2   Light acclimation strategies change from summer green to spring ephemeral as

3   wild leek plants age.[1]

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ACKNOWLEDGMENTS

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ABSTRACT

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23 *Premise of the study* - Spring ephemeral forest herbs emerge early to take advantage of the high

24 light conditions preceding canopy closure; they complete their life cycle in a few weeks, then

25 senesce as the tree canopy closes. Summer greens acclimate their leaves to shade and thus

26 manage to maintain a net carbon gain throughout summer. Differences in phenology among life

27 stages within a species have been reported in tree saplings, whose leaf activity may extend

28 beyond the period of shade conditions caused by mature trees. Similar phenological acclimation

29 has seldom been studied in forest herbs.

30   *Methods* - We compared wild leek bulb growth and leaf phenology among plants from

31 seedling to maturity and under 4 to 60 % natural light availability. We also compared leaf

32 chlorophyll content and chl a/b ratio among seedlings and adult plants in a natural population as

33 an indicator of photosynthetic capacity and acclimation to light environment.

34   *Key results* - Overall, younger plants senesced later than mature ones. Increasing light

35 availability delayed senescence in mature plants, while hastening seedling senescence. In natural

36 populations, only seedlings acclimated to the natural reduction in light availability through time.

37   *Conclusions* - Wild leek seedlings exhibit a summer green phenology, whereas mature

38 plants behave as true spring ephemerals. Growth appears to be more source limited in seedlings

39 than in mature plants. This modulation of phenological strategy, if confirmed in other species,

40 would require a review of the current classification of species as either spring ephemerals,

41 summer greens, wintergreens or evergreens.

42 **Keywords (3-10):** *Allium tricoccum*; Juvenile character; Leaf life span; Light; Phenology; Spring

43 ephemeral; Summer green; Understory herb.

INTRODUCTION

Growth of understory forest plants is mainly limited by light availability (Neufeld and Young, 2014). The light environment varies greatly in forests from one site to another, depending on canopy structure and tree species, and can influence growth of understory herbs (Barbier et al., 2008). Indeed, many forest herbs respond positively to increased light availability (Fournier et al., 2004; Naud et al., 2010).

Plants of the understory strata have developed various phenological and physiological strategies, either to avoid shade or to acclimate to the light environment as it changes throughout the growing season (ex. Landhausser et al., 1997; Rothstein and Zak, 2001; Katahata et al., 2005; Tessier, 2008). The most commonly encountered herbs of temperate deciduous forests are summer greens (Neufeld and Young, 2014), which unfold their leaves in spring shortly before or during canopy closure. Although most of their carbon fixation occurs prior to canopy closure, their leaves acclimate to canopy-induced shading by reducing photosynthetic capacity and respiration rate, thus achieving a net carbon gain during summer (Rothstein and Zak, 2001). Their leaves exhibit high plasticity in response to light availability. Spring ephemerals avoid shade by taking advantage of the short period of high light conditions in spring to accumulate carbon in their belowground storage organ through photosynthesis (Lapointe, 2001). Their leaves unfold immediately after snowmelt and senesce shortly after canopy closure. They produce typical sun leaves, i.e. with a high photosynthetic capacity and respiratory rate (Rothstein and Zak, 2001).

Phenological differences have been reported between juvenile and mature individuals in tree species. A common strategy adopted by juveniles is to expand leaf activity, most often by earlier leaf flushing in spring before canopy closure, but also by later senescence in autumn after canopy reopening, which increases their annual carbon assimilation (Augspurger and Bartlett,

67   2003; Jolly et al., 2004; Vitasse, 2013). The higher light availability in spring and fall is crucial

68   for sugar maple (*Acer saccharum* Marsh.) and Ohio buckeye (*Aesculus glabra* Willd.) saplings,

69   which may not even achieve positive net growth without this high light period (Augspurger,

70   2008).

71   Similar acclimation may be possible in herbaceous species. Juveniles of the ferns

72   *Dryopteris filix-mas* (L.) Schott. and *D. dilatata* (Hoffm.) retain their leaves throughout the year,

73   including winter (wintergreens), and replace them with new leaves in spring, whereas mature

74   plants develop leaves in spring that will senesce at the end of summer (summer greens; Willmot,

75   1989). This difference in phenology may, however, be a response to the light environment

76   provided by either the tree canopy or mature ferns. To our knowledge, no other study has

77   compared the phenology of seedlings and mature individuals of an understory herbaceous

78   species. If this modulation of phenological strategy occurs in other species, phenological

79   strategies could then in fact be plastic, rather than predetermined, as suggested by the usual

80   classification of species as either spring ephemerals, summer greens, wintergreens or evergreens.

81   The present study aimed to determine whether age-related changes in phenological

82   strategy can also be observed in another forest herb group, the spring ephemerals. We posited that

83   seedlings and juveniles would acclimate their leaves to lower light availability and prolong leaf

84   life span, thus behaving as summer green plants. In contrast, mature plants would behave as true

85   spring ephemerals, senescing their leaves when subjected to lower light conditions rather than

86   shade acclimating them. To test these hypotheses, wild leek plants of different ages were

87   subjected to the same three light regimes induced by a shade house environment. We also

88   compared leaf chlorophyll content and chl a/b ratio between seedlings and adult plants in a

89   natural population as indicators of photosynthetic capacity (Nobel, 1991) and of acclimation to

90   light environment throughout the season (Dale and Causton, 1992).

MATERIAL AND METHODS

91

92 *Studied species*—Wild leek (*Allium tricoccum* Ait.) is a spring ephemeral geophyte that grows in

93 hardwood forests in eastern North America. The plant is extensively harvested for consumption

94 in some areas due to the characteristic taste of its leaves and bulbs. It is mostly found in sugar

95 maple forests, on rich brunisol with a pH between 6 and 6.6 (Lavallée, 1978). Its one to three

96 leaves unfold early in spring right after snowmelt (late April in Québec) and senesce a few weeks

97 later, following canopy closure (late May), thus taking advantage of the high light conditions in

98 spring. Anthesis occurs in July and seeds are mature in late August (Jones, 1979), but most

99 reproductive effort is through asexual reproduction by division of the bulb (Nault and Gagnon,

100 1993). It is not uncommon for natural populations to reach densities higher than 350 bulbs $\cdot$ m^{-2}

101 (pers. obs.; see also Dagenais, 1985). Seedlings are regularly found underneath mature plant

102 foliage.

103 *Shade house experiment*—Three randomized complete blocks were set up at the Montreal

104 Botanical Garden. Each contained three $2 \times 1 \times 1$ m (length $\times$ width $\times$ height) shade houses

105 transmitting three different light levels, for a total of nine plots. Shade houses were set up in order

106 to provide light levels of 4 %, 15 % and 60 %, simulating respectively a dense canopy, sparse

107 canopy and large canopy clearing. Shade houses consisted of a tubular frame, to which sheets of

108 translucent synthetic material were tied. The 60 % plots were not covered on top, but had shading

109 material on the sides in order to reduce overall light level and provide the same protection against

110 wind as the other treatments. Light measurements were performed throughout the growing season

111 using a quantum sensor interfaced with a LI-1600 Steady State Porometer (LI-COR Biosciences,

112 Lincoln, NE, USA) and revealed an effective light transmittance of respectively 5.2, 12.9 and

113 58.7 % in comparison with light availability outside the shade houses.

114   Each plot contained one-year-old seedlings, two- and three-year-old juveniles, and mature

115   plants. One-year old seedlings were sown the previous autumn; two and three year-old juveniles

116   have been through one and two growing seasons respectively before the experiment began,

117   whereas mature plants were much older. Mature bulbs were harvested in June 2011, after their

118   leaves had senesced, in a forest near Gatineau, QC, Canada. Their diameter was measured and

119   only intact bulbs with a diameter > 9 mm were selected; flowering plants were discarded. Bulbs

120   were transplanted in 8.9 cm × 8.9 cm pots containing a mixture of 90% Promix HP (Premier

121   Tech Horticulture, Rivière-du-Loup, QC, Canada) and 10% coarse sand, fertilized with an

122   organic fertilizer Bio-Garden 4-3-6 (McInnes Natural Fertilizers Inc). The pots were

123   overwintered outside from late November 2011 to late March 2012, covered by a thick layer of

124   leaf litter and *Winter Max ™* wintering fabric.

125   Seeds for bulbs aged 3, 2 and 1 years were sown respectively in December 2009, 2010

126   and 2011 in 15 × 7.6 × 5.1 cm (L×W×H) polyethylene containers filled with the growing medium

127   Pro-Mix® HP (Premier Tech, Rivière-du-Loup, QC, Canada) and cold stratified at 2°C in a cold

128   room during the following winter. Seedlings that were to become the two- and three-year-old

129   juveniles were subjected to respectively one and two growth cycles in greenhouses in spring and

130   summer 2010 (three-year-old juveniles only) and 2011 (two- and three-year-old juveniles) prior

131   to the experiment. They were also cold stratified at 2°C in a cold room each winter. In mid-April

132   2012, all seedlings and juveniles were transplanted in 40 mL and 60 mL cells respectively, using

133   the same growing medium and fertilization as mature plants. They were then placed outside in

134   order to acclimate to the spring temperature. Six mature plants, 12 one-year-old seedlings, 6 two-

135   year-old juveniles and 6 three-year-old juveniles were randomly allocated to each of the 9 plots.

136   At this point, the shade house structures were set up on the plots, and shading material was

137   attached only to the sides of the structures (~ 60 % light transmitting) in order to simulate the

138   light conditions prevailing in forests before canopy closure, while protecting plants from the

139   wind. The light treatments were applied randomly within each block from May 16th onward, by

140   affixing the appropriate shade cloth to the top of the shade house frame.

141   Phenological surveys were performed weekly from May 17th onward, during which

142   phenological stage was determined for each plant. Seven phenological stages were defined: 6 =

143   fully unfolded, green leaf; 5 = yellow apex only; 4 = 5-10 % leaf area senesced; 3 = 10-25 %; 2 =

144   25-50 %; 1 = 50-100 %; 0 = fully senesced leaf. Mean phenological stage was then calculated for

145   each plot, at each survey. The area under the curve (AUC) for the progression of leaf senescence

146   (see Fig. 1 for the curves) was calculated for each plot based on the phenological data recorded

147   from the first survey until complete leaf senescence. The area under the curve was estimated

148   following the trapezoid rule. We chose to present AUC rather than leaf life span because leaf

149   senescence occurs gradually over a few weeks and the rate of progression can vary with the rate

150   of aboveground canopy closure. Although a very gradual decrease in AUC could translate into a

151   longer leaf life span and lower AUC than would be the case with very rapid senescence, in the

152   case of wild leek, AUC and leaf life span were positively and linearly correlated $(r^2 = 0.91; P <$

153   0.001; not shown).

154   Gas exchange was recorded weekly on mature plants using a LI-6400 portable

155   photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA). Measurement conditions were

156   saturating PPFD (2940 µmol photon·m^{-2}·s^{-1}) and ambient PPFD (respectively 60, 240 and 960

157   µmol photon·m^{-2}·s^{-1} for the 4%, 15% and 60% light regimes), at ambient CO_2 of 400 µmol·mol^{-1}.

158   Although the photosynthetic rate started to plateau well under a PPFD of 2000 µmol·m^{-2}·s^{-1}, in

159   most plants, it kept increasing, albeit very slowly, up to 2940 µmol·m^{-2}·s^{-1}. Hence, for

160   comparison purposes, saturating photosynthetic capacity (A_{sat}) was measured at 2940 µmol·m^{-2}·s^{-}

161   ^{1} for all individuals. Air temperature and humidity were similar to those of ambient air. During

162   senescence, only green parts of the leaves were measured. Gas exchange could not be recorded

163   on the other age groups, due to the small size of the leaf. Bulb width was measured on July 27th,

164   i.e. after leaf senescence was completed in all plots and for all plant ages, while total leaf width

165   was measured at the end of May before any sign of senescence.

166   *Natural population*—Sampling was performed in a large natural population of wild leek located

167   in a deciduous forest of the Basses-Laurentides (Lower Laurentian) region in southern Québec,

168   Canada. The canopy was mainly composed of *Acer saccharum* Marsh., *Carya cordiformis*

169   (Wangenh.) K.Koch and *Ostrya virginiana* (Miller) K.Koch. The exact location will not be

170   disclosed in order to prevent unauthorized harvesting.

171   Four sampling sites were selected within the wild leek population in spring 2013, each

172   presenting abundant mature plants but also some seedlings which were not directly located

173   underneath mature plant leaves. We wanted to ensure that seedlings and mature plants received

174   similar light levels to characterize the age effect on photosynthetic capacity in the absence of

175   differential light acclimation. We established a 2-meter linear transect at each site. Samplings

176   were performed before tree budburst (May 5th), during tree leaf unfolding but before wild leek

177   senescence (May 12th), and after complete canopy closure and wild leek mature plant senescence,

178   but before seedling senescence (May 29th). At each sampling date, leaves from five adults and

179   five seedlings closest to each transect were harvested. Leaves were kept frozen at -20°C until

180   analysis. Whole leaves were used for chlorophyll extraction from seedlings. On mature leaves,

181   two discs of 1.8 cm diameter were cut out using a punch. Following acetone extraction on leaves

182   ground in liquid nitrogen, chlorophyll absorbance was measured by spectrophotometry and

183   quantified using the equations of Porra and Grimme (1974). We then calculated the chlorophyll

184   a/b ratio (chl a/b) and the total chlorophyll a and b content (mg chl·g fw^{-1}). Chlorophyll a/b ratio

185   is expected to decrease under shade conditions as light harvesting complexes of photosystem II

186   become larger to improve light capture (Chow et al., 1990; Ballottari et al., 2007). Light

187   harvesting complexes contain both chlorophyll a and b, whereas the core of the photosystems

188   contains mainly chlorophyll a, which explains why the chl a/b ratio decreases as light harvesting

189   complexes enlarge.

190   *Statistical analyses*—Statistical analyses were performed using *R* 3.1.1 software (R Core Team,

191   2014) and the *R* packages *lme4* (Bates et al., 2014), *multcomp* (Hothorn et al., 2008) and *outliers*

192   (Komsta, 2011). Prior to all analyses, residuals were tested for normality (Shapiro-Wilk test) and

193   homogeneity of variance (Bartlett test). Appropriate transformations were applied when

194   necessary.

195   We performed a linear mixed effects analysis of the fixed effects of plant age (A), light

196   availability (L) and their interaction (A × L) on leaf phenology (AUC), bulb width (BW) and

197   total leaf width (TLW):BW ratios, with the block as a random effect in the shade house

198   experiment. TLW alone was not considered in the analyses since leaves had already unfolded at

199   the time of treatment application. *P*-values were obtained through likelihood ratio tests. Since the

200   A × L interaction was significant for all response variables, further likelihood ratio tests were

201   performed among light availability levels within each age, and among ages within each light

202   availability level. Multiple comparisons were then performed using the Tukey HSD test. When

203   comparing mature bulb width among light treatments, bulb width recorded in 2011 (i.e. the year

204   before the treatments were applied) was integrated in the model as a covariate.

205   A linear regression of A_{sat} and A_{amb} against the number of days following shade cloth

206   installation was performed for each plot. At the last survey (June 14th), all plants under the 4%

207   light availability treatment were completely senesced. No measurements were thus taken for this

208   treatment on this date. The slope and intercept of the function were then compared among light

209   availability levels using the likelihood ratio and Tukey HSD tests.

210   The chl a/b and total chl content of the samples harvested from the natural population

211   were compared among all five dates and age combinations using likelihood ratio and Tukey HSD

212   tests, considering sampling site as a random factor. One outlier point was removed from the chl

213   content dataset prior to the analysis. We suspected a weighing error and we also performed the

214   Grubbs test for confirmation of one outlier point ($G = 4.55$; $P < 0.001$). The chl a/b ratio from

215   this sample was included in the statistical analysis, because the sample weight did not influence

216   this ratio.

217   **RESULTS**

218   Seedlings and two-year-old juveniles had a delayed senescence compared with the three-year-old

219   juveniles and mature plants, under all light regimes. Indeed, three-year-old juveniles and mature

220   plants completed senescence before June 28th and June 23th respectively, whereas seedlings and

221   two-year-old juveniles had not yet fully senesced 29 days later, on July 26th (Fig. 1). There was a

222   significant interaction between plant age and light availability effects on AUC (Fig. 2a; statistical

223   results in Table 1a). Senescence was delayed for mature plants and three-year-old juveniles as

224   light availability increased, as indicated by the 111 % and 75 % higher AUC for the progression

225   of leaf senescence, under 60 % light availability compared to 4 % (Fig 2a). The seedlings

226   exhibited the opposite: AUC was 20% lower under 4% light availability than under 60% light

227   availability. Light availability did not significantly affect the AUC of the two-year-old juveniles.

228   Wild leek bulbs increased in size with light availability during the growing season (Fig.

229   2b). There was a significant interaction between plant age and light availability effects on bulb

230   width (Table 1a). The variation indeed appeared to be more pronounced for seedlings and mature

231   plants, as suggested by the larger difference between 4 % and 60 % treatments (Fig. 2b), and the

232 higher χ^2 values for the effect of light within seedlings ($\chi_2^2 = 30.5$) and mature ($\chi_2^2 = 12.8$) plants

233 than within either two- ($\chi_2^2 = 7.92$) or three-year-old ($\chi_2^2 = 6.10$) juveniles (Table 1b).

234 The TLW:BW ratio was 76 to 161 % higher in mature plants than in the younger ones

235 (Fig. 2c; Table 1), which means that mature plants produced less bulb biomass per leaf area than

236 younger ones. Higher light availability decreased the TLW:BW ratio, but only in seedlings. Since

237 TLW was not significantly affected by light availability within a single season (data not shown),

238 it was expected that TLW:BW ratio would decrease as final bulb size increased (Fig. 2b).

239 A_{sat} of mature plants decreased steadily in all plots following the reduction in light

240 availability (Fig. 3a), reflecting the change in leaf phenological stage reported based on visual

241 assessment (Fig 1d). However, A_{sat} decreased more rapidly under the 4% light regime, which had

242 a mean slope steeper than under the 15% and 60% light regimes (Fig. 3). The intercept of the

243 regression equation, i.e. the initial A_{sat}, was not significantly different among treatments, as

244 expected, since they were all exposed to the same initial conditions. A_{amb} also decreased steadily

245 until complete senescence under the 60% and 15% light regimes, but did not vary significantly

246 through time under 4% in two of the three plots (Fig. 4b).

247 Before canopy closure (May 5th), mature plants sampled in natural stands had a chl a/b

248 ratio similar to that of seedlings (Fig. 4a; likelihood ratio test: $\chi_4^2 = 68.3$; $P < 0.001$). One week

249 later, as tree leaf canopy was closing, chl a/b remained constant in mature plants, whereas it had

250 decreased significantly in seedlings. The chl a/b ratio of seedlings continued to decrease until

251 May 29th. At the beginning of the season, seedlings had a 27 % lower chlorophyll concentration

252 (mg chl·g fw^{-1}) than mature plants (Fig. 4b; likelihood ratio test: $\chi_4^2 = 30.8$; $P < 0.001$). However,

253 their chlorophyll concentration increased during the season to reach that of mature plants, which,

254 on the contrary, did not modulate their chlorophyll concentrations.

DISCUSSION

255

256   ***Phenology***—Young plants presented a phenology different from that of older plants. Overall,

257   senescence of seedlings and two-year-old juveniles occurred later, compared to three-year-old

258   juveniles and adults, long after being shaded. This phenology supports our hypothesis that wild

259   leek seedlings adopt the strategy of a summer green, which is the most common phenology

260   encountered among forest herbs (Neufeld and Young, 2014). This is consistent with (1) the

261   previously reported capacity of sapling leaves to unfold earlier and senesce later than canopy

262   closure, which was found to improve growth and survival of the tree species *Acer saccharum*

263   Marsh. and *Aesculus glabra* Willd. (Augspurger, 2008; Vitasse, 2013), and with (2) findings

264   regarding young plants of the two summer green ferns *Dryopteris filix-mas* (L.) Schott. and *D.*

265   *dilatata* (Hoffm.) A. Gray, which exhibit a wintergreen phenology (Willmot, 1989). Since wild

266   leek plants unfold their leaves very early in the season, the only way for seedlings to increase leaf

267   life span is to delay leaf senescence until later in the season. Moreover, early leafing increases the

268   risk of leaf damage by freezing (Jones, 1979; Lopez et al., 2008; Vitasse et al., 2014).

269   ***Acclimation***— Three-year-old juveniles and mature plants delayed their senescence under higher

270   light availability. This is not surprising, considering that spring ephemerals are adapted to take

271   advantage of the higher light availability in spring (Lapointe, 2001). Such plasticity was

272   previously observed in experiments studying the responses of wild leek (Dion, 2015) and

273   *Erythronium japonicum* (Balrer) Decne. (Kim et al., 2015) to the timing of canopy closure.

274   Higher light availability after canopy closure, such as that found in small gaps, may thus favor

275   longer leaf life span in mature wild leek plants. However, the response of leaf senescence to a

276   change in light availability might also be due to the inability of spring ephemerals to acclimate

277   their leaves to lower light conditions as the season progresses. We observed some plasticity of the

278   chl a/b ratio within a growing season, but only for seedlings, whereas mature wild leek plants can

279   modulate their chl a/b ratio in response to alteration of the light environment, but only from one

280   season to another (Bernatchez et al., 2013). We would also expect shade-acclimating leaves to

281   improve their ambient photosynthetic activity slightly within a few weeks of exposure to low

282   light conditions, as do summer greens (Rothstein and Zak, 2001), but mature wild leek plants did

283   not exhibit any improvement (Fig. 3b). Moreover, the sun leaves of spring ephemerals most

284   likely have respiratory rates that would be too high (Gutjahr and Lapointe, 2008; Gandin et al.,

285   2011) for the plants to maintain under low light conditions.

286   The increase in chlorophyll concentration observed in leaves of seedlings is also an

287   indication of their acclimation to low light conditions (Neufeld and Young, 2014). Seedlings that

288   we sampled in the natural population did not grow directly under mature plant leaves, meaning

289   that light availability was similar for mature plants and seedlings. This allows us to confirm that

290   the summer green phenology and the capacity of seedlings to light acclimate both stem from

291   ontogenic effects (Vitasse, 2013). Overall, seedling summer-green phenology and shade-

292   acclimation capacity may explain why many can be found directly under the dense canopy

293   formed by adults.

294   The leaves of wild leek seedlings are proportionally smaller than those of larger plants, as

295   indicated by their low TLW:BW ratio. We hypothesize that seedlings might thus be more source

296   limited. Source limited plants are expected to use extra light more efficiently than more sink

297   limited plants (Li et al., 2015), such as mature plants of this species, which have a high TLW:BW

298   ratio. Accordingly, seedlings completed their life cycle faster under a higher light regime, and

299   nevertheless produced a larger bulb than under lower light conditions. Growth in mature plants is

300   most likely limited by bulb growth rate (sink limitation), as reported in other spring ephemerals

301   (Badri et al., 2007; Gutjahr and Lapointe, 2008; Sunmonu and Kudo, 2014). This would explain

302   why higher light conditions and the resulting higher photosynthetic rates (Fig. 3b) did not

303   translate into a shorter growing season in adults, as seen in seedlings, but instead to a delay in

304   leaf senescence. While bulb size increased, more days were required to reach this larger size, as if

305   bulb growth could not be hastened. Actually, the high TLW:BW ratio of mature plants may have

306   played a key role in the evolution of the spring ephemeral habit. The plants can rapidly fill up

307   their carbohydrate reserves, but they also rapidly become sink limited, which induces leaf

308   senescence (Lapointe, 2001). Such a drastic reduction in leaf life span can be advantageous, since

309   it spares the plant from having to undergo biochemical changes to acclimate existing leaves to

310   new light conditions (Neufeld and Young, 2014). Juveniles seem to be in a transition from

311   summer green (more source limited) to spring ephemeral characteristics (more sink limited;

312   Lapointe, 2001), senescing sooner than seedlings, but without a TLW:BW ratio as high as adult

313   plants.

314   *Conclusion*— Our results clearly outline the differences in phenology and acclimation to light

315   availability between wild leek seedlings and mature plants. Seedlings behave as summer greens,

316   senescing later than mature plants, but hasten their senescence in response to higher light

317   availability. In contrast, mature plants behave like spring ephemerals, senescing early, but delay

318   their senescence under increased light. Few studies have explored phenological differences

319   among life stages in a single herbaceous species, and to our knowledge, this is the first to report

320   phenological differences between seedlings and mature plants for spring ephemeral herbs. Our

321   results also provide evidence that this phenological plasticity is partly a response to light

322   environment and partly due to changes in source to sink size ratio. Spring ephemeral phenology

323   might have been achieved as a result of a high leaf to bulb ratio, which enables fast accumulation

324   of resources. This study further demonstrates that both adults and seedlings benefit from

325   increased light availability, such as that which can occur under late forest canopy closure or in

326 forest gaps. Further research should focus on the factors that limit photosynthetic capacity in

327 seedlings, as well as on the factors that explain the slow growth of the bulb in larger plants. The

328 two- and three-year-old juveniles, which exhibit a transition from source to sink limitation, might

329 be very useful subjects in which to investigate these questions.

330   Our study thus calls for consideration of differences in phenology and light acclimation

331 strategy among life stages when classifying plant species according to their phenology. Juvenile

332 phenology is currently unaccounted for in ecological surveys, while it may help improve our

333 understanding of the ecological role of forest herbs throughout their life cycle.

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433 **Table 1.** Likelihood ratio tests of (a) the effects of bulb age, light availability and their interaction on leaf phenology, bulb width and
434 total leaf width: bulb width ratio (TLW:BW). (b) The effects of light availability within each plant age and of plant age within each
435 light regime were also computed, because the age × light interaction was significant in the general model for all response variables.
436

		Leaf phenology (AUC)				Bulb width[†] (mm)				TLW:BW ratio			
a) Model	**Effect**	SE	d.f.	χ^2	P	SE	d.f.	χ^2	P	SE	d.f.	χ^2	P
All effect	Age	20.67	3	88.1	< 0.001	0.49	3	128.8	< 0.001	0.12	3	95.2	< 0.001
levels	Light	20.67	2	4.45	0.108	0.49	2	21.7	< 0.001	0.12	2	12.2	0.002
included	Age × Light	20.67	6	24.2	< 0.001	0.49	6	45.7	< 0.001	0.12	6	22.3	0.001
b) Effect of	**Within**												
	Seedlings	15.4	2	11.0	0.004	0.12	2	30.5	< 0.001	0.07	2	24.8	< 0.001
Light	2 yr-old juv.	25.2	2	4.04	0.133	0.19	2	7.92	0.019	0.05	2	3.80	0.150
availability	3 yr-old juv.	17.0	2	9.64	0.008	0.17	2	6.10	0.047	0.15	2	4.30	0.117
	Mature plants	4.5	2	20.3	< 0.001	0.83	2	12.8[‡]	0.002	0.16	2	2.66[‡]	0.265
	4 % light	13.0	3	51.9	< 0.001	0.61	3	58.0	< 0.001	0.08	3	50.2	< 0.001
Plant age	15 % light	23.8	3	35.7	< 0.001	0.53	3	57.9	< 0.001	0.14	3	34.9	< 0.001
	60 % light	22.2	3	29.8	< 0.001	0.26	3	58.7	< 0.001	0.13	3	36.8	< 0.001

437
438
439 Note:
440 Abbreviations: Standard error (SE); juveniles (juv.).
441 Results of multiple comparisons by Tukey HSD test are presented in Figure 2.
442 [†] Bulb width was log transformed prior to likelihood ratio test in the general model including all effect levels to respect postulates of normality and homogeneity
443 of variance.
444 [‡] Effect of light availability within mature plants for bulb width and TLW:BW ratio: results from the likelihood ratio test using bulb width of previous year as a
445 covariate.

446

447   **Figure 1.** Progression of leaf senescence in wild leek plants of different ages, under varying light

448   conditions induced by use of shade cloths beginning May 16th. Phenological stages: 6 = green

449   leaf; 5 = yellow apex; 4 = 5-10 % leaf area senesced; 3 = 10-25 %; 2 = 25-50 %; 1 = 50-100%; 0

450   = fully senesced leaf. Values presented are averages of the mean phenological stage per plot (±

451   SE).

452

453   **Figure 2.** Wild leek leaf phenology (a), bulb width after one growing season (b) and total leaf

454   width : bulb width ratio (TLW:BW) (c) as influenced by plant age, light availability and their

455   interaction. Mean ± SE. Leaf phenology represents the area under the curve of phenological stage

456   progression as a function of time (see Fig. 1). A higher AUC means that the leaves senesced later.

457   Within a same plant age (capital letters) or light regime (lower-case letters), values with a same

458   letter are not significantly different according to the Tukey HSD test ($\alpha = 0.05$). For TLW:BW,

459   the effect of light availability was significant only among one-year bulbs, hence the absence of

460   capital letters for other plant ages. Results of likelihood ratio tests of all effects, and of the effects

461   of light and age within each level of age and light, are presented in Table 1.

462

463   **Figure 3.** Reduction of saturating photosynthetic activity (a; A_{sat}) and photosynthetic activity at

464   ambient light (b; A_{amb}) following application of shade cloth that induced three different light

465   regimes over wild leek mature plants (mean ± SE). Linear regression equations are: $A_{sat} = -0.27t$

466   + 11.8 (60%), $A_{sat} = -0.27t + 10.4$ (15%) and $A_{sat} = -0.51t + 12.1$ (4%); $A_{amb} = -0.26t + 11.0$;

467   (60%); $A_{amb} = -0.17t + 7.3$ (15%) and $A_{amb} = -0.05t + 2.63$ (4%). There is no significant linear

468   relationship for A_{amb} at 4% light availability in two of the three plots. For A_{sat}, slope of the 4%

469   light availability treatment is significantly steeper than the two others according to Tukey HSD

470   test ($\alpha = 0.05$; result from likelihood ratio test: $\chi_2^2 = 17.3$; $P < 0.001$). The intercept was not

471   significantly different among the three treatments (result from likelihood ratio test: $\chi_2^2 = 3.05$; $P =$

472   0.218). For A_{amb}, the slope of the 60% treatment was significantly steeper than that of the 15%

473   treatment ($\chi_2^2 = 27.9$; $P < 0.001$). All intercepts were significantly different from each other ($\chi_2^2 =$

474   27.4; $P < 0.001$).

475

476   **Figure 4.** Chlorophyll a/b ratio (a) and total chlorophyll concentration (b; mg chl·g fw^{-1}) of

477   leaves of seedlings and mature wild leek plants harvested in natural populations before, during

478   and after tree leaf canopy closure. Mean ± SE. Data points accompanied by the same letter are

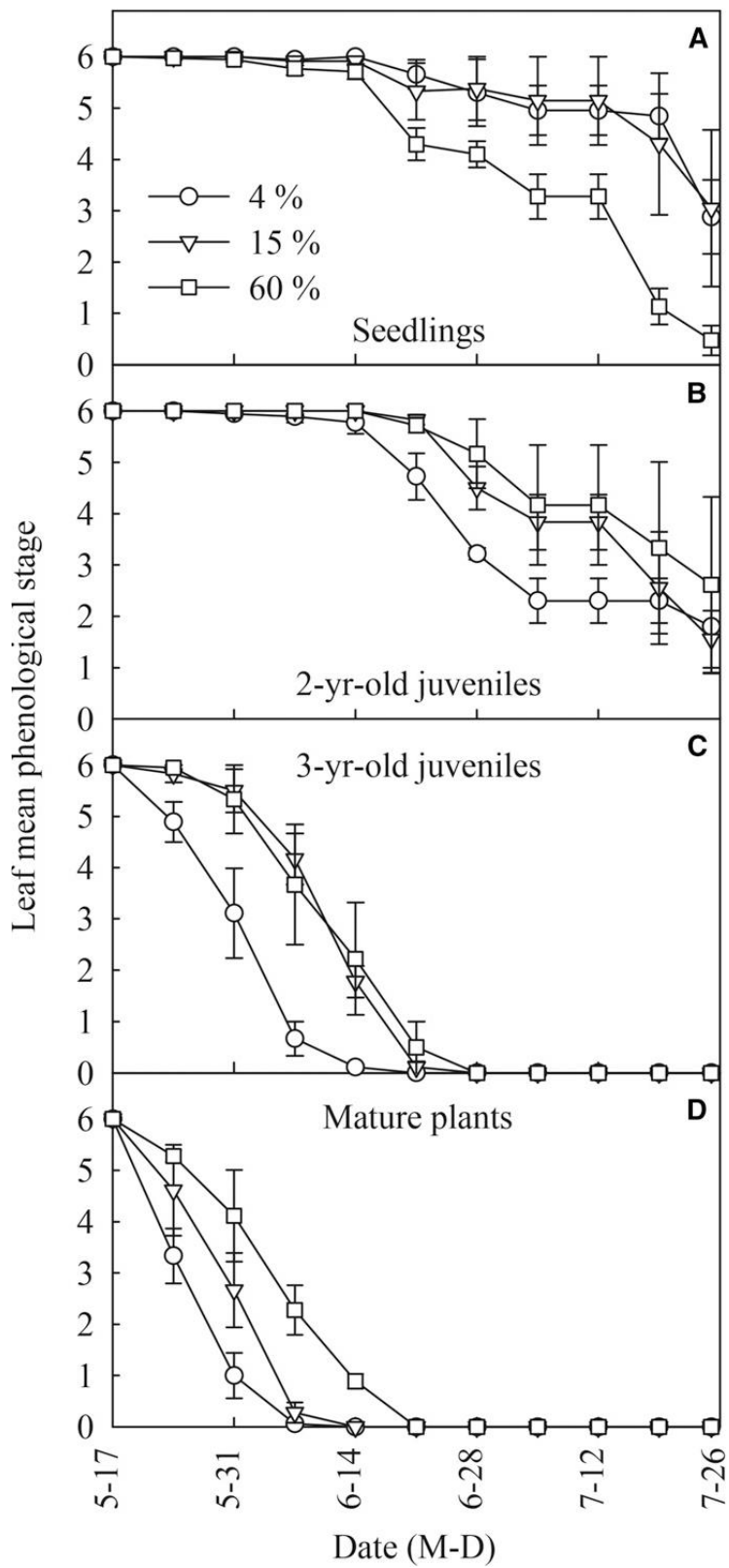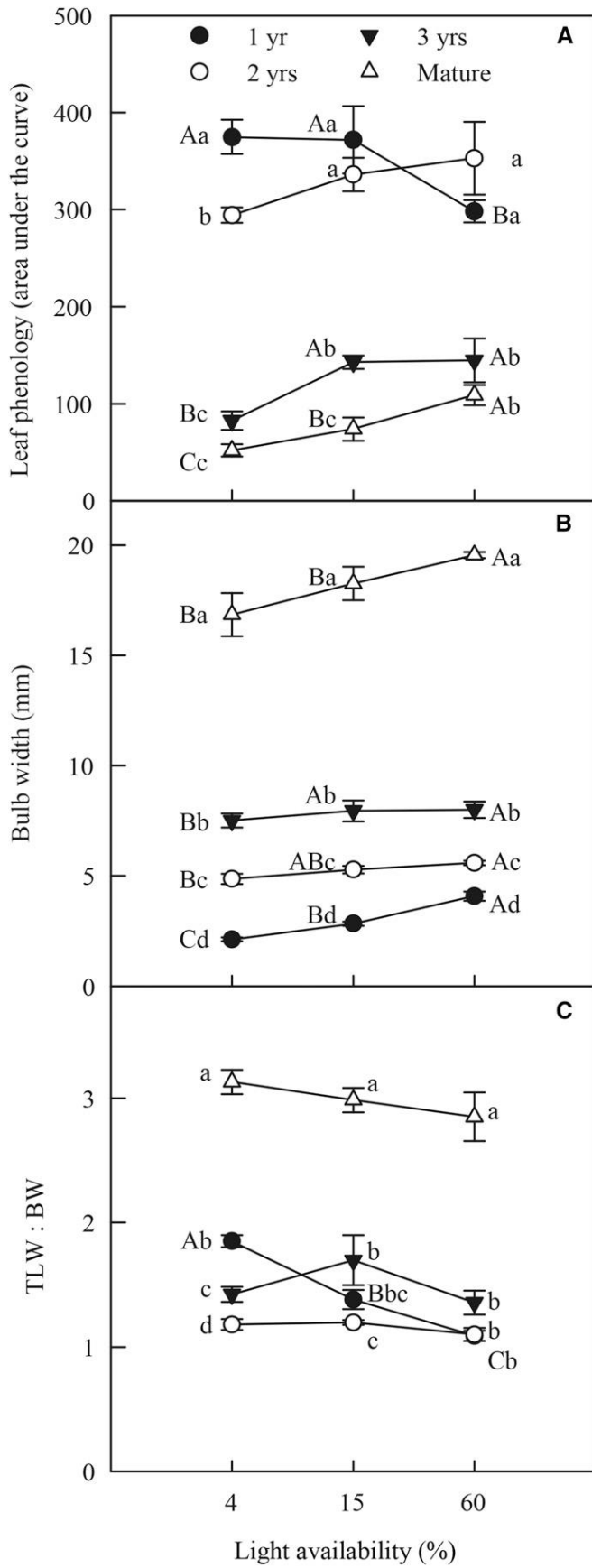479   not significantly different according to the Tukey HSD test ($\alpha = 0.05$).
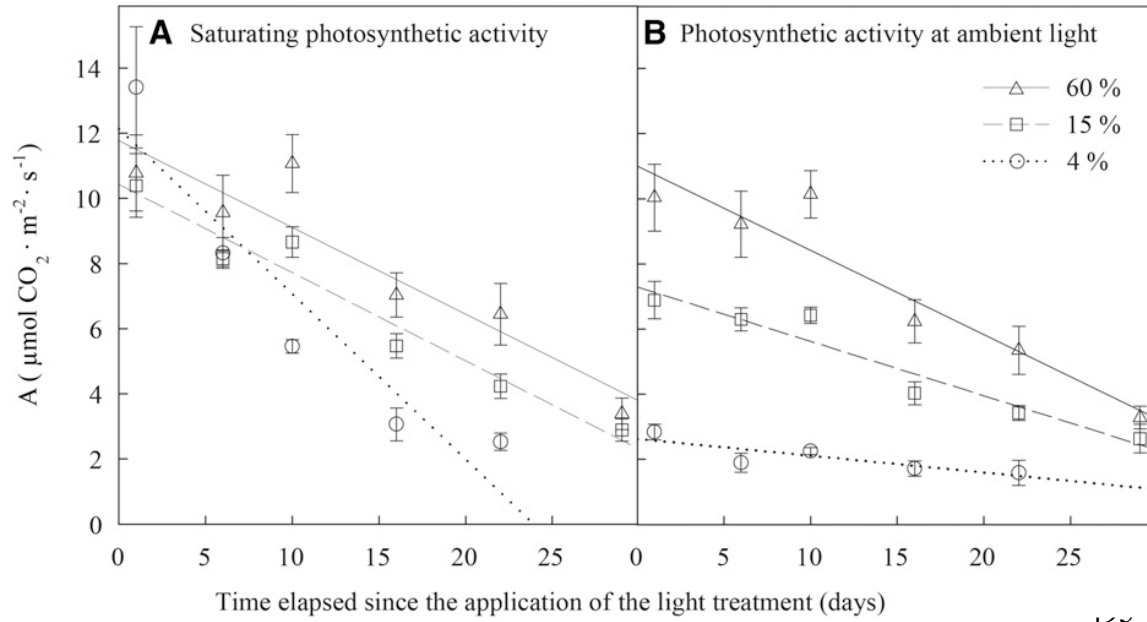
480

Figure 1

Figure 2

486   Figure 3

A Saturating photosynthetic activity

B Photosynthetic activity at ambient light

60 %
15 %
4 %

$A\ (\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1})$

Time elapsed since the application of the light treatment (days)

496

497

498

Figure 4