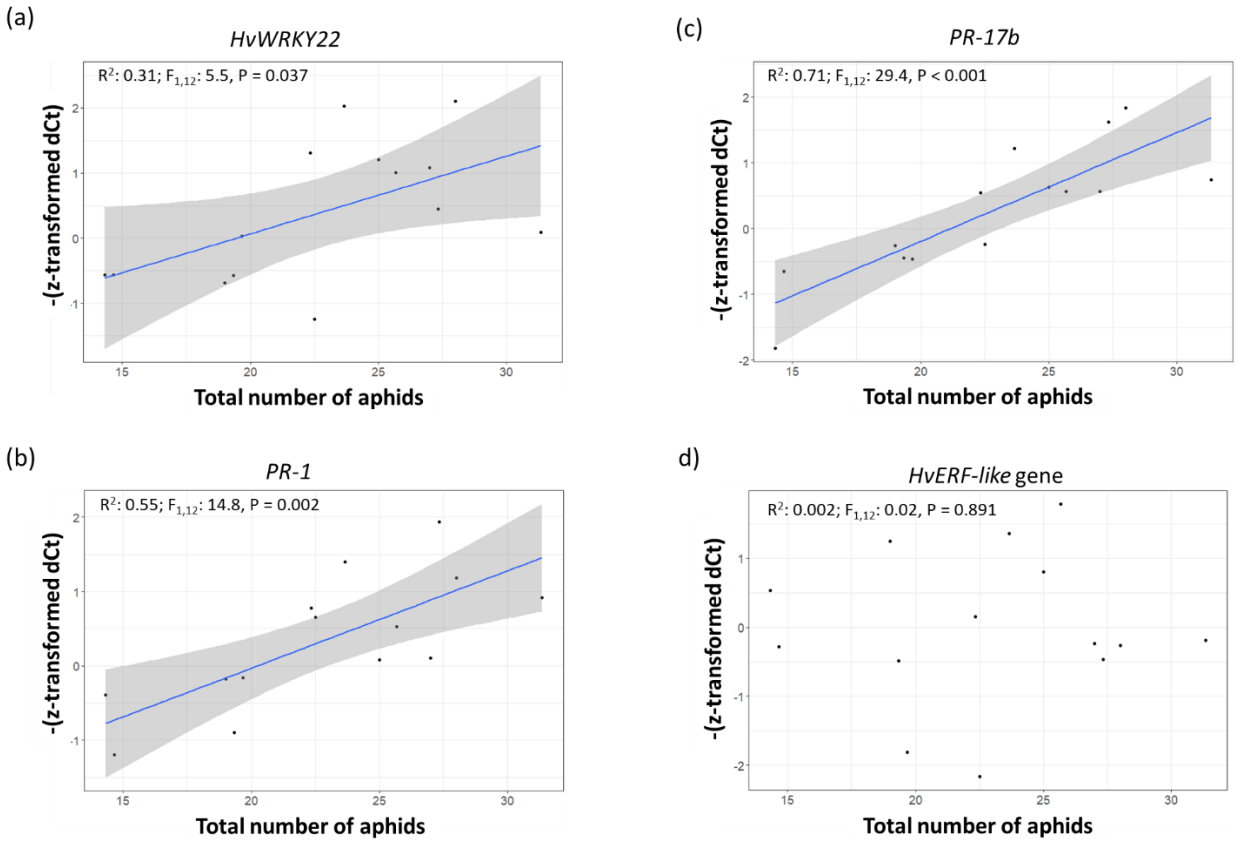


540

541

542

543 Figure 5:



544

545 **References**

546 Ahmad, M., Grancher, N., Heil, M., Black, R. C., Giovani, B., Galland, P., & Lardemer, D. (2002).
547 Action spectrum for cryptochrome-dependent hypocotyl growth inhibition in *Arabidopsis*. *Plant*
548 *Physiology*, *129*(2), 774-785.

549 Alkhedir, H., Karlovsky, P., & Vidal, S. (2010). Effect of light intensity on colour morph
550 formation and performance of the grain aphid *Sitobion avenae* F.(Homoptera: Aphididae). *Journal of*
551 *Insect Physiology*, 56(12), 1999-2005.

552 Aphalo, P. J., A., McLeod, A., Heikkilä, A., Gómez, I., López Figueroa, F., Robson, M. T., & Strid,
553 Å. (2012). Beyond the visible: a handbook of best practice in plant UV photobiology. Helsingfors
554 Universitet. p. 35-70.

555 Ayalon, I., de Barros Marangoni, L. F., Benichou, J. I., Avisar, D., & Levy, O. (2019). Red Sea
556 corals under Artificial Light Pollution at Night (ALAN) undergo oxidative stress and photosynthetic
557 impairment. *Global Change Biology*, 25(12), 4194-4207.

558 Ballare, C. L. (2009). Illuminated behaviour: phytochrome as a key regulator of light foraging
559 and plant anti-herbivore defence. *Plant, Cell & Environment*, 32(6), 713-725.

560 Ballaré, C. L. (2014). Light regulation of plant defense. *Annual Review of Plant Biology*, 65.

561 Ballaré, C. L., & Pierik, R. (2017). The shade-avoidance syndrome: Multiple signals and
562 ecological consequences. *Plant, Cell & Environment*, 40(11), 2530-2543.

563 Baraniya, D., Nannipieri, P., Kublik, S., Vestergaard, G., Schloter, M., & Schöler, A. (2018). The
564 impact of the diurnal cycle on the microbial transcriptome in the rhizosphere of barley. *Microbial*
565 *ecology*, 75(4), 830-833.

566 Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models
567 Using lme4. Journal of Statistical Software, 67(1), 1-48.

568 Beer, K., Joschinski, J., Sastre, A. A., Krauss, J., & Helfrich-Förster, C. (2017). A damping
569 circadian clock drives weak oscillations in metabolism and locomotor activity of aphids (*Acyrthosiphon*
570 *pisum*). *Scientific Reports*, 7(1), 1-9.

571 Bennie, J., Davies, T. W., Cruse, D., & Gaston, K. J. (2016). Ecological effects of artificial light at
572 night on wild plants. *Journal of Ecology*, 104(3), 611-620.

573 Bennie, J., Davies, T.W., Cruse, D., Inger, R. & Gaston, K.J. (2015b) Cascading effects of artificial
574 light at night: resource-mediated control of herbivores in a grassland ecosystem. *Philosophical*
575 *Transactions of the Royal Society of London B*, 370, 20140131

576 Briggs, W. R., Beck, C. F., Cashmore, A. R., Christie, J. M., Hughes, J., Jarillo, J. A., ... & Okada,
577 K. (2001). The phototropin family of photoreceptors. *The Plant Cell*, 13(5), 993-997.

578 Briggs, W. R., & Christie, J. M. (2002). Phototropins 1 and 2: versatile plant blue-light receptors.
579 *Trends in Plant Science*, 7(5), 204-210.

580 Chory, J., Chatterjee, M., Cook, R. K., Elich, T., Fankhauser, C., Li, J., ... & Reed, J. (1996). From
581 seed germination to flowering, light controls plant development via the pigment phytochrome.
582 *Proceedings of the National Academy of Sciences*, 93(22), 12066-12071.

583 Christie, J.M. (2007) Phototropin blue-light receptors. *Annual Review of Plant Biology*, 58, 21–
584 45.

585 Day, J., Baker, J., Schofield, H., Mathews, F., & Gaston, K. J. (2015). Part-night lighting:
586 Implications for bat conservation. *Animal Conservation*, 18(6), 512-516.

587 Dey, S., Wenig, M., Langen, G., Sharma, S., Kugler, K. G., Knappe, C., ... & Vlot, A. C. (2014).
588 Bacteria-triggered systemic immunity in barley is associated with WRKY and ETHYLENE RESPONSIVE
589 FACTORs but not with salicylic acid. *Plant Physiology*, 166(4), 2133-2151.

Desouhant, E., Gomes, E., Mondy, N., & Amat, I. (2019). Mechanistic, ecological, and evolutionary consequences of artificial light at night for insects: review and prospective. *Entomologia Experimentalis et Applicata*, 167(1), 37-58.

Dominoni, D., Quetting, M., & Partecke, J. (2013). Artificial light at night advances avian reproductive physiology. *Proceedings of the Royal Society B: Biological Sciences*, 280(1756), 20123017.

Donohue, K., Heschel, M. S., Chiang, G. C., Butler, C. M., & Barua, D. (2007). Phytochrome mediates germination responses to multiple seasonal cues. *Plant, Cell & Environment*, 30(2), 202-212.

Douglas, A. (2006). Phloem-sap feeding by animals: problems and solutions. *Journal of experimental botany*, 57(4), 747-754.

Duarte, C., Quintanilla-Ahumada, D., Anguita, C., Manríquez, P. H., Widdicombe, S., Pulgar, J., Silva-Rodríguez, E. A., Miranda, C., Manríquez, K., & Quijón, P. A. (2019). Artificial light pollution at night (ALAN) disrupts the distribution and circadian rhythm of a sandy beach isopod. *Environmental Pollution*, 248, 565-573.

Falcón, J., Torriglia, A., Attia, D., Viénot, F., Gronfier, C., Behar-Cohen, F., Martinsons, C., & Hicks, D. (2020). Exposure to artificial light at night and the consequences for flora, fauna, and ecosystems. *Frontiers in Neuroscience*, 1183.

Fox, J., & Weisberg, S. (2019). An {R} Companion to Applied Regression, Third Edition. Thousand Oaks CA: Sage. URL: https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Gaston, K. J. (2018). Lighting up the nighttime. *Science*, 362(6416), 744-746.

Gaston, K. J., Visser, M. E., & Hölker, F. (2015). The biological impacts of artificial light at night: the research challenge. *Philosophical Transactions of the Royal Society B,* 370(1667), 20140133.

van Geffen, K. G., van Grunsven, R. H., van Ruijven, J., Berendse, F., & Veenendaal, E. M. (2014). Artificial light at night causes diapause inhibition and sex-specific life history changes in a moth. *Ecology and Evolution*, 4(11), 2082-2089.

Grubisic, M., van Grunsven, R. H., Manfrin, A., Monaghan, M. T., & Hölker, F. (2018). A transition to white LED increases ecological impacts of nocturnal illumination on aquatic primary producers in a lowland agricultural drainage ditch. *Environmental Pollution*, 240, 630-638.

Grubisic, M., Haim, A., Bhusal, P., Dominoni, D. M., Gabriel, K., Jechow, A., Kupprat, F., Lerner, A., Marchant, P., Riley, W., Stebelova, K., van Grunsven, R. H. A., Zeman, M., Zubidat, A. E. & Hölker, F. (2019). Light pollution, circadian photoreception, and melatonin in vertebrates. *Sustainability*, 11(22), 6400.

Howe, G. T., Gardner, G., Hackett, W. P., & Furnier, G. R. (1996). Phytochrome control of short-day-induced bud set in black cottonwood. *Physiologia Plantarum*, 97(1), 95-103.

Joschinski, J., Beer, K., Helfrich-Förster, C., & Krauss, J. (2016). Pea aphids (Hemiptera: Aphididae) have diurnal rhythms when raised independently of a host plant. *Journal of Insect Science*, 16(1).

International Darksky Association (2020). LED Practical Guide. Tucson, AZ, USA https://www.darksky.org/our-work/lighting/lighting-for-citizens/led-guide/

Kami, C., Lorrain, S., Hornitschek, P., & Fankhauser, C. (2010). Light-regulated plant growth and development. In *Current Topics in Developmental Biology* (Vol. 91, pp. 29-66). Academic Press.

Kimura, M., & Kagawa, T. (2006). Phototropin and light-signaling in phototropism. *Current Opinion in Plant Biology,* 9(5), 503-508.

Kraiselburd, I., Moyano, L., Carrau, A., Tano, J., & Orellano, E. G. (2017). Bacterial photosensory proteins and their role in plant–pathogen interactions. *Photochemistry and Photobiology*, 93(3), 666-674.

Kyba, C. C. M., Ruhtz, T., Fischer, J., & Hölker, F. (2011). Cloud coverage acts as an amplifier for ecological light pollution in urban ecosystems. *PloS one*, 6(3), e17307.

Kyba, C. C. M., Kuester, T., Sánchez de Miguel, A., Baugh, K., Jechow, A., Hölker, F., Bennie, J., Elvidge, C. J., Gaston, K. J. & Guanter, L. (2017). Artificially lit surface of Earth at night increasing in radiance and extent. *Science Advances*, 3(11), e1701528.

Kwak, M. J., Lee, S. H., Khaine, I., Je, S. M., Lee, T. Y., You, H. N., ... & Woo, S. Y. (2017). Stomatal movements depend on interactions between external night light cue and internal signals activated by rhythmic starch turnover and abscisic acid (ABA) levels at dawn and dusk. *Acta Physiologiae Plantarum*, 39(8), 1-12.

Kwak, M. J., Je, S. M., Cheng, H. C., Seo, S. M., Park, J. H., Baek, S. G., ... & Woo, S. Y. (2018). Night light-adaptation strategies for photosynthetic apparatus in yellow-poplar (*Liriodendron tulipifera* L.) exposed to artificial night lighting. *Forests*, 9(2), 74.

Lenk, M., Wenig, M., Bauer, K., Hug, F., Knappe, C., Lange, B., Timsy, Häußler, F., Mengel, F., Dey, S., Schäffner, A., & Vlot, A. C. (2019). Pipecolic acid is induced in barley upon infection and triggers immune responses associated with elevated nitric oxide accumulation. *Molecular Plant-Microbe Interactions*, 32(10), 1303-1313.

Matzke, E. B. (1936). The effect of street lights in delaying leaf-fall in certain trees. *American Journal of Botany*, 446-452.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7(9), 405-410.

Moreno, J. E., Tao, Y., Chory, J., & Ballaré, C. L. (2009). Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. *Proceedings of the National Academy of Sciences*, 106(12), 4935-4940.

Nalam, V., Isaacs, T., Moh, S., Kansman, J., Finke, D., Albrecht, T., & Nachappa, P. (2021). Diurnal feeding as a potential mechanism of osmoregulation in aphids. *Insect Science*, 28(2), 521-532.

Narayandas, G. K., & Alyokhin, A. V. (2006). Diurnal patterns in host finding by potato aphids, *Macrosiphum euphorbiae* (Homoptera: Aphididae). *Journal of Insect Behavior*, 19(3), 347.

Perkin, E. K., Hölker, F., Richardson, J. S., Sadler, J. P., Wolter, C., & Tockner, K. (2011). The influence of artificial light on stream and riparian ecosystems: questions, challenges, and perspectives. *Ecosphere*, 2(11), 1-16.

Poppe, C., Sweere, U., Drumm-Herrel, H., & Schäfer, E. (1998). The blue light receptor cryptochrome 1 can act independently of phytochrome A and B in *Arabidopsis thaliana*. *The Plant Journal*, 16(4), 465-471.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Rich, C., & Longcore, T. (Eds.). (2013). *Ecological consequences of artificial night lighting*. Island Press.

Roden, L. C., & Ingle, R. A. (2009). Lights, rhythms, infection: the role of light and the circadian clock in determining the outcome of plant–pathogen interactions. *The Plant Cell*, 21(9), 2546-2552.

Roy, J., Rineau, F., De Boeck, H. J., Nijs, I., Pütz, T., Abiven, S., et al. (2021). Ecotrons: powerful and versatile ecosystem analysers for ecology, agronomy and environmental science. *Global Change Biology*, 27(7), 1387-1407.

Sanders, D., Kehoe, R., Cruse, D., van Veen, F. F., & Gaston, K. J. (2018). Low levels of artificial light at night strengthen top-down control in insect food web. *Current Biology*, 28(15), 2474-2478.

Schulz, E.-D., Beck, E., Buchmann, N., Clemens, S., Müller-Hohenstein, K., & Scherer-Lorenzen, M. (2019). *Plant Ecology, Second Edition*. Springer.

Senzaki, M., Barber, J. R., Phillips, J. N., Carter, N. H., Cooper, C. B., Ditmer, M. A., Fristrup, K. M., McClure, C. J. W., Mennitt, D. J., Tyrrell, L. P., Vukomanovic, J., Wilson, A. A., & Francis, C. D. (2020). Sensory pollutants alter bird phenology and fitness across a continent. *Nature*, 587(7835), 605-609.

Shinomura, T., Nagatani, A., Hanzawa, H., Kubota, M., Watanabe, M., & Furuya, M. (1996). Action spectra for phytochrome A-and B-specific photoinduction of seed germination in *Arabidopsis thaliana. Proceedings of the National Academy of Sciences*, *93*(15), 8129-8133.

Shrestha, A., Elhady, A., Adss, S., Wehner, G., Böttcher, C., Heuer, H., Ordon, F., & Schikora, A. (2019). Genetic differences in barley govern the responsiveness to N-acyl homoserine lactone. *Phytobiomes Journal*, 3(3), 191-202.

Smith, H. L., McAusland, L., & Murchie, E. H. (2017). Don't ignore the green light: exploring diverse roles in plant processes. *Journal of Experimental Botany*, 68(9), 2099-2110.

Somers, D. E., Devlin, P. F., & Kay, S. A. (1998). Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* circadian clock. Science, 282(5393), 1488-1490.

Stone, E. L., Harris, S., & Jones, G. (2015). Impacts of artificial lighting on bats: a review of challenges and solutions. *Mammalian Biology*, 80(3), 213-219.

Tjallingii, W. F. (1978). Electronic recording of penetration behaviour by aphids. *Entomologia Experimentalis et Applicata*, 24(3), 721-730.

Wang, X. D., Bi, W. S., Jing, G., Yu, X. M., Wang, H. Y., & Liu, D. Q. (2018). Systemic acquired resistance, NPR1, and pathogenesis-related genes in wheat and barley. *Journal of Integrative Agriculture*, 17(11), 2468-2477.

H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

Will, T., & van Bel, A. J. (2006). Physical and chemical interactions between aphids and plants. *Journal of experimental botany*, 57(4), 729-737.

704 Wit, M. de, Spoel, S. H., Sanchez-Perez, G. F., Gommers, C. M., Pieterse, C. M., Voesenek, L.
705 A., & Pierik, R. (2013). Perception of low red: far-red ratio compromises both salicylic acid-and
706 jasmonic acid-dependent pathogen defences in *Arabidopsis*. *The Plant Journal*, 75(1), 90-103.

707 Wu, M. C., Hou, C. Y., Jiang, C. M., Wang, Y. T., Wang, C. Y., Chen, H. H., & Chang, H. M. (2007).
708 A novel approach of LED light radiation improves the antioxidant activity of pea seedlings. *Food*
709 *Chemistry*, 101(4), 1753-1758.

710 Yang, Y. X., Wang, M. M., Yin, Y. L., Onac, E., Zhou, G. F., Peng, S., ... & Zhou, Y. H. (2015a).
711 RNA-seq analysis reveals the role of red light in resistance against *Pseudomonas syringae* pv. *tomato*
712 DC3000 in tomato plants. *BMC Genomics*, 16(1), 120.

713 Yang, Y. X., Wang, M. M., Ren, Y., Onac, E., Zhou, G., Peng, S., ... & Yu, J. Q. (2015b). Light-
714 induced systemic resistance in tomato plants against root-knot nematode *Meloidogyne incognita*.
715 *Plant Growth Regulation*, 76(2), 167-175.