

[Click here to view linked References](#)

1 **JULIA JACA<sup>1\*</sup>, MANUEL NOGALES<sup>2</sup> & ANNA TRAVESET<sup>1</sup>**

2

3 **Effect of diurnal vs. nocturnal pollinators and flower position on the**  
4 **reproductive success of *Echium simplex***

5

6 <sup>1</sup>Global Change Research Group, Institut Mediterrani d'Estudis Avançats IMEDEA

7 (CSIC- UIB), C/ Miquel Marqués 21, E07190-Esporles, Mallorca, Balearic Islands,

8 Spain

9 <sup>2</sup>Island Ecology and Evolution Research Group, Instituto de Productos Naturales y

10 Agrobiología (CSIC-IPNA), C/ Astrofísico Fco. Sánchez 3, E38206-La Laguna, Canary

11 Islands, Spain

12 \*corresponding author: [JuliaJaca@hotmail.com](mailto:JuliaJaca@hotmail.com), +34 657462349

13 MS. JULIA JACA ESTEPA (Orcid ID : 0000-0002-4061-1629)

14 DR. MANUEL NOGALES (Orcid ID : 0000-0002-5327-3104)

15 DR. ANNA TRAVESET (Orcid ID : 0000-0002-1816-1334)

16

17

18 **Abstract**

19

20 Nocturnal pollination plays an important role in sexual plant reproduction but has been  
21 overlooked, partially because of intrinsic difficulties in field experimentation. Even less  
22 attention has received the effect of within-inflorescence spatial position (distal or  
23 proximal) on nocturnal pollinators of columnar plants, despite there have been  
24 numerous studies examining the relationship between such position and reproductive  
25 success. Woody endemic *Echium simplex* possesses large erect inflorescences bearing  
26 thousands of flowers which are visited by a wide array of diurnal and nocturnal animals.  
27 In this study, we identified nocturnal visitors and compared their pollination  
28 effectiveness with that of diurnal pollinators in different inflorescence sections by  
29 means of selective exclosures in NE Tenerife (Canary Islands). Nocturnal visitors  
30 included at least ten morphospecies of moths (such as *Paradrina rebeli* and *Eupithecia*  
31 sp.), two coleopteran species (mainly *Alloxantha* sp.), neuropterans (*Chrysoperla*  
32 *carnea*), dictyopterans (*Phyllodromica brullei*), dermapterans (*Guanchia* sp.) and  
33 julidans (*Ommatoiulus moreletii*). In general, plants excluded from pollinators set less  
34 fruits than open-pollination (control) plants which set fruits homogeneously across  
35 sections. Diurnally-pollinated plants set more fruit in their upper parts whereas  
36 nocturnally-pollinated plants set fruit in both upper and bottom sections. We conclude  
37 that although the frequency and diversity of diurnal pollinators is far higher than that of  
38 nocturnal pollinators, both exhibit different foraging behaviour that generates  
39 complementary effects on the reproductive success of *E. simplex*.

40

41 **Keywords:** Canary Islands; pollination effectiveness; reproductive biology; resource  
42 allocation

43

44 **Introduction**

45 Plant reproductive success is the result of the interactions of both biotic (e.g.  
46 pollination, herbivory, disease) and abiotic (e.g. resource availability,  
47 physical environment) components of the ecological context with maternal constraints  
48 (Lee 1988). Mutualistic interactions between plants and their pollinators are of  
49 particular interest. Although most studies have focused on diurnal pollinators, nocturnal  
50 pollination plays a more important role in sexual plant reproduction than previously  
51 suspected, since pollen is carried over greater distances by moths than by diurnal insect  
52 pollinators (Macgregor et al. 2018). Nocturnal pollination has been overlooked partially  
53 because of the intrinsic difficulty of field experimentation at night; moreover, such  
54 process may easily be affected by artificial light at night (Knop et al. 2017).  
55 Nocturnal pollinators include a variety of taxa including insects, bats, birds, and even  
56 rodents (Baker 1961; von Helversen and Winter 2003; Knop et al. 2017). Some floral  
57 traits are usually associated with nocturnal pollination and form a particular pollination  
58 syndrome (Faegri and van der Pijl 1971; Fenster et al. 2004; Reynolds et al. 2009). This  
59 idea has been a central theme in pollination biology for many years (Faegri and van der  
60 Pijl 1966) and suggests that certain floral traits enhance the pollination efficiency of a  
61 particular pollinator type, leading to specialization in that pollination type. The flower  
62 characteristics traditionally associated with nocturnal pollination syndrome include:  
63 opening at dusk/night (Baker 1961; Van Doorn and Van Meeteren 2003), pale colour or  
64 white (Baker 1961; Lunau and Maier 1995), attracting scent (Jürgens et al. 2002;  
65 Raguso 2008) and copious nectar (Fenster et al. 2004). However, most plants are visited  
66 by a broad range of morphologically and taxonomically diverse species (Waser 1982;  
67 Elam and Linhart 1988; Haber and Frankie 1989; Thompson and Pellmyr 1992; Sahley

68 1996; Nassar et al. 1997), indicating that flower morphology may not be an accurate  
69 predictor of the type of animal visiting the flowers. Moreover, further observations and  
70 experiments addressed at evaluating the contribution of pollination to plant fitness are  
71 needed in order to differentiate pollinators from other visitors, since many species are  
72 nectar and/or pollen thieves (Schemske and Horvitz 1984; Waser et al. 1996).

73 In plants in which the flowers are grouped in inflorescences, numerous studies have  
74 examined the relationship between reproductive success and flower anthesis (early or  
75 late) and/or within-inflorescence spatial position (distal or proximal) (for a review, see  
76 Stephenson 1981; Wyatt 1982; Lee 1988; or Diggle 1995). For example, in species with  
77 columnar inflorescences with acropetal flower opening, higher fruit and seed set are  
78 often found in proximal flowers (Solomon 1988; Herrera 1991; Ehrlén 1992, 1993;  
79 Karoly 1992; Guitian 1994; Guitián and Navarro 1996; Navarro 1996) than in  
80 intermediate flowers (Sutherland 1987) or proximal flowers (Goldingay and Whelan  
81 1993). Three non-exclusive hypotheses have been proposed to explain these patterns of  
82 within- inflorescence variation regarding reproductive success:

- 83 1) The ‘resource competition hypothesis’, focused on abiotic components,  
84 postulates that the ovaries compete for a limited amount of resources  
85 (Stephenson 1981 and references therein; Klein et al. 2015).
- 86 2) The ‘architectural effects hypothesis’, related to maternal constraints, postulates  
87 that there is a constraint on the translocation of nutrients to reproductive organs  
88 due to the inherent structural features of an inflorescence, such as the waning of  
89 the vasculature in distal structures or the variation in the diameter of supporting  
90 structures (Diggle 1995 and references therein).
- 91 3) The ‘non-uniform pollination hypothesis’, with biotic components, postulates  
92 that there is a variation in pollen receipt along the inflorescence and differences

93 may be attributable to insufficient quantity or quality of pollen (Lee 1988;  
94 Thomson 1989a; Berry and Calvo 1991; Goldingay and Whelan 1993; Kudo et  
95 al. 2001).

96  
97 Woody endemic *Echium* species in the Canary Islands, both candelabra shrubs and  
98 monocarpic rosette ‘trees’, possess large erect inflorescences often carrying thousands  
99 of flowers visited by a wide range of animals. The patterns of female reproductive  
100 success within inflorescences have never been assessed. Previous studies with *Echium*  
101 *simplex* revealed that despite being visited by diurnal insects, birds and lizards, flying  
102 insects were responsible for most of the pollination (Jaca et al. 2019). However, *E.*  
103 *simplex* might also be visited at night, as its flowers possess traits associated with the  
104 moth pollination syndrome (phalaenophily): they open at night, produce pale-coloured  
105 or white flowers with a heavy scent, offering rewards (nectar and pollen) in tubular  
106 corollas (Baker 1961; Kevan and Baker 1983).

107 In the present study, we aimed to investigate the reproductive success of both nocturnal  
108 and diurnal pollinators in different inflorescence sections. Our specific questions were:  
109 (1) what are the nocturnal pollinators of *E. simplex* in each inflorescence section and  
110 how frequent are they relative to diurnal pollinators? (2) what is the pollination  
111 effectiveness of nocturnal and diurnal pollinators in each inflorescence section, in terms  
112 of fruit and seed set, seed weight, and germination?

113

## 114 **Materials and methods**

### 115 *Study species*—

116 The giant rosette plant *E. simplex* DC. (Boraginaceae), locally known as ‘tajinaste  
117 blanco’, is endemic to the Anaga Biosphere Reserve in NE Tenerife (Canary Islands).

118 This area encompasses a 4.9-3.9 million-year-old basaltic massif (Guillou et al. 2004).  
119 It is considered a vulnerable species in the red list of Spanish vascular flora (Moreno,  
120 2008), with very few, reduced and isolated populations. The species is one of the three  
121 monocarpic *Echium* species in the Canary Islands, together with *E. wildpretii* on La  
122 Palma and Tenerife, and *E. pininana* on La Palma, and it grows for 5-9 years before  
123 producing a single large inflorescence (Stöcklin and Lenzin 2013). Reproductive  
124 individuals reach a height of up to 3 m, of which the prolonged inflorescence -  
125 composed of scorpioid cymes - can contribute up to 1.5 m. The inflorescence height is  
126 directly proportional to the rosette diameter and it flowers acropetally (from  
127 bottom/proximal to upper/distal parts). The cymes are double-coiled and the largest  
128 plants may show 3-4 branches per cyme. After a successful pollination event, a flower  
129 develops into a fruit which consists of a maximum of four nutlets. The number of cymes  
130 and flowers per cyme increases along the inflorescence. The smallest of our examined  
131 plants had an average of 12 flowers per cyme whilst the largest had 51. The number of  
132 mature subfruits per flower (from one to three, on average) also increased along the  
133 inflorescence. Hence, the number of potential seeds produced increases enormously  
134 with the size of the inflorescence, ranging from 4,560 to 234,000 (Stöcklin and Lenzin  
135 2013).

136         Flowers are protandrous and are open for two to three days. The carpel elongates  
137 and splits, becoming taller than the anthers during the female phase. The flowers open  
138 successively from the proximal to the distal part of the cyme. The total flowering time  
139 of an individual plant is 3-5 weeks. Nectar standing crop varies during flower ontogeny  
140 with male and transitional flowers producing more nectar than in the female phase  
141 (approx. 2 ml vs. 1ml) but sugar concentration remains constant (~17%) (Jaca *et al.*  
142 2019).

143

144 ***Study area***—

145 The study site is located at the north-west of Chamorga village, northeastern Tenerife  
146 (Canary Islands). The population of *E. simplex* is found at an altitude around 250 m  
147 a.s.l. and occupies an area of about 1 km<sup>2</sup>. There are also scattered individuals along the  
148 north coast trails. The location has a warm coastal climate with average temperatures  
149 between 17 and 19 °C in winter and 20 and 25 °C in summer. The summer is very dry  
150 and most rain falls in winter, but only in small quantities. The area is exposed to the  
151 moist northeastern trade-wind, which is responsible for the lush green vegetation of  
152 Anaga mountains. The vegetation is shrubby-herbaceous, dry-Mediterranean and  
153 characterized by numerous endemic species such as *Artemisia thuscula*, *Descurainia*  
154 *millefolia*, *Aeonium canariense*, *Asphodelus tenuifolius*, *Achyranthes aspera* and  
155 *Galactites tomentosa*. Fieldwork was conducted once a week during a five-week period  
156 at the peak of the flowering season of *E. simplex*, between 10<sup>th</sup> May and 8<sup>th</sup> June 2016.

157

158 ***Flower visitors and visitation frequency***—

159 Data on diurnal visitors and visitation frequency was available from our previous study  
160 on this plant (Jaca et al. 2019). To identify nocturnal flower visitors and determine their  
161 visitation frequency, a total of 18 haphazardly chosen individual plants were observed  
162 during focal censuses for a total of 35 h. Individual plants were observed for 60 min per  
163 census (ca. 2 h observation per plant) at a shorter distance (0.5 m) from dusk to  
164 midnight. Insects of all species or morphospecies were captured and taken to the lab for  
165 identification. Animals were considered as flower visitors whenever they touched the  
166 flower, as the sexual organs are exerted from the corolla. For each flower visitor, we

167 recorded species identity (sometimes at family or order level), number of flowers and  
168 section of the plant visited (i.e. high, intermediate or low section).

169

170 ***Relative effectiveness of night and day flower visitors as pollinators—***

171 We conducted experiments to study the importance of pollination by diurnal and  
172 nocturnal flower visitors. Prior to flowering, the inflorescences of 21 haphazardly  
173 selected plants were bagged with muslin cloth to exclude any type of flower visitor and  
174 randomly assigned to day ('diurnally pollinated plants') or night ('nocturnally  
175 pollinated plants') time exposure treatment. Once per week, diurnally pollinated plants  
176 were unbagged during all the hours of the day (from 6:00 am to 9:00 pm), while  
177 nocturnally pollinated plants were unbagged all the hours of the night (from 9:00 pm to  
178 6:00 am the next day), and kept bagged the rest of the time. Additionally, 12 plants were  
179 permanently bagged to assess the level of autogamy, while 13 individuals were left  
180 open to pollinators, i.e. acting as a control group.

181 Five cymes from upper, intermediate and lower sections of each inflorescence and plant  
182 were collected once ripe and taken to the laboratory. Fruit set was calculated as the  
183 proportion of flowers that develop into fruits, and seed set as the amount of viable seeds  
184 produced per fruit. Seeds were regarded as non-viable (aborted) based on a  
185 characteristic smaller size and greyness. Previous germination trials confirmed that such  
186 seeds are indeed not viable (Jaca et al. 2019).

187 Germination trials were later carried out to test for differences among treatments (i.e.,  
188 control, autogamy, diurnal pollination and nocturnal pollination). A total of 1105 viable  
189 seeds (at least 18 seeds per plant, i.e., six seeds per inflorescence section per plant) were  
190 sown in early October 2016 into trays filled with a 1.2.1 mixture of peat, common  
191 agricultural soil and ravine sand in a greenhouse in Tacoronte (North Tenerife), as in



192 Jaca et al. (2019). Trays were watered every two days to ensure that the soil was  
193 constantly moist, and seedling emergence was registered every five days for three  
194 months until January 2017, when the germination experiment concluded after no seeds  
195 germinated during the next 25 days. Germinability (fraction of seeds that germinate)  
196 and germination rate (days to germination) were recorded for each seed (although we  
197 use the term germination we actually refer to the seedling time emergence). Seeds sown  
198 under each treatment were previously weighed to the nearest 0.1 mg.

199

200 *Statistical analyses*—

201 We used generalized linear mixed models (GLMM) in R software version 3.5.0 (R Core  
202 Team 2018), followed by a Tukey test of multiple comparisons. Census observations  
203 were clustered into functional groups of visitors for the analysis. The model was  
204 adjusted to a gamma error distribution, using the number of probed flowers per unit  
205 time and per flower as response variables and observation ID, nested in individual plant,  
206 as random effect. For the diurnal *vs.* nocturnal pollination and germination experiments,  
207 each estimate of plant reproductive success (i.e. fruit set, seed set, seed weight,  
208 germinability, and germination rate) was analysed separately as a dependent variable.  
209 Differences in fruit set and germinability were estimated using a binomial error  
210 distribution and logit link function, whereas a Poisson family was used to test for  
211 differences in seed set and germination rate (as the data were a discrete count of seeds  
212 or days, respectively). Seed weight was normally distributed and, for this variable, we  
213 thus adjusted errors to a Gaussian distribution. In all of these models, individual plant  
214 was used as random effect to control for lack of independence among flowers on the  
215 same individual plant.

216

217 **Results**

218 ***Floral visitors and visitation rates—***

219 Nocturnal insects visiting flowers of *E. simplex* were clustered into 6 groups: (1) moths,  
220 at least ten morphospecies, of which only two (*Paradrina rebeli* and *Eupithecia* sp.)  
221 could be identified, (2) beetles, mainly *Alloxantha* sp., with one unidentified, (3)  
222 neuropterans (*Chrysoperla carnea*, F. Chrysopidae), (4) dictyopterans (*Phyllodromica*  
223 *brullei*, F. Blattellidae), (5) dermapterans, (*Guanchia* sp. F. Forficulidae), and (6)  
224 julidans (*Ommatouilus moreletii*, F. Julidae) (Table 1). Visitation rates exhibited  
225 differences among insect groups ( $\chi^2 = 142.03$ ,  $df = 5$ ,  $P < 0.001$ ). The most frequent  
226 insect groups were lepidopterans (Fig. 1), visiting higher (distal) sections within the  
227 inflorescences, followed by coleopterans at intermediate and low positions, and other  
228 species mainly at the low sections (Table 1).

229

230 ***Comparative reproductive effectiveness of nocturnal and diurnal pollination in the***  
231 ***three inflorescence sections—***

232 Fruit set was affected by pollination treatment and inflorescence section (pollination  
233 treatment x section:  $\chi^2 = 33.34$ ,  $df = 6$ ,  $P < 0.001$ , Fig. 2). The number of fruits produced  
234 per flower was higher in the control plants open to pollinators, compared to those  
235 excluded from all pollinators and to those only visited by nocturnal pollinators. Within a  
236 plant, the number of fruits produced was higher in upper and bottom inflorescence  
237 sections in nocturnally-pollinated plants, whereas it was higher in the upper section in  
238 diurnally-pollinated plants (Fig. 2).

239 There was no interaction effect of pollination treatment x inflorescence section on seed  
240 set ( $\chi^2 = 12.38$ ,  $df = 6$ ,  $P = 0.054$ ). Seed set was influenced by pollination treatment ( $\chi^2$   
241  $= 17.25$ ,  $df = 3$ ,  $P < 0.001$ , Fig. 3) but not by inflorescence section ( $\chi^2 = 1.93$ ,  $df = 2$ ,  $P =$

242 0.38). Diurnally pollinated plants produced more seeds per fruit than nocturnally  
243 pollinated ones and also than control plants (Fig. 3).

244 Similarly, there was no interactive effect on seed weight between pollination treatment  
245 and inflorescence section ( $\chi^2 = 10.67$ ,  $df = 6$ ,  $P = 0.10$ ). Seed weight was affected by  
246 both pollination treatment and inflorescence section ( $\chi^2 = 8.96$ ,  $df = 3$ ,  $P = 0.03$ ; and  $\chi^2 =$   
247  $24.51$ ,  $df = 2$ ,  $P < 0.01$ , respectively, Fig. 4). Seeds from selfed flowers were  
248 significantly heavier than those from control flowers (Fig. 4A). Moreover, bottom  
249 inflorescence sections produced lighter seeds than upper and intermediate sections (Fig.  
250 4B).

251 Regarding germination patterns, both germinability and germination rate were  
252 influenced by an interactive effect among seed weight, inflorescence section and  
253 pollination treatment ( $\chi^2 = 16.01$ ,  $df = 6$ ,  $P < 0.05$ , and  $\chi^2 = 104.30$ ,  $df = 6$ ,  $P < 0.001$ ,  
254 respectively, Figs. 5 and 6).

255 In all inflorescence sections, most of the heavier seeds from control plants germinated.  
256 However, seeds from other treatments and inflorescence sections behaved differently.  
257 The heavier seeds of the diurnally pollinated plants germinated more when seeds were  
258 from the high sections of the inflorescence. The opposite occurred with seeds from the  
259 intermediate and low inflorescence sections, i.e. heavier seeds germinated less.

260 Furthermore, the heavier seeds of the nocturnally pollinated plants in the high and  
261 intermediate sections germinated slightly more than the lighter ones, whereas the  
262 opposite happened with seeds from the low sections, i.e. germinated less than lighter  
263 ones. Finally, for the autogamy treatment, we found that the heavier seeds had a higher  
264 germinability than the lighter ones, but this was only with seeds from the intermediate  
265 section and we found the opposite in the low and high sections, i.e. lighter seeds  
266 germinated more (Fig. 5).

267 Regarding germination rate, heavier control seeds from the upper and intermediate  
268 sections germinated earlier, whereas those from the bottom section were later. The  
269 germination rate of seeds in relation to their weight in diurnally vs nocturnally plants  
270 showed the opposite patterns, i.e. heavier seeds from the upper and bottom sections of  
271 diurnally pollinated plants took germinated faster, but not those from intermediate  
272 sections, and heavier seeds from the upper and bottom sections of nocturnally pollinated  
273 plants took longer to germinate, while those from intermediate sections germinated  
274 faster (Fig. 6). Finally, heavier selfed seeds germinated faster than the lighter ones from  
275 all sections of the plant.

276

## 277 **Discussion**

278 Ours is the first study that combines the effect of type of pollinators (nocturnal vs.  
279 diurnal) and inflorescence section on the reproductive success of a plant species.  
280 *Echium simplex* exhibited a uniform fruit set along the inflorescence, suggesting  
281 absence of competition among sections or maternal constraints, and uniform pollination.  
282 Although the species is mostly pollinated during the day, we found that nocturnal and  
283 diurnal pollinators displayed a complementary pollination behavior which translated  
284 into a complementary reproductive success.

285

### 286 ***Diversity of flower visitor groups***—

287 At night, *E. simplex* flowers are visited by six different functional groups of animals.  
288 This is a higher number than the usually reported in nocturnal pollination studies, where  
289 mostly moth visits are reported (Stephenson and Thomas 1977; Jennersten and Morse  
290 1991; Jürgens *et al.* 1996; Ghazoul 1997; Groman and Pellmyr 1999; Martinell *et al.*  
291 2010, but see Brantjes and Leemans 1976). However, the attractiveness of this plant for

292 insect visitors is greater during daytime, with up to 90 species of flower visitors  
293 identified (Jaca et al. 2018). This pattern of higher species diversity during the day is  
294 found in some plants (Jennersten and Morse 1991; Ghazoul 1997), though diversity is  
295 higher at night in others (Brantjes and Leemans 1976; Stephenson and Thomas 1977;  
296 Jürgens et al. 1996; Groman and Pellmyr 1999; Martinell et al. 2010). Some nocturnal  
297 insects are also observed in day censuses (Knop et al. 2017), as in our study. Indeed  
298 *Chrysoperla carnea*, *Guanchia* sp. and *Phyllodromica brullei* were also recorded in  
299 diurnal censuses (Jaca et al. 2019), as these animals can have diurnal activity or  
300 rest/hide within the flowers.

301 The most common nocturnal visitors in *E. simplex* were moths and the beetle  
302 *Alloxantha* sp. (Oedemeridae). This contrasts with other studies that report beetle visits  
303 as merely anecdotal (Stephenson and Thomas 1977; Groman and Pellmyr 1999;  
304 Martinell et al. 2010, but see Knop et al. 2017). When moths land on the inflorescence  
305 of *E. simplex* they sometimes walk over the flowers while probing them, and may  
306 remain on them for a short period. All body parts can contact the exerted anthers and  
307 pistils, and thus they are potentially effective pollinators (Ghazoul 1997). The moth  
308 diversity we found on *E. simplex* is much lower than that reported in other studies in  
309 both paleartic and nearctic realms, such as those on *Manfreda virginica* or *Silene otitis*  
310 and *S. sennenii* (Brantjes and Leemans 1976; Groman and Pellmyr 1999; Martinell et al.  
311 2010), but is similar to *Catalapa speciosa* or *S. vulgaris* and others (Stephenson and  
312 Thomas 1977; Jürgens et al. 1996). Beetles feed on pollen and move within the flowers  
313 but are probably irrelevant pollinators. In fact, their presence may indeed be deleterious,  
314 reducing final reproductive success by removing pollen from the stigmas (Kevan and  
315 Baker 1983; Jaca et al. 2019). As for other flower visitors, these nocturnal beetles were  
316 seen only anecdotally in other studies on night pollination, without being considered as

317 pollinators (Crumb et al. 1941; Brantjes and Leemans 1976; Thien 1980; Schneemilch  
318 et al. 2011; Knop et al. 2017).  
319 Regarding visitation frequency, nocturnal visitors were less frequent than diurnal ones  
320 (Jaca et al. 2019). This pattern is consistent with that found in most nocturnal  
321 pollination studies, despite the target species having a nocturnal syndrome (Stephenson  
322 and Thomas 1977; Ghazoul 1997; Young 2002 for a comparative table among studies;  
323 Martinell et al. 2010). It has been suggested that nocturnal visitors are scarcer because  
324 of their energetics, as they might require a larger nectar reward because of the cooler  
325 night temperatures (Morse and Fritz 1983); it has also been suggested that they could  
326 increase their length of visit during the night compared to diurnal pollinators  
327 (McMullen 2009).

328

329 ***Reproductive effectiveness of night and day pollination in the inflorescence***  
330 ***sections—***

331 In our previous studies on *E. simplex* we found that diurnal flying hymenopterans are  
332 the main pollinators responsible for its reproductive success (Jaca et al. 2019). In  
333 general, control plants set more fruits than diurnally or nocturnally pollinated, and than  
334 selfed plants, while diurnally pollinated plants set more fruits than nocturnally  
335 pollinated and selfed plants. This result is consistent with other studies (Bertin and  
336 Willson 1980; Morse and Fritz 1983; Jennersten and Morse 1991; Guitian et al. 1993;  
337 Navarro 1999), but not with others in which no differences have been found (McMullen  
338 2009) or where a higher fruit set in nocturnally pollinated plants compared to diurnally  
339 pollinated plants has been reported (Martinell et al. 2010).  
340 There was no difference in fruit set among plant sections in either control or selfed  
341 plants, suggesting absence of competition among sections or maternal constraints, and

342 uniform pollination in *E. simplex*, unlike most studies of reproductive success patterns  
343 in inflorescences (Diggle 1995 for a review; Tremblay 2006; Torices and Méndez  
344 2010). It is generally thought that perennial monocarpic species use stored reserves for  
345 fruit development rather than resources obtained during the flowering season, even  
346 more so than annually fruiting species (Stephenson 1981; Udovic and Aker 1981).  
347 However, day- and night-pollinated plants showed a fruit production pattern indicating  
348 non-uniform pollination (Karoly 1992; Kudo et al. 2001; Tremblay 2006). Some studies  
349 (Lee 1988; Tremblay 2006) have reported higher reproductive success in bottom  
350 positions due to the behaviour of pollinators; these move distally upward on  
351 inflorescences, may become satiated with the resources and thus may leave the plant  
352 before visiting the upper flowers; alternatively, the bottom of the inflorescence may be  
353 more likely than the distal parts to receive cross pollen. We found that diurnally  
354 pollinated plants set more fruits in upper inflorescence sections. One explanation might  
355 be that if diurnal insects (mostly bees) do indeed move upwards, upper positions may  
356 avoid stigma clogging to some extent (Brown and Mitchell 2001) if *E. simplex*  
357 competes with other co-flowering plants for pollinators. By contrast, other studies found  
358 higher pollen deposition in the upper flowers of inflorescences, with no relation to  
359 directional pollinator foraging and bee preference for higher flowers (Roubik et al.  
360 1982; Lortie and Aarssen 1999). The deposition of large amounts of self-pollen,  
361 however, may also clog up the stigma and prevent effective pollination (Kikuzawa  
362 1989; Thomson 1989b).  
363 Nocturnally pollinated plants were found to set less fruits in intermediate compared to  
364 bottom and upper parts. The presence of *Alloxantha* sp. consuming the pollen in  
365 intermediate sections might reduce final reproductive success; previous studies have

366 documented beetles reducing plant fitness due to pollen consumption (Kevan and Baker  
367 1983).

368 Diurnally pollinated plants set more seeds per fruit than control plants. This finding in  
369 *E. simplex* is consistent with studies by Jennersten (1988) and Martinell et al. (2010)  
370 who found higher or equal seed set in controls and day-pollinated plants. However, the  
371 reduced seed set in control plants may be compensated by the greater fruit production  
372 Although some studies also found higher seed set in diurnally compared with  
373 nocturnally pollinated flowers (Jennersten 1988), most studies actually found the  
374 opposite (Jürgens et al. 1996; Groman and Pellmyr 1999; Young 2002; Martinell et al.  
375 2010) or no effect (Jennersten and Morse 1991). In addition, no differences were  
376 detected between seed set of nocturnal and selfed fruits, indicating a low effectiveness  
377 of nocturnal pollinators, as documented by Jennersten (1988) for *Viscaria vulgaris*.  
378 Seeds of *E. simplex* coming from selfed flowers were heavier than those resulting from  
379 cross-pollination. The reason is that the former have a thicker coat, whilst embryo size  
380 is similar between the two treatments (Jaca et al. 2019). Comparing seed weight  
381 between inflorescence sections, bottom seeds were lighter than upper and intermediate  
382 ones. This contrast with other studies that have found basal seeds to be heavier (Byrne  
383 and Mazer 1990; Navarro 1996; Vallius 2000).

384 In accordance with findings from other germination studies (Schemske 1983; Navarro  
385 and Guitián 2002), heavy seeds showed higher germinability and germinated faster than  
386 light ones in all treatments, except those from the bottom sections of inflorescences. The  
387 thicker seed coat produced by selfed flowers is probably what slows germination  
388 (Crocker 1906; Miyoshi and Mii 1988). Indeed, this was previously reported in at least  
389 one species, *Sinapis arvensis* (Paolini et al. 2001).

390



391 ***Concluding remarks***—

392 Despite the relatively abundant literature on nocturnal vs. diurnal pollination, and on  
393 fruiting patterns along the inflorescences, this is the first study that examined both  
394 effects simultaneously. We found that *E. simplex* was visited at night -mainly by moths  
395 and beetles-, but at lower rates than during the day. The exclusion experiment indicated  
396 that fruiting patterns along the inflorescences in open-pollinated plants show no  
397 variation, but nocturnally and diurnally exposed plants do. This indicates different  
398 visiting behaviour between nocturnal and diurnal pollinators generating  
399 complementarity effects in *E. simplex* pollination services. Both germinability and  
400 germination rate were influenced by seed weight, inflorescence section and pollination  
401 treatment.

402

403 **Acknowledgements**

404 The authors thank Marcos Báez and Antonio Pérez Delgado for insect identification,  
405 Benito Pérez Vispo for his technical assistance in the field, and Juana Pérez López for  
406 providing logistical support in Chamorga. We are also grateful to Servicio  
407 Administrativo de Medio Ambiente, Excmo. Cabildo Insular de Tenerife for permission  
408 (2016-01704) to work in Anaga Biosphere Reserve, Tenerife. The company Tagoro  
409 Medioambiente provided its greenhouse to perform the seedling emergence  
410 experiments; Manuel Martín helped us in the follow-up of the experiment. Julia Jaca  
411 was funded by a predoctoral fellowship from the Ministerio de Educación, Cultura y  
412 Deporte (FPU13/05880) and by the unemployment benefit from the Ministerio de  
413 Trabajo, Migraciones y Seguridad Social. The study was framed within a project  
414 financed by the Ministerio de Economía, Industria y Competitividad (CGL2017-88122-  
415 P) to Anna Traveset.

416

417

418

419 **Conflict of Interest**

420 The authors declare that they have no conflict of interest.

421

422 **References**

423 Baker HG (1961) The adaptation of flowering plants to nocturnal and crepuscular  
424 pollinators. *Q Rev Biol* 36:64–73

425 Berry PE, Calvo RN (1991) Pollinator limitation and position dependent fruit set in the  
426 high Andean orchid *Myrosmodes cochleare* (Orchidaceae). *Plant Syst Evol*  
427 174:93–101.

428 Bertin RI, Willson MF (1980) Effectiveness of diurnal and nocturnal pollination of two  
429 milkweeds. *Can J Bot* 58:1744–1746.

430 Brantjes NBM, Leemans JAAM (1976) *Silene otites* (Caryophyllaceae) pollinated by  
431 nocturnal lepidoptera and mosquitoes. *Acta Bot Neerl* 25:281–295

432 Brown BJ, Mitchell RJ (2001) Competition for pollination: effects of pollen of an  
433 invasive plant on seed set of a native congener. *Oecologia* 129:43–49.

434 Byrne M, Mazer SJ (1990) The effect of position on fruit and of yield in relationships  
435 among components of yield in *Phytolacca rivinoides* (Phytolaccaceae). *Biotropica*  
436 22:353–365

437 Crocker W (1906) Role of seed coats in delayed germination. Contributions from the  
438 Hull Botanical Laboratory. LXXXV. *Bot Gaz* 42:265–291

439 Crumb SE, Eide PM, Bonn AE (1941) The European earwig. *USDA Tech Bull* 766:76.

440 Diggle PK (1995) Architectural effects and the interpretation of patterns of fruit and

441 seed development. *Annu Rev Ecol Syst* 26:531–552

442 Ehrlén J (1992) Proximate limits to seed production in a herbaceous perennial legume,  
443 *Lathyrus vernus*. *Ecology* 73:1820–1831

444 Ehrlén J (1993) Ultimate functions of non-fruiting flowers in *Lathyrus vernus*. *Oikos*  
445 68:45–52

446 Elam DR, Linhart YB (1988) Pollination and seed production in *Ipomopsis aggregata*:  
447 differences among and within Flower color morphs. *Am J Bot* 75:1262–1274.

448 Faegri K, van der Pijl L (1971) The principles of pollination ecology. Pergamon Press,  
449 New York

450 Faegri K, van der Pijl L (1966) The Principles of Pollination Ecology. Pergamon Press,  
451 Oxford, UK

452 Fenster CB, Armbruster WS, Wilson P, et al (2004) Pollination syndromes and floral  
453 specialization. *Annu Rev Ecol Evol Syst* 35:375–403

454 Ghazoul J (1997) The pollination and breeding system of *Dipterocarpus obtusifolius*  
455 (Dipterocarpaceae) in dry deciduous forests of Thailand. *J Nat Hist* 31:901–916

456 Goldingay RL, Whelan RJ (1993) The influence of pollinators on fruit positioning in  
457 the Australian shrub *Telopea speciosissima* (Proteaceae). *Oikos* 68:501–509

458 Groman JD, Pellmyr O (1999) The pollination biology of *Manfreda virginica*  
459 (Agavaceae): Relative contribution of diurnal and nocturnal visitors. *Oikos* 87:373

460 Guillou H, Carracedo JC, Paris R, Torrado FJP (2004) Implications for the early shield-  
461 stage evolution of Tenerife from K/Ar ages and magnetic stratigraphy. *Earth Planet*  
462 *Sci Lett* 222:599–614.

463 Guitián J (1994) Selective fruit abortion in *Prunus mahaleb* (Rosaceae). *Am J Bot*  
464 81:1555–1558.

465 Guitián J, Navarro L (1996) Allocation of reproductive resources within inflorescences

466 of *Petrocoptis grandiflora* (Caryophyllaceae). Can J Bot 74:1482–1486

467 Guitian P, Guitian J, Navarro L (1993) Pollen transfer and diurnal versus nocturnal  
468 pollination in *Lonicera etrusca*. Acta Oecologica 14:219–227

469 Haber WA, Frankie GW (1989) A tropical hawkmoth community: Costa Rican dry  
470 forest sphingidae. Biotropica 21:155–172

471 Herrera J (1991) Allocation of reproductive resources within and among inflorescences  
472 of *Lavandula stoechas* (Lamiaceae). Am J Bot 78:789–794.

473 Jaca J, Nogales M, Traveset A (2018) Reproductive success of the Canarian *Echium*  
474 *simplex* (Boraginaceae) mediated by vertebrates and insects. Plant Biol.

475 Jennersten O (1988) Pollination of *Viscaria vulgaris* (Caryophyllaceae): The  
476 contributions of diurnal and nocturnal insects to seed set and seed predation. Oikos  
477 52:319–327

478 Jennersten O, Morse DH (1991) The quality of pollination by diurnal and nocturnal  
479 insects visiting common milkweed, *Asclepias syriaca*. Am Midl Nat 125:18–28.

480 Jürgens A, Witt T, Gottsberger G (2002) Flower scent composition in night-flowering  
481 *Silene* species (Caryophyllaceae). Biochem Syst Ecol 30:383–397.

482 Jürgens A, Witt T, Gottsberger G (1996) Reproduction and pollination in Central  
483 European populations of *Silene* and *Saponaria* species. Bot Acta 109:316–324

484 Karoly K (1992) Pollinator limitation in the facultatively autogamous annual, *Lupinus*  
485 *nanus* (Leguminosae). Am J Bot 79:49–56

486 Kevan PG, Baker HG (1983) Insects as flower visitors and pollinators. Annu Rev  
487 Entomol 28:407–453.

488 Kikuzawa K (1989) Floral biology and evolution of gynodioecism in *Daphne*  
489 *kamtchatica* var. *jezoensis*. Oikos 56:196

490 Klein AM, Hendrix SD, Clough Y, et al (2015) Interacting effects of pollination, water

491 and nutrients on fruit tree performance. *Plant Biol* 17:201–208

492 Knop E, Zoller L, Ryser R, et al (2017) Artificial light at night as a new threat to  
493 pollination. *Nature* 548:206–209

494 Kudo G, Maeda T, Narita K (2001) Variation in floral sex allocation and reproductive  
495 success within inflorescences of *Corydalis ambigua* (Fumariaceae): pollination  
496 efficiency of resource limitation? *J Ecol* 89:48–56.

497 Lee TL (1988) Patterns of fruit and seed production. In: Lovett Doust J, Lovett Doust L  
498 (eds) *Plant Reproductive Ecology: Patterns and Strategies*. Oxford University  
499 Press, New York, USA, pp 179–202

500 Lortie CJ, Aarssen LW (1999) The advantage of being tall: Higher flowers receive more  
501 pollen in *Verbascum thapsus* L. (Scrophulariaceae). *Ecoscience* 6:68–71.

502 Lunau K, Maier EJ (1995) Innate colour preferences of flower visitors. *J Comp Physiol*  
503 A 177:1–19

504 Macgregor CJ, Kitson JJN, Fox R, et al (2018) Construction, validation, and application  
505 of nocturnal pollen transport networks in an agro-ecosystem: a comparison using  
506 light microscopy and DNA metabarcoding. *Ecol Entomol*

507 Martinell CC, Dötterl S, Blanché C, et al (2010) Nocturnal pollination of the endemic  
508 *Silene sennenii* (Caryophyllaceae): An endangered mutualism? *Plant Ecol*  
509 211:203–218.

510 McMullen CK (2009) Pollination biology of a night-flowering Galápagos endemic,  
511 *Ipomoea habeliana* (Convolvulaceae). *Bot J Linn Soc* 160:11–20.

512 Miyoshi K, Mii M (1988) Ultrasonic treatment for enhancing seed germination of  
513 terrestrial orchid, *Calanthe discolor*, in asymbiotic culture. *Sci Hortic*  
514 (Amsterdam) 35:127–130.

515 Moreno JC (2008) Lista Roja 2008 de la Flora Vascular Española. Dirección General de

516 Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural  
517 y Marino, y Sociedad Española de Biología de la Conservación de Plantas),  
518 Madrid, Spain

519 Morse DH, Fritz RS (1983) Contributions of diurnal and nocturnal insects to the  
520 pollination of common milkweed (*Asclepias syriaca* L.) in a pollen-limited system.  
521 *Oecologia* 60:190–197

522 Nassar JM, Ramírez N, Linares O (1997) Comparative pollination biology of  
523 Venezuelan columnar cacti and the role of nectar-feeding bats in their sexual  
524 reproduction. *Am J Bot* 84:918–927.

525 Navarro L (1996) Fruit-set and seed weight variation in *Anthyllis vulneraria* subsp.  
526 *vulgaris* (Fabaceae). *Plant Syst Evol* 201:139–148.

527 Navarro L (1999) Pollination ecology and effect of nectar removal in *Macleania bullata*  
528 (Ericaceae). *Biotropica* 31:618–625.

529 Navarro L, Guitián J (2002) The role of floral biology and breeding system on the  
530 reproductive success of the narrow endemic *Petrocoptis viscosa* rothm.  
531 (Caryophyllaceae). *Biol Conserv* 103:125–132.

532 Paolini R, Bàrberi P, Rocchi C (2001) The effect of seed mass, seed colour, pre-chilling  
533 and light on the germination of *Sinapis arvensis* L. *Ital J Agron* 5:39–46

534 R Core Team (2018) R: A language and environment for statistical computing

535 Raguso RA (2008) Wake up and smell the roses: the ecology and evolution of floral  
536 scent. *Annu Rev Ecol Evol Syst* 39:549–569

537 Reynolds RJ, Westbrook MJ, Rohde AS, et al (2009) Pollinator specialization and  
538 pollination syndromes of three related North American *Silene*. *Ecology* 90:2077–  
539 2087

540 Roubik DW, Ackerman JD, Copenhaver C, Smith BH (1982) Stratum, tree, and flower

541 selection by tropical bees: implications for the reproductive biology of outcrossing  
542 *Cochlospermum vitifolium* in Panama. *Ecology* 63:712–720.

543 Sahley CT (1996) Bat and hummingbird pollination of an autotetraploid columnar  
544 cactus, *Weberbauerocereus weberbaueri* (Cactaceae). *Am J Bot* 83:1329–1336.

545 Schemske DW (1983) Breeding system and habitat effects on fitness components in  
546 three Neotropical *Costus* (Zingiberaceae). *Evolution* (N Y) 37:523–539

547 Schemske DW, Horvitz CC (1984) Variation among floral visitors in pollination ability:  
548 a precondition for mutualism specialization. *Science* (80- ) 225:519–521

549 Schneemilch M, Williams C, Kokkinn M (2011) Floral visitation in the Australian  
550 native shrub genus *Acrotriche* R.Br (Ericaceae): An abundance of ants  
551 (Formicidae). *Aust J Entomol* 50:130–138.

552 Solomon BP (1988) Patterns of pre- and postfertilization resource allocation within an  
553 inflorescence: Evidence for interovary competition. *Am J Bot* 75:1074–1079.

554 Stephenson AG (1981) Flower and fruit abortion : proximate causes and ultimate  
555 functions. *Annu Rev Ecol Syst* 12:253–279

556 Stephenson AG, Thomas WW (1977) Diurnal and nocturnal pollination of *Catalpa*  
557 *speciosa* (Bignoniaceae). *Syst Bot* 2:191–198

558 Stöcklin J, Lenzin H (2013) *Echium simplex*, ein seltener Schopfrosettenbaum auf  
559 Teneriffa. *Bauhinia* 24:23–37

560 Sutherland S (1987) Why hermaphroditic plants produce many more flowers than fruits:  
561 experimental tests with *Agave mackelveyana*. *Evolution* (N Y) 41:750–759

562 Thien LB (1980) Patterns of pollination in the primitive angiosperms. *Biotropica* 12:1–  
563 13

564 Thompson JN, Pellmyr O (1992) Mutualism with pollinating seed parasites amid co-  
565 pollinators : constraints on specialization. *Ecology* 73:1780–1791

- 566 Thomson JD (1989a) Deployment of ovules and pollen among flowers within  
567 inflorescences. *Evol Trends Plants* 3:65–68
- 568 Thomson JD (1989b) Germination schedules of pollen grains : Implications for pollen  
569 selection. *Evolution* (N Y) 43:220–223
- 570 Torices R, Méndez M (2010) Fruit size decline from the margin to the center of capitula  
571 is the result of resource competition and architectural constraints. *Oecologia*  
572 164:949–958
- 573 Tremblay RL (2006) The effect of flower position on male and female reproductive  
574 success in a deceptively pollinated tropical orchid. *Bot J Linn Soc* 151:405–410.
- 575 Udovic D, Aker C (1981) Fruit abortion and the regulation of fruit number in *Yucca*  
576 *whipplei*. *Oecologia* 49:245–248
- 577 Vallius E (2000) Position-dependent reproductive success of flowers in *Dactylorhiza*  
578 *maculata* (Orchidaceae). *Funct Ecol* 14:573–579
- 579 Van Doorn WG, Van Meeteren U (2003) Flower opening and closure: a review. *J Exp*  
580 *Bot* 54:1801–1812
- 581 von Helversen O, Winter Y (2003) Glossophagine bats and their flowers: costs and  
582 benefits for plant and pollinators. In: Kunz TH, Fenton MB (eds) *Bat ecology*. The  
583 University of Chicago Press, Chicago, pp 346–397
- 584 Waser NM (1982) A comparison of distances flown by different visitors to flowers of  
585 the same species. *Oecologia* 55:251–257
- 586 Waser NM, Chittka L, Price M V., et al (1996) Generalization in pollination systems,  
587 and why it matters. *Ecology* 77:1043–1060
- 588 Wyatt R (1982) Inflorescence architecture: how flower number, arrangement, and  
589 phenology affect pollination and fruit-set. *Am J Bot* 69:585–594.
- 590 Young HJ (2002) Diurnal and nocturnal pollination of *Silene alba* (Caryophyllaceae).





[Click here to view linked References](#)

Table 1: Number of visits per inflorescence section by each visitor group

Visitor group	No. Visits	Position within the inflorescence		
		Upper	Intermediate	Bottom
Lepidoptera (at least 10 moth morphospecies)	69	41	23	5
Coleoptera (mainly <i>Alloxantha</i> sp)	19	0	8	11
<i>Chrysoperla carnea</i> (Neuroptera)	1	0	1	0
<i>Phyllodromica brullei</i> (Blattaria)	1	0	0	1
<i>Guanchia</i> sp (Dermaptera)	4	0	1	3
<i>Ommatouilus moreletii</i> (Julidae)	3	0	0	3

[Click here to view linked References](#)

Figure 1. Flower night visitation rate (visits  $\cdot$  h<sup>-1</sup>  $\cdot$  flower<sup>-1</sup>) of *E. simplex* by different insect groups (CO: coleoptera, mainly *Alloxantha* sp., LE: lepidoptera). Letters indicate significant differences using Tukey's test after GLM.

Figure 2. Mean (+SE) proportion of flowers that set fruit per pollination treatment and inflorescence section. Numbers inside each bar are sample sizes (number of cymes). Lower case letters indicate significant differences between inflorescence sections within each treatment, and capital letters indicate significant differences between treatments within inflorescence sections using Tukey's test after GLM.

Figure 3. Mean (+SE) number of seeds per fruit for each pollination treatment. Numbers inside each bar are fruit sample sizes. Letters indicate significant differences using Tukey's test after GLM.

Figure 4. Mean (+SE) seed weight per (a) pollination treatment and (b) inflorescence section. Numbers in each bar are sample sizes. Letters indicate significant differences using Tukey's test after GLM.

Figure 5. GLMM predicted probabilities and confidence intervals of germinability along seed weight for each exclusion treatment and inflorescence section. Numbers besides each line are sample sizes.

Figure 6. GLMM predicted probabilities and confidence intervals of germination rate according to seed weight for each exclusion treatment and inflorescence section.

Numbers beside each line are sample sizes

Figure 1

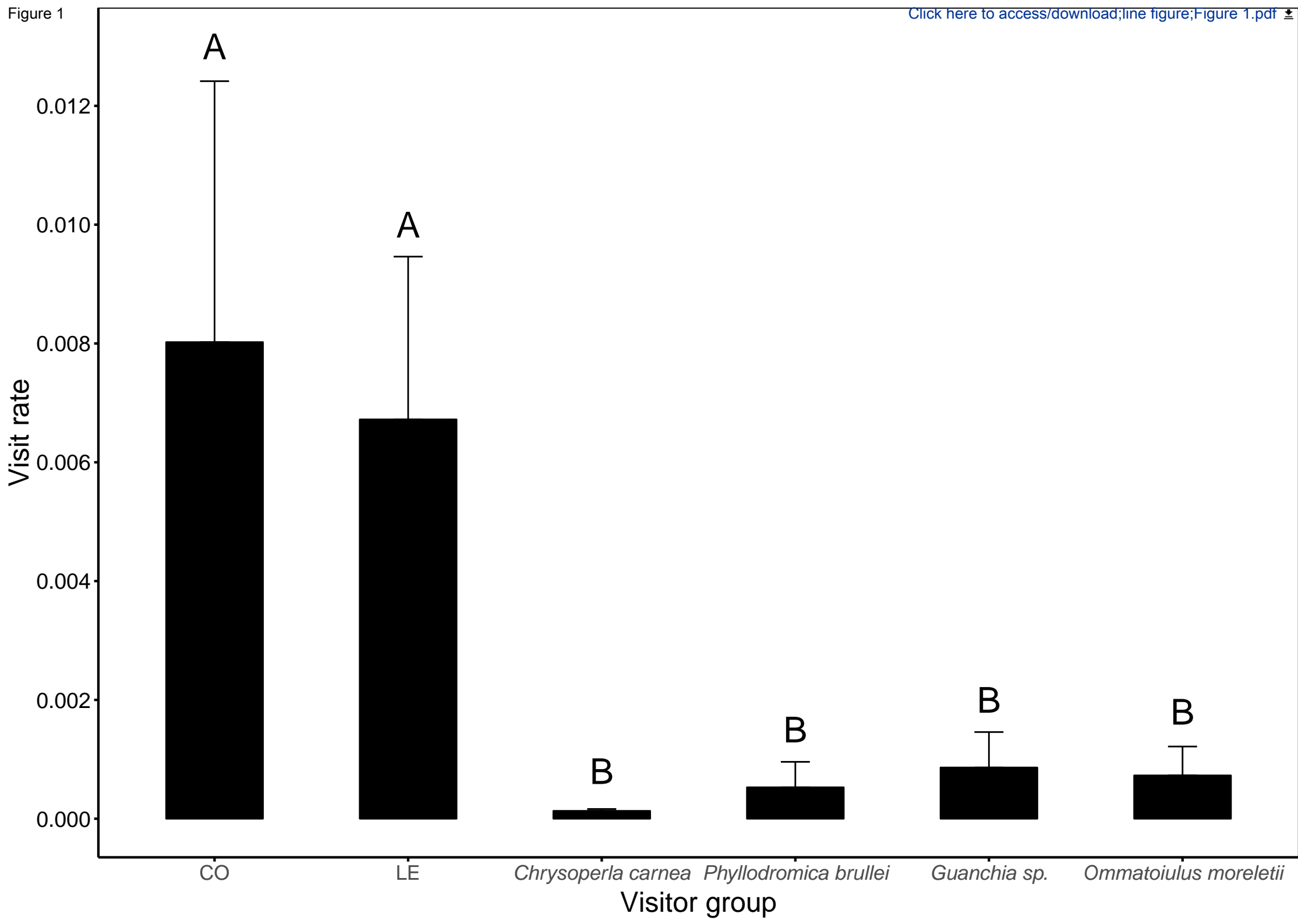


Figure 2

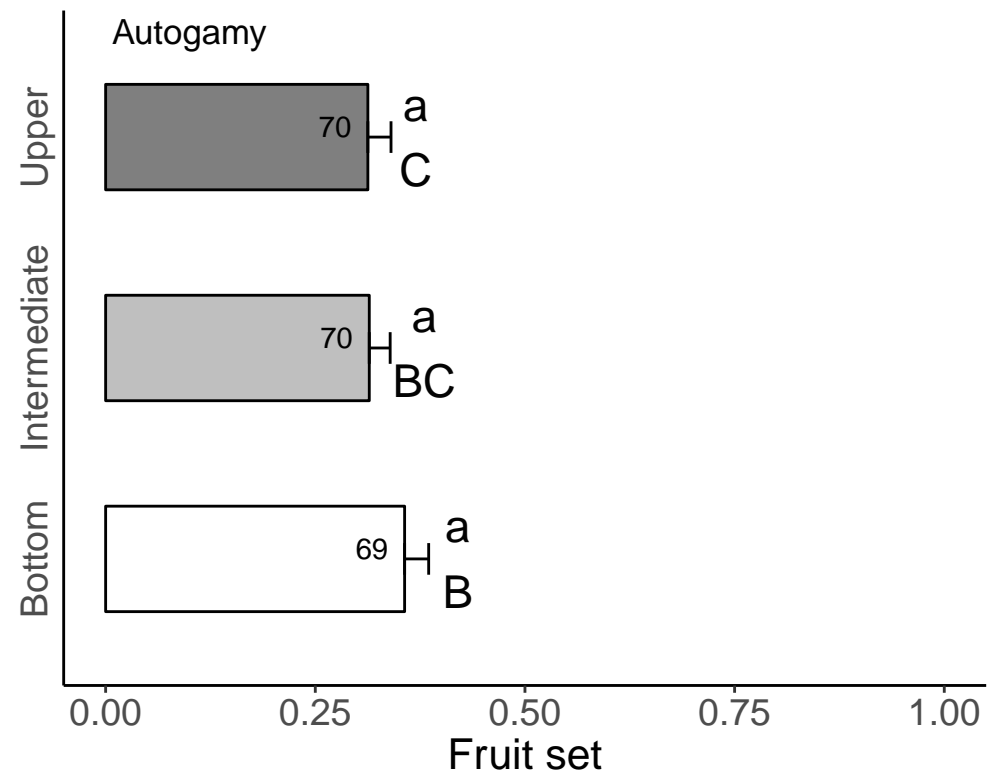
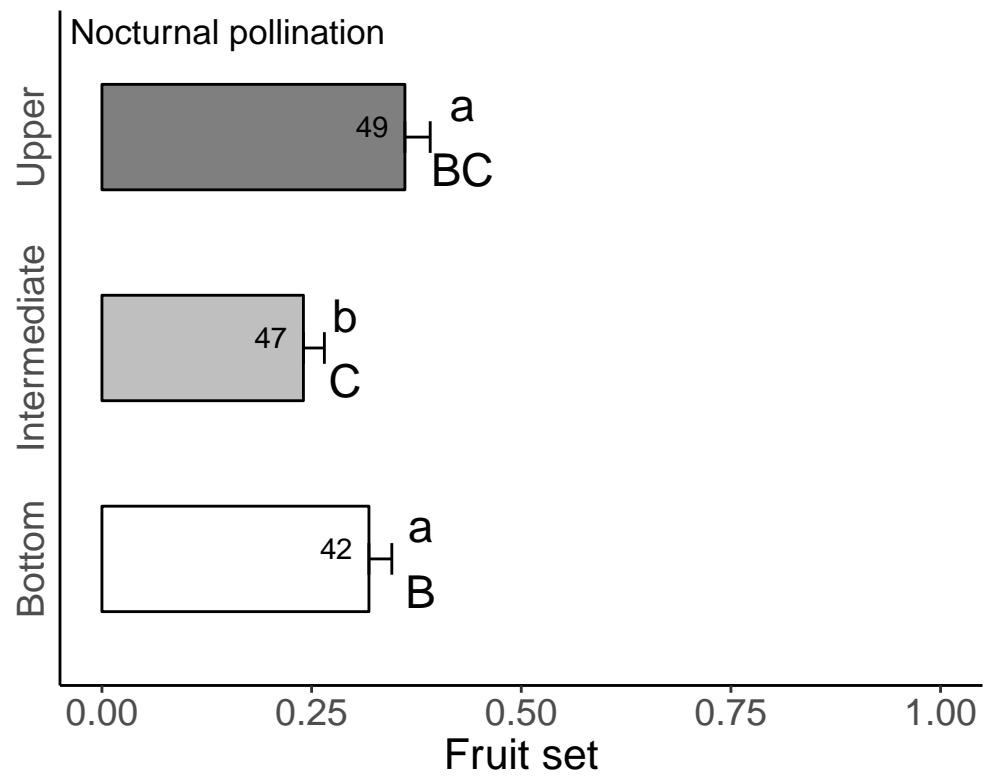
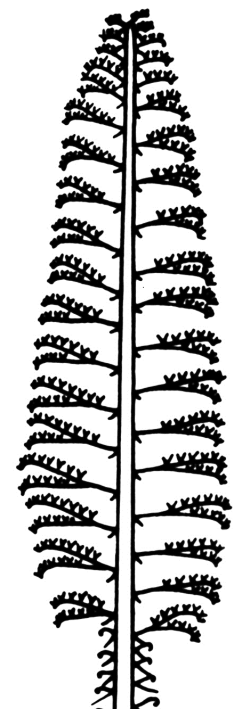
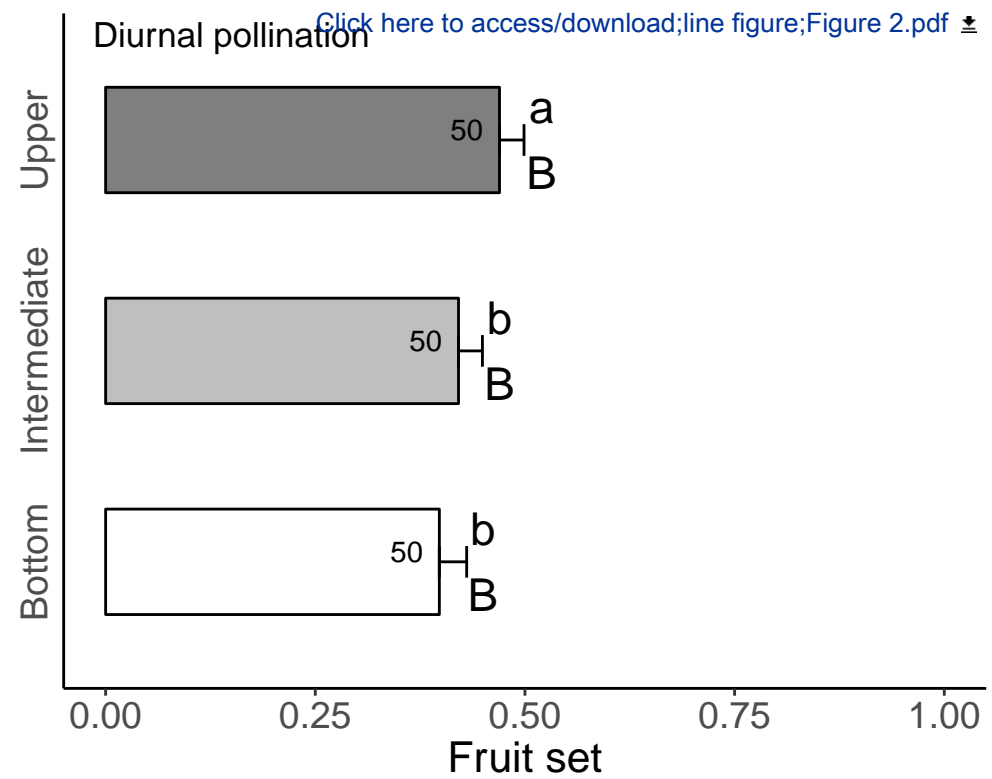
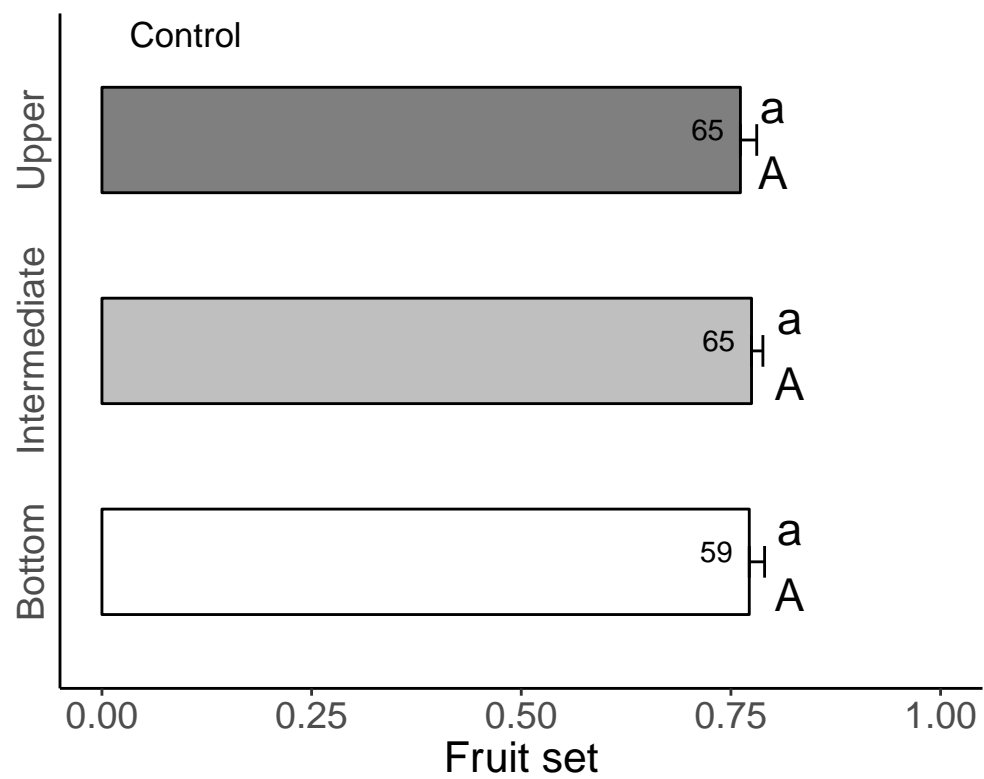
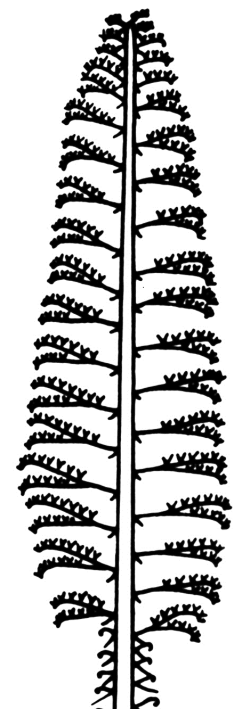
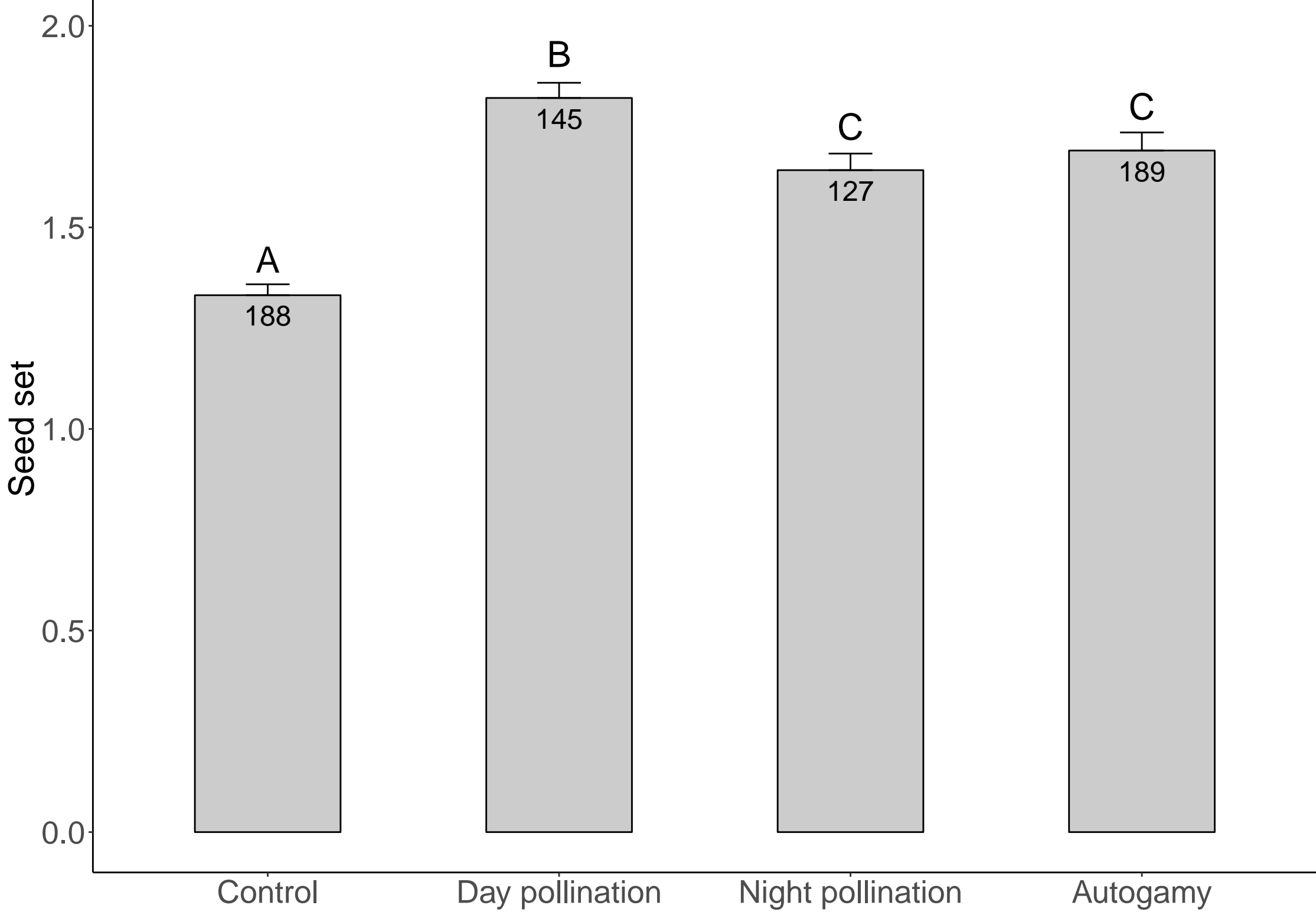
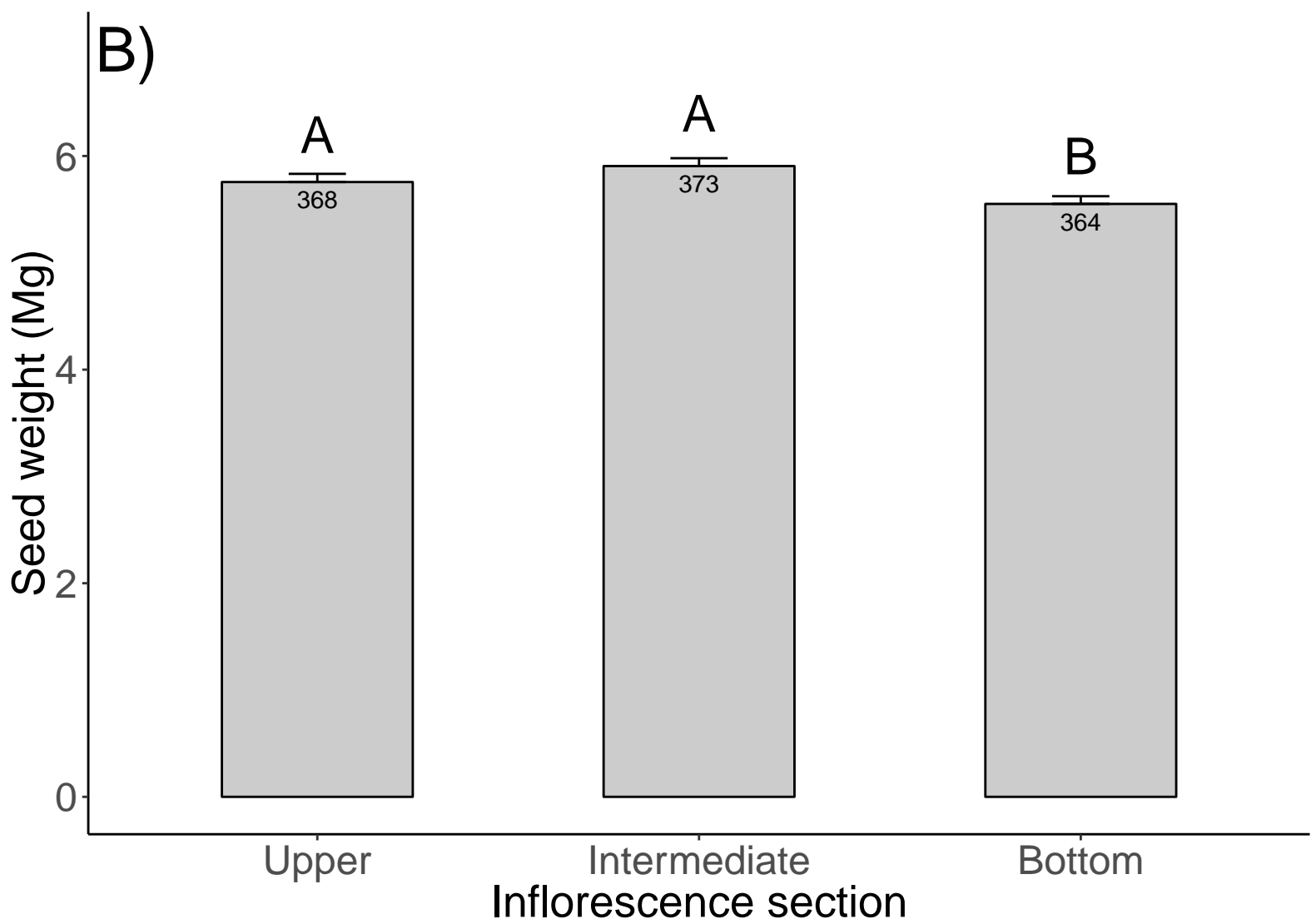
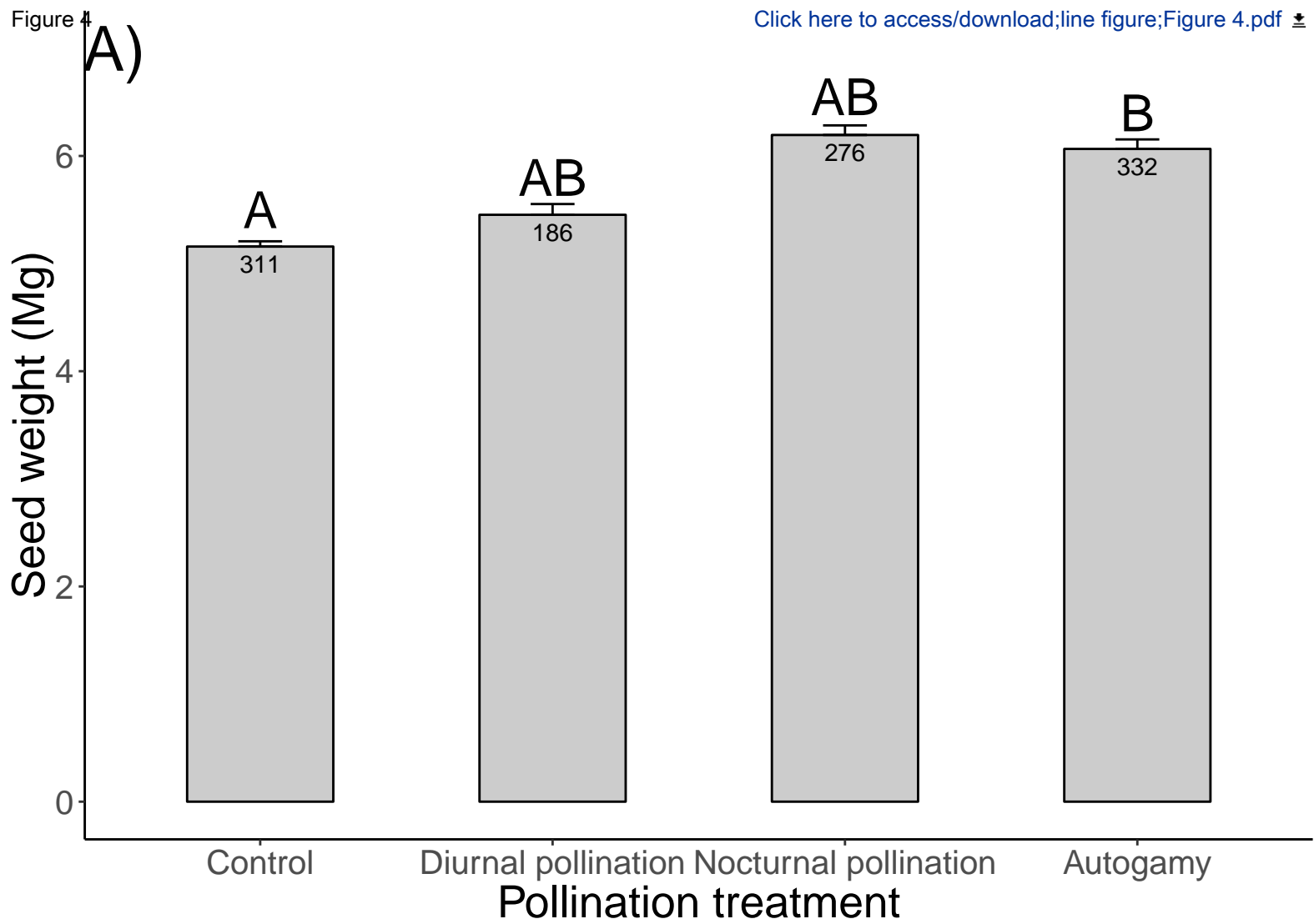
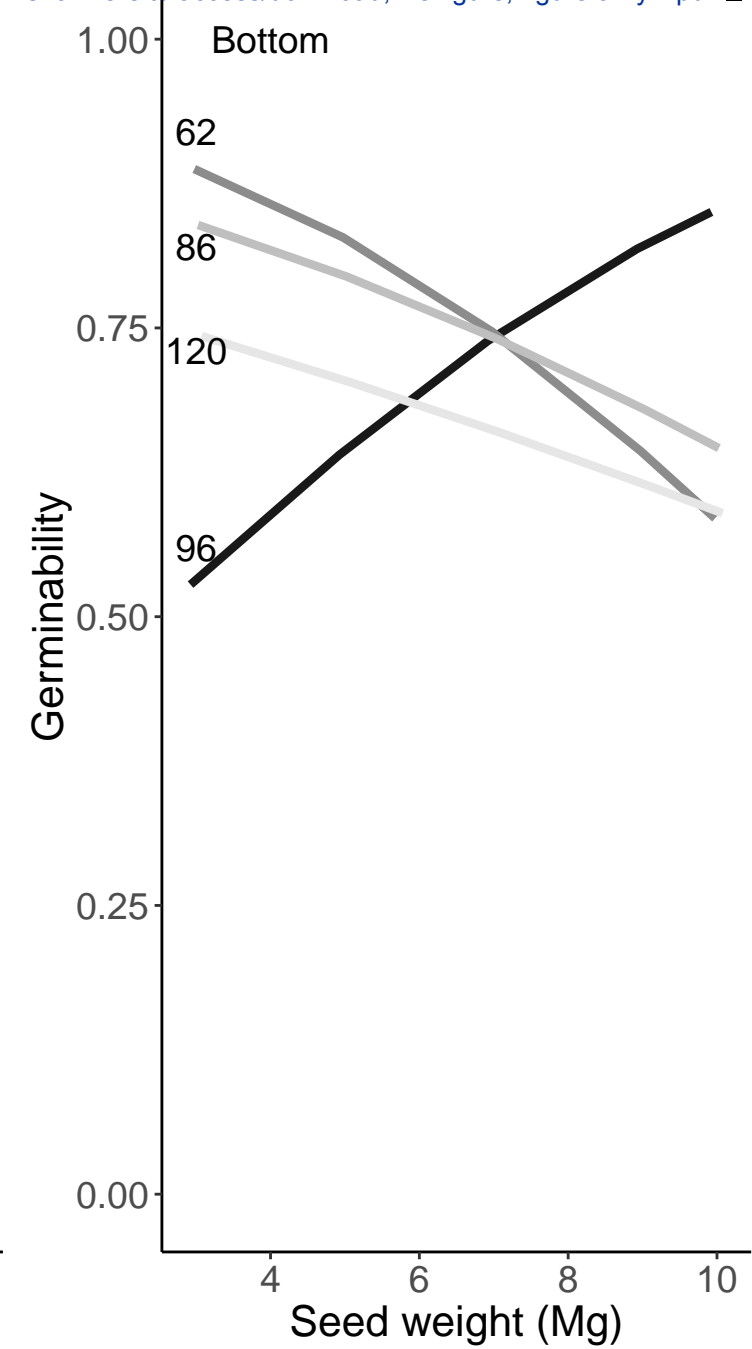
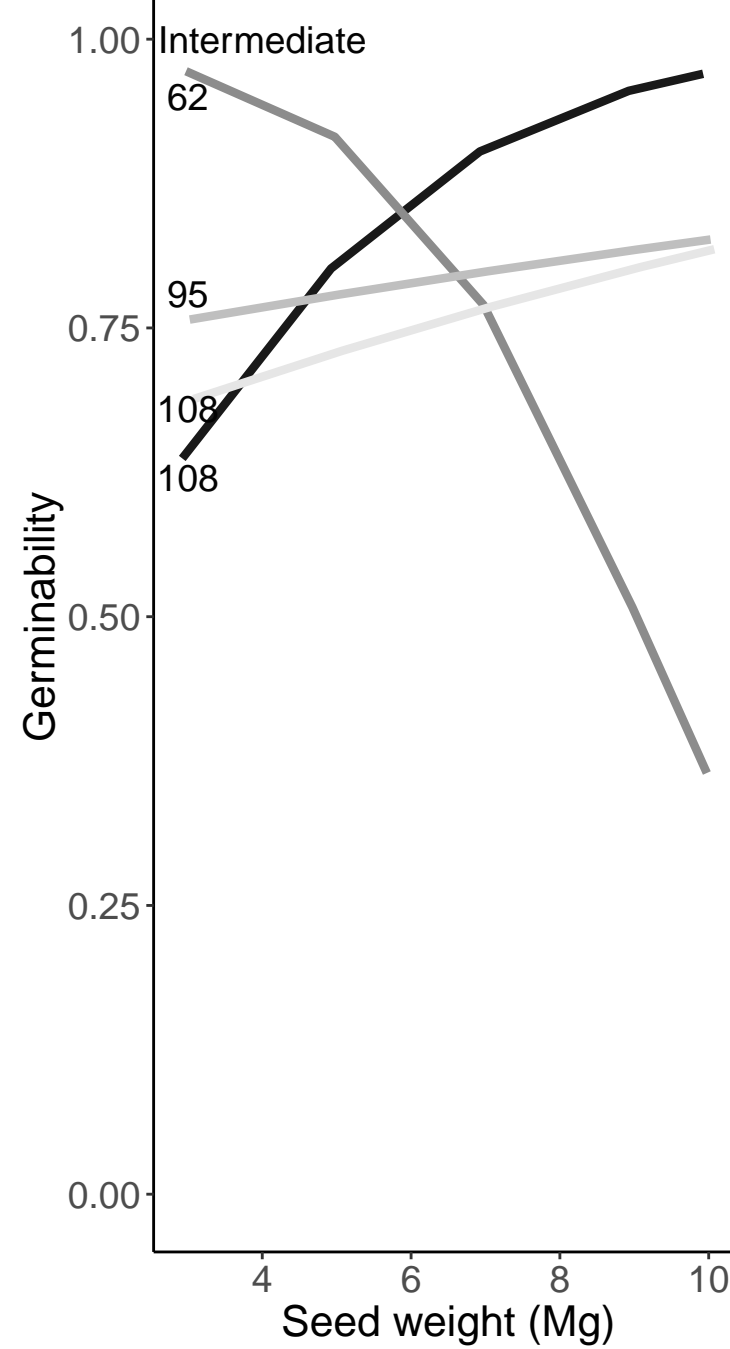
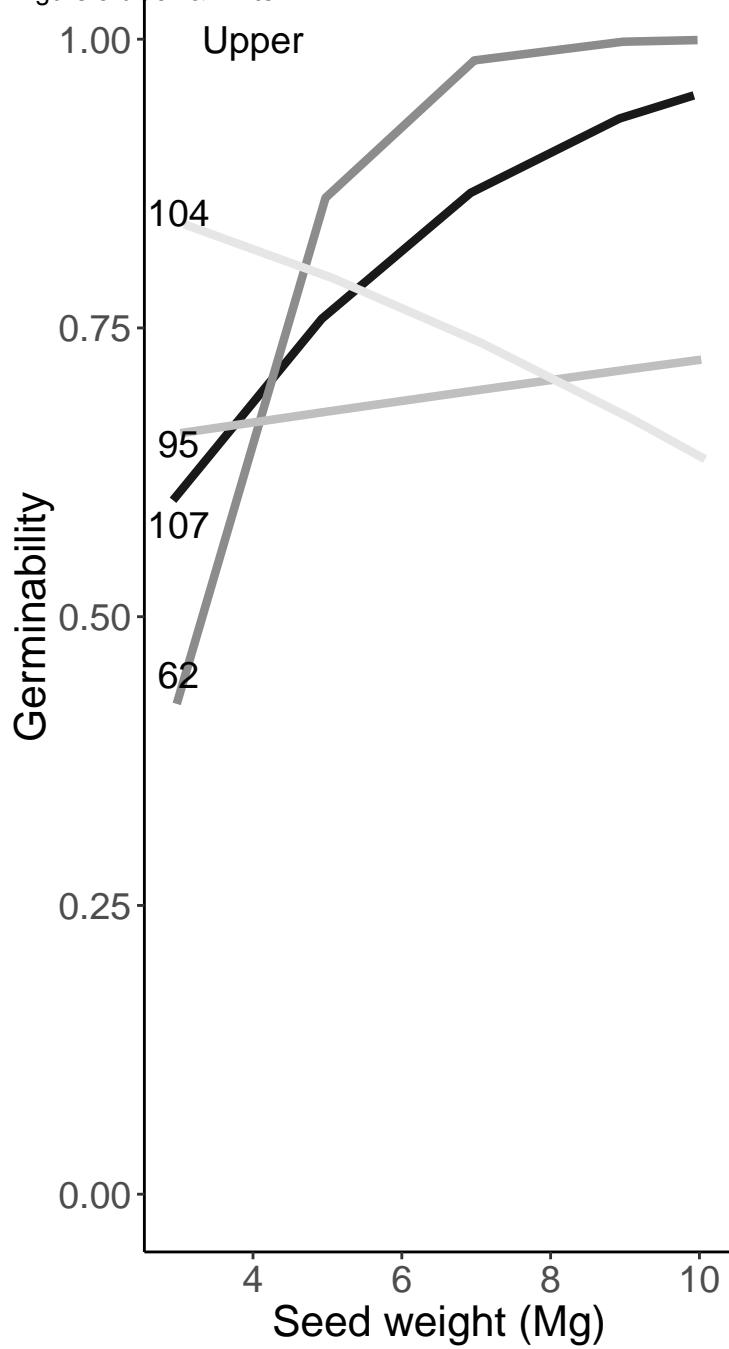


Figure 3





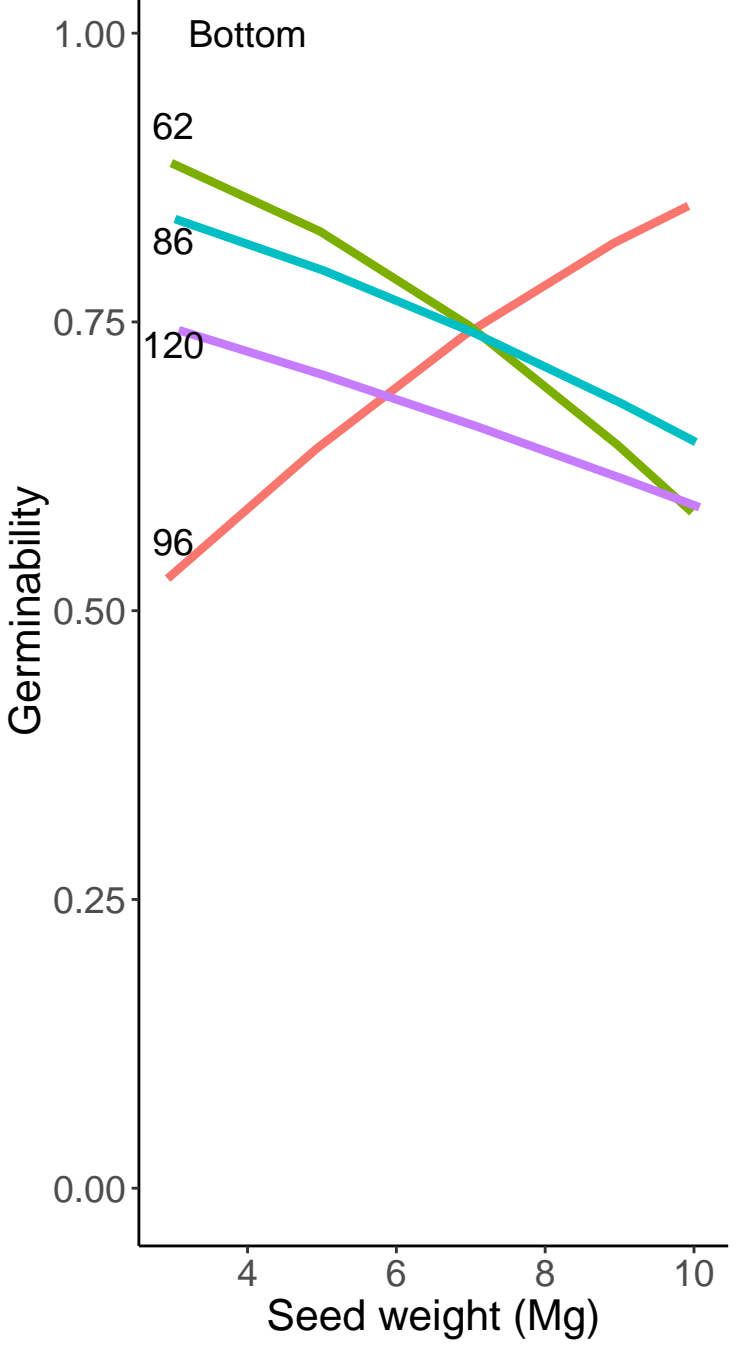
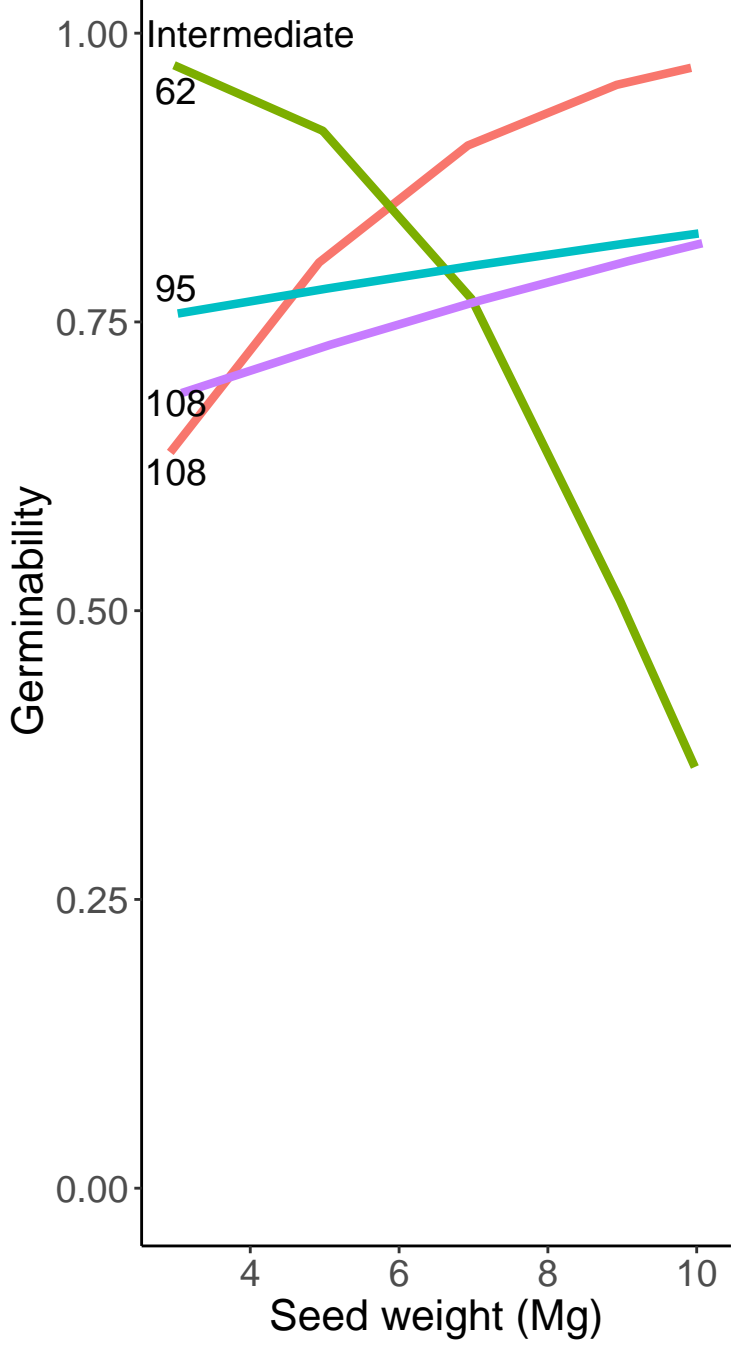
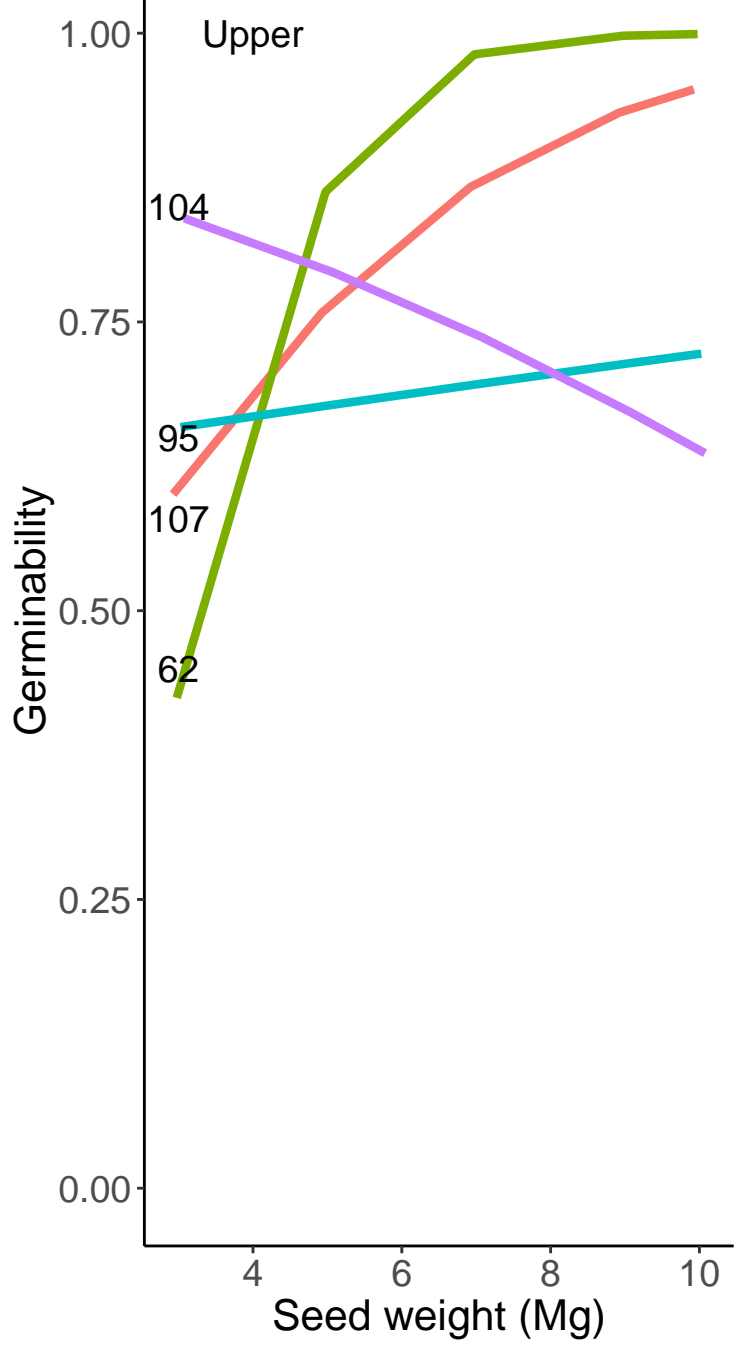




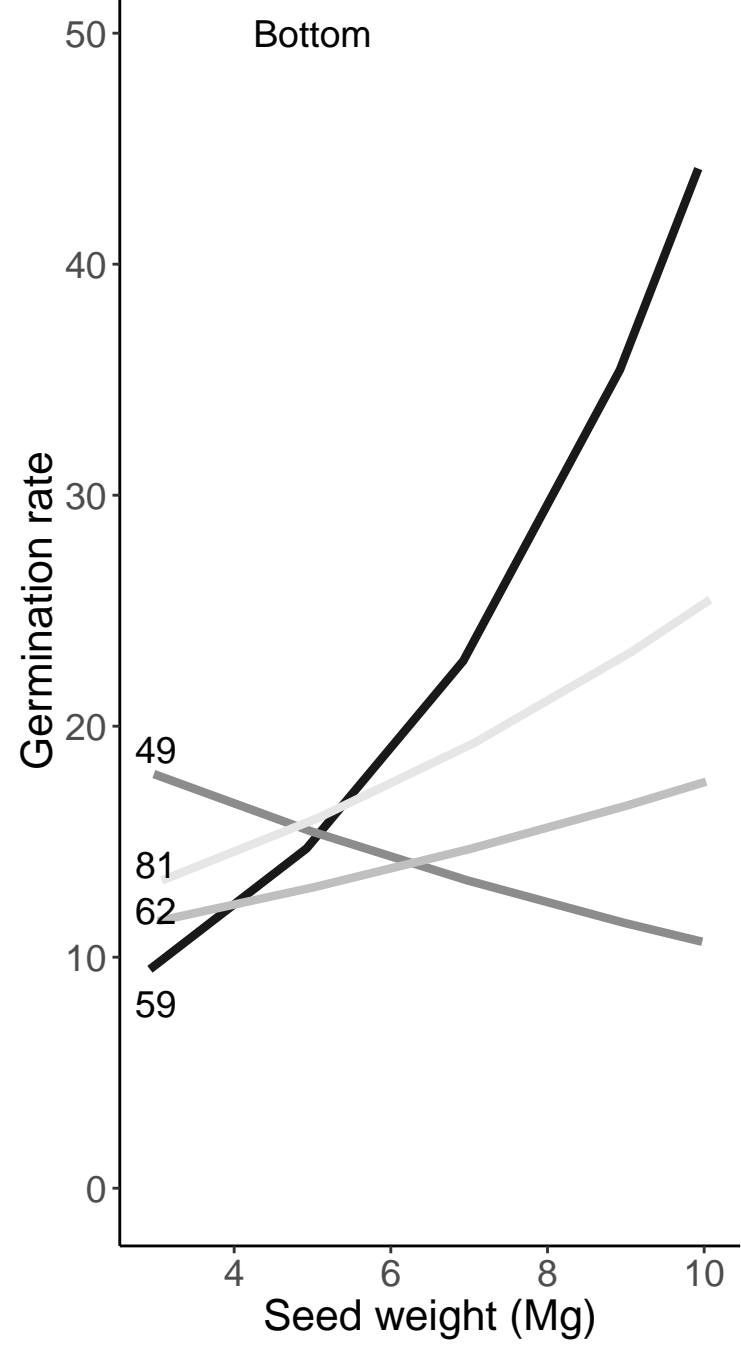
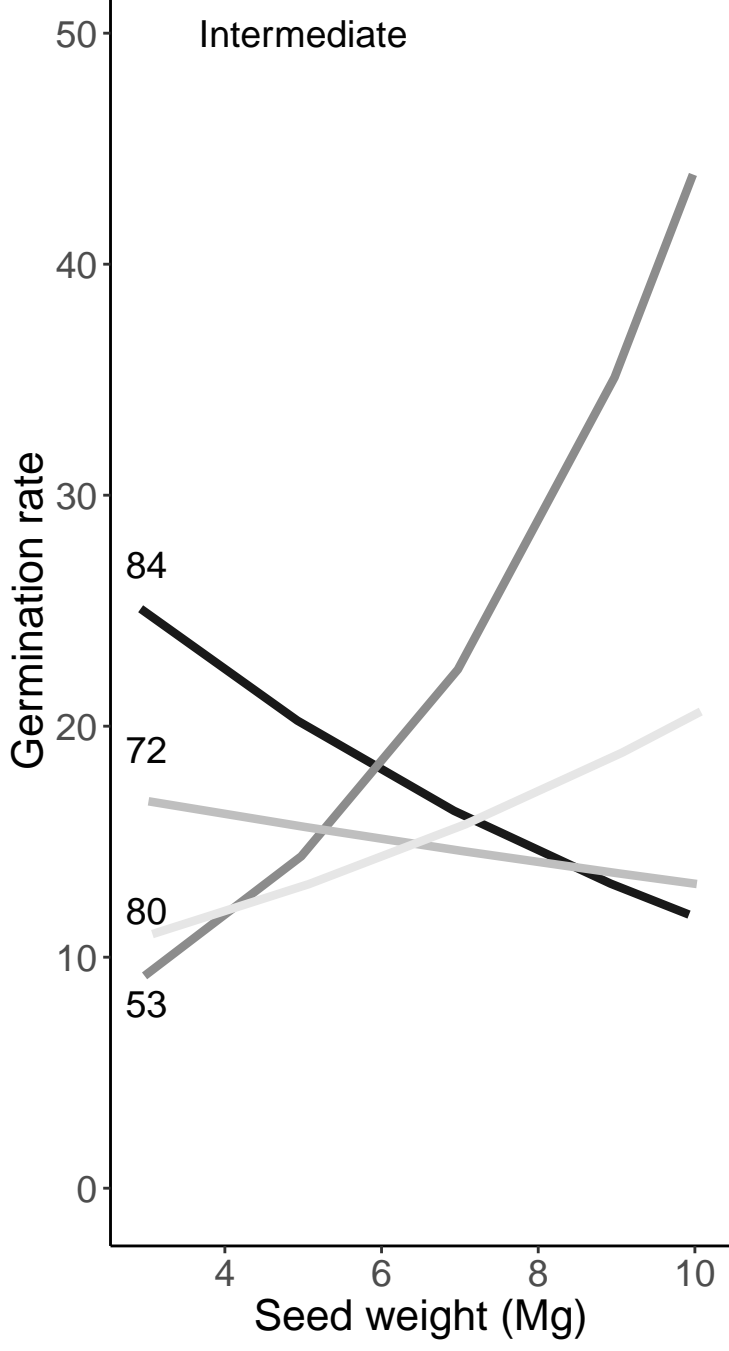
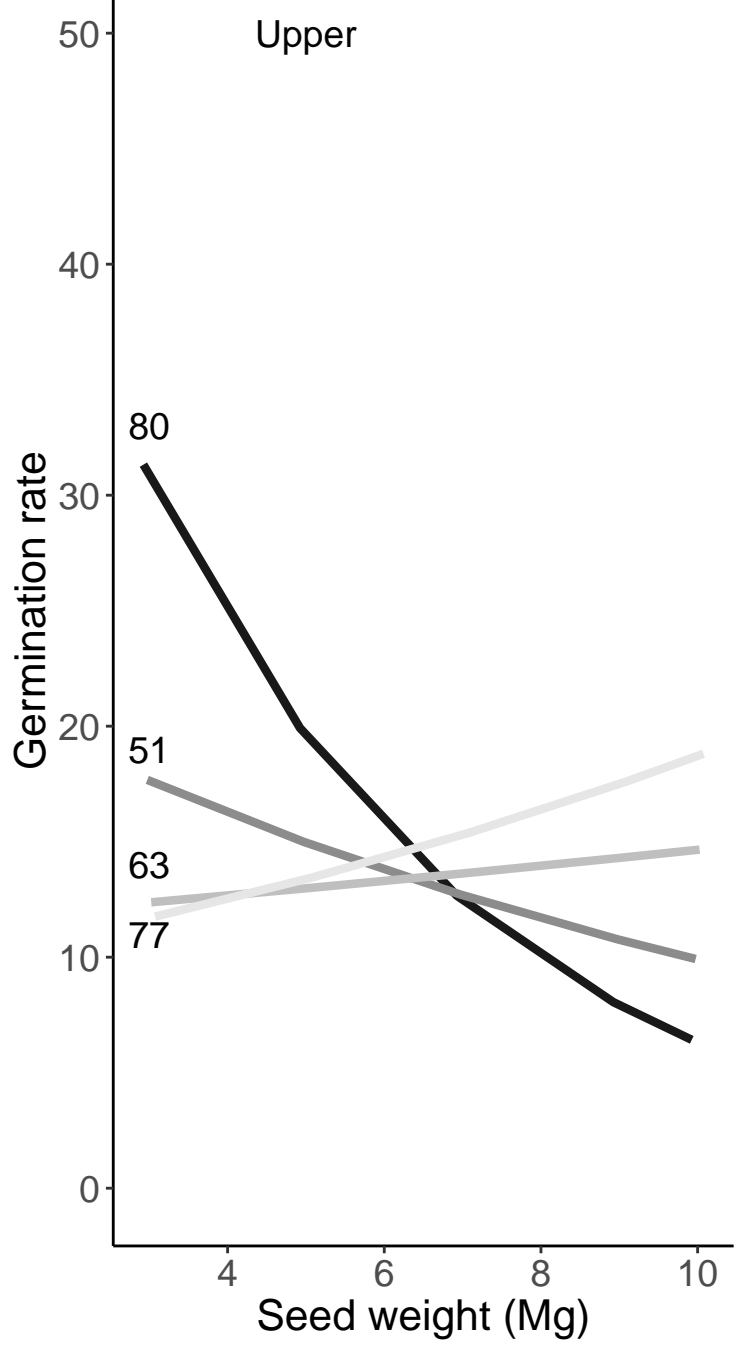
Treatment ● Control ● Diurnal pollination ● Nocturnal pollination ● Autogamy

Figure 5

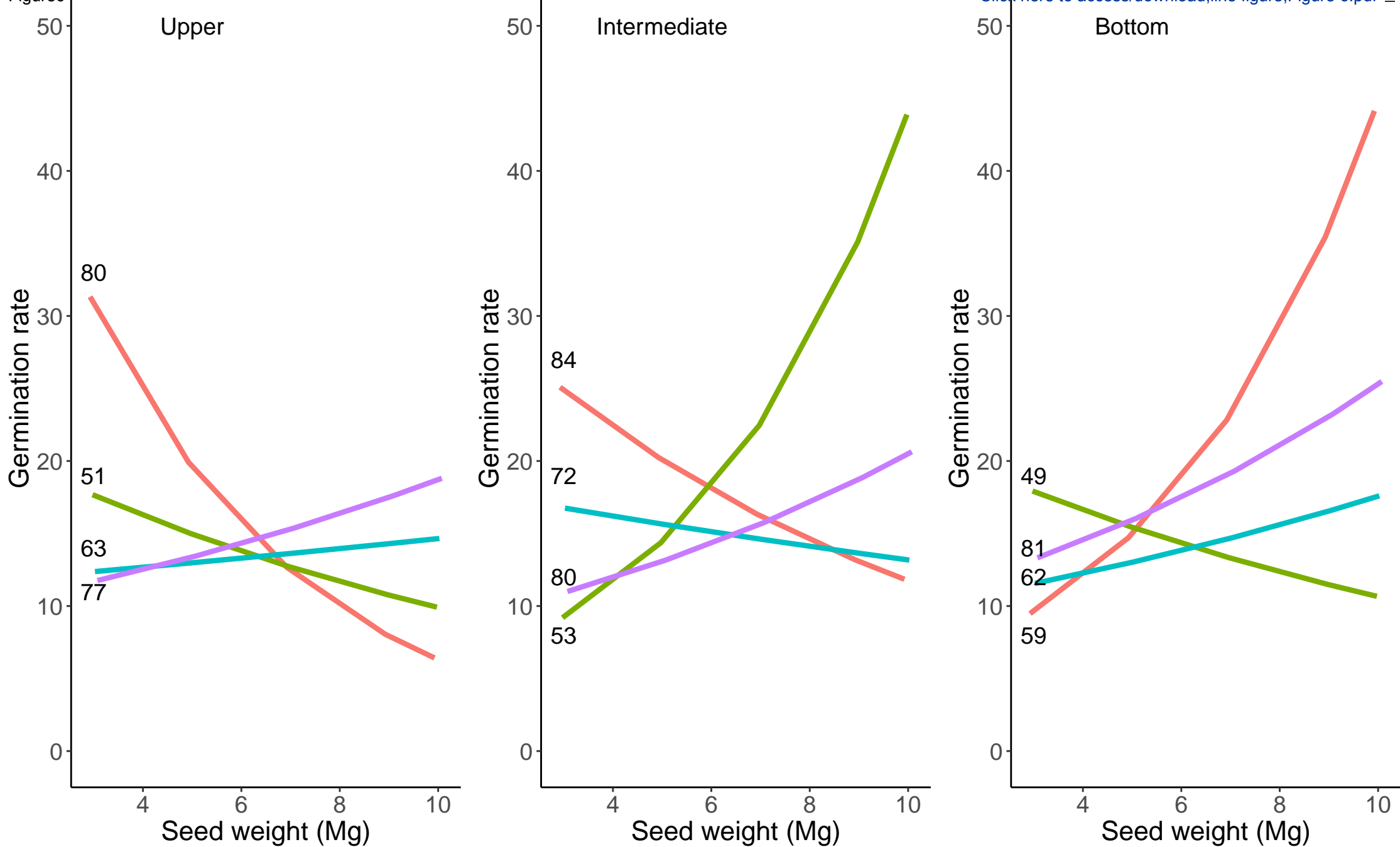
[Click here to access/download;line figure;Figure 5.pdf](#)



Treatment ● Control ● Diurnal pollination ● Nocturnal pollination ● Autogamy



Treatment ● Control ● Diurnal pollination ● Nocturnal pollination ● Autogamy



Treatment ● Control ● Diurnal pollination ● Nocturnal pollination ● Autogamy