1 **Immediate and lag effects of hydrological change on floodplain grassland plants**

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15

16 **Abstract**

17 Hydrological alteration due to climate change events such as floods and drought is a significant threat to

18 globally important wetlands, including floodplain wet grasslands. This research incorporated two field

19 experiments with the aim to assess immediate and longer-term functional responses of floodplain plants to

20 hydrological change. Plant introductions and transplants between a wetter riparian and a drier site in southern

21 England were used to simulate hydrological change. Species showed immediate and differential responses to

22 contrasting hydrologies. *Rhinanthus minor*, a hemi-parasitic annual species with ruderal traits, was lost from the

23 riparian grassland within four weeks. The survival and production of a leguminous perennial, *Lathyrus*

24 *pratensis*, in high groundwater levels soon decreased. However, the perennial *Primula veris* mostly functioned

25 well in contrasting hydrological regimes, possibly because it can tolerate stress. The perennial wetland species

26 *Caltha palustris* showed lag effects, over three years, when its hydrology was altered to a sub-optimal drier

27 scenario by transplantation, with declining survival and a sustained reduction in leaf production and flowering.

28   Disturbance caused by transplantation and weather conditions also affected its performance. Thus, this study

29   shows that some functionally important floodplain species may succumb within weeks to a hydrological event

30   facilitated by climate change, unless they are able to tolerate the challenging conditions, while the performance

31   of other characteristic species could decline and continue to show constrained performance for years as a

32   consequence of altered hydrology.

33

34   **Keywords**

35   Climate change; Flowering; Plant traits; Production; Survival; Wetlands

36

37   **Introduction**

38   Hydrological alteration due to climate change is predicted to be one of the most significant threats to wetlands

39   such as floodplains, wet grasslands, and marshes (Joyce et al. 2016). Wetlands are defined by their hydrology,

40   with plants and associated biodiversity adapted to particular hydrological regimes. Thus, climate change

41   predictions for increased temperatures, altered precipitation patterns and more extreme events such as prolonged

42   floods and droughts will fundamentally influence wetland ecosystems. Consequently, wetland services that

43   millions of humans depend upon are threatened by climate events, including agricultural and fisheries

44   production, flood attenuation and aquifer recharge, nutrient and carbon cycling, and sediment deposition (Zedler

45   and Kercher 2005). Extreme climate events are increasing in intensity and frequency (IPCC 2012), such that the

46   rapid and substantial changes in hydrology they cause are likely to induce novel but persistent states (Brotherton

47   and Joyce 2015). For example, drought can quickly reduce soil moisture and limit long-term water availability

48   in wetlands (Thompson et al. 2009; Garssen et al. 2014) while major flooding can raise groundwater levels,

49   sustain saturated soils, and lead to paludification (Crawford et al. 2003). Moreover, flooding is becoming

50   increasingly prevalent at atypical periods of the year, including the temperate growing season when plants are

51   more sensitive to its effects (Van Eck et al. 2006).

52

53   Floodplain grasslands may be good indicators of hydrological change because they are dynamic and

54   heterogenous, composed of a mosaic of plant communities reflecting their hydrology and management

55   (Toogood et al. 2008). Floodplains are characterised by variable water levels, with seasonal or episodic

56   inundation, permanent or temporarily high groundwater, and periodic or persistently saturated soils. Floodplain

57   patches exposed to higher water levels and a greater frequency and duration of flooding are more subject to the

58   direct effects of inundation and waterlogging, such as anoxia stress. The indirect effects of anaerobic conditions

59   on soils, including limiting nutrient availability and increasing soil toxicity, increase stress for plants in

60   waterlogged floodplain environments. Variable conditions filter plant community composition because each

61   species has a defined hydrological niche (Silvertown et al. 1999), mediated by its functional traits (Brotherton

62   and Joyce 2015). Plants able to survive inundation have adaptations, such as a capacity for anaerobic

63   metabolism or oxygen transfer by aerenchyma (Armstrong et al. 1994), and life strategies that enable them to

64   persist (Grime 1977).

65

66   Climate events can lead to increased or reduced water levels in floodplains, and both can drive plant

67   performance and composition in floodplain grasslands. Heatwaves and drought cause a reduction in

68   groundwater levels and soil water availability. Evidence on the impacts of low water levels in wetlands due to

69   extreme climate events suggests likely increases in more terrestrial plant species, coupled with a loss of species

70   better adapted to wetter conditions (Thompson et al. 2009; Herrera-Pantoja et al. 2012). Plant growth,

71   production and species richness can be severely constrained by a shortage of available water (Grime 1977;

72   Garssen et al. 2014). Flood events may not only result in inundation but also rapidly and substantially raise

73   groundwater levels through recharge, causing waterlogging over a prolonged period. Extreme flooding and

74   waterlogging can delay and reduce flowering, and limit growth and biomass, although some stressed plants

75   over-compensate by rapid shoot elongation and accelerated or increased flower production (Brotherton and

76   Joyce 2015). Raised water levels and increased flooding can prompt shifts in species composition (Toogood

77   and Joyce 2009; Garssen et al. 2015), potentially with the loss of functionally dominant or rare species. The

78   effects of extreme flooding may include a subsequent reduction in plant species diversity, although this does not

79   necessarily affect productivity (Ilg et al. 2008), with species possessing traits for tolerating submersion and soil

80   saturation most likely to survive (Wright et al. 2016).

81

82   Plant responses to climate events may be either immediate, where a significant impact is observed relatively

83   quickly, or lagged, whereby effects are not readily detectable within the same growing season as the event

84   (Smith 2011). Some species may be sensitive to hydrological change and are rapidly extirpated, while others

85 may initially exhibit resilience to extreme weather events (Kreyling et al. 2008), although longer-term

86 performance may be affected. Monitoring of a floodplain before and after an extreme flood event suggests that

87 plants less adapted to flooding are more likely to be immediately affected, leading to changes in distribution and

88 loss of biodiversity (Ilg et al. 2008). However, the reorganisation of plant distribution following a flood event

89 may last several years (Vervuren et al. 2003). Lagged effects due to climate change stressors may take time to

90 exert themselves on plant performance, however eventually they can become apparent as reduced growth and

91 biomass, delayed flowering, or phenological changes (Sherry et al. 2007; Jentsch et al. 2009; Sherry et al. 2011).

92

93 The immediate and lag effects of hydrological events have not been considered for floodplain wet grasslands as

94 research has tended to focus on incremental climate change in mesic grasslands (Thompson et al. 2013;

95 Kreyling et al. 2014; Ludewig et al. 2014). Moreover, there has been little research investigating the role plant

96 traits play in determining responses to changed hydrology under field conditions (but see Oddershede et al.

97 2018). The overarching aim of this field research was to examine how floodplain grassland plants respond

98 immediately and in subsequent growing seasons to hydrological change. The study comprised two

99 complementary experiments incorporating two floodplain grasslands with contrasting hydrological regimes to

100 serve as a proxy for climatic scenarios. Plant introductions and transplants were used to assess species responses

101 to hydrological change. Nooten and Hughes (2017) concluded from a review of 47 studies that field

102 transplantation is a powerful, direct method of predicting how species and communities will respond to climate

103 change. Aims of the two experiments were to:

104   1. Assess immediate (within-year) impacts of contrasting hydrologies on three floodplain grassland plant

105   species with different functional traits, and

106   2. Evaluate immediate and longer-term (three-year) effects of altered hydrology on a characteristic wet

107   floodplain plant species.

108 It was expected that a) species would respond differently to hydrology dependent upon their traits, and b) the

109 effects of altered hydrology would become more apparent over time.

110

111   **Methods**

112   Study area

113   The field study was located at Amberley Wildbrooks in southern England (50^0 55' 2.291", 0^0 32' 11.692")

114   because it offered the opportunity to utilise two different floodplain grassland sites at close proximity to each

115   other with intra-site vegetation homogeneity but contrasting inter-site hydrologies, especially in relation to

116   groundwater level and inundation. Amberley Wildbrooks is a wet grassland landscape intersected by drainage

117   ditches situated adjacent to the river Arun and prone to extensive flooding in winter. It is within a Ramsar

118   wetland of international importance due to its rare and diverse plant and animal species. The Wildbrooks are

119   grazed mostly by cattle at relatively low-intensity from approximately April to October, contingent on water

120   levels.

121

122   There were two experimental sites at Amberley Wildbrooks, each of approximately 20 m x 40 m, located 15 m

123   apart, separated by a human-made embankment that contains overbank flooding from the river and therefore

124   controls water regimes. Soil classification was the same at both sites, being stoneless clayey alluvial gley soils

125   of the Fladbury series (National Soil Resouces Institute 2013). Between the embankment and the river channel,

126   the site is inundated frequently by the river from early winter to spring with high groundwater levels and

127   saturated soil for most of the year. This location is referred to as the riparian site and supports an inundation

128   grassland community with *Agrostis stolonifera* and *Alopecurus geniculatus,* and tussocks of coarse vegetation of

129   *Deschampsia cespitosa*, *Juncus effusus* and *Juncus inflexus*. In contrast, the locality inland of the embankment,

130   referred to as the interior site, represents the former floodplain that is inundated only when flooding in the

131   catchment upstream breaches the flood defences. Consequently, it is a grassland of winter-saturated soil that

132   rarely floods and dries rapidly in spring. The short sward here is characterised by *Lolium perenne* and

133   *Cynosurus cristatus*, with *J. effusus* and *Carex* species forming larger tussocks.

134

135   The field study was conducted in three spring-summer growing seasons from 2014-16, with each year showing

136   considerable climatic variability. For southern England, 2014 was a much warmer and wetter year than the

137   long-term average with a mean annual temperature of 11.5^0C and total annual rainfall of 1042mm compared to

138   9.6^0C and 788mm for 1981-2010, respectively. This was followed by two warmer and slightly drier years than

139   the long-term average in 2015 (10.9^0C, 740mm) and 2016 (10.8^0C, 759mm) (Met Office 2019). Water levels

140 were monitored manually each growing season at one-two week intervals using a network of up to nine dipwells

141 across each site. Overall, the interior site had lower groundwater levels than the riparian site, and interior water

142 levels receded more rapidly during the season, in all three years (Fig. 1). During the growing seasons, flooding

143 was recorded only once, on the riparian site in early March 2016. Soil nutrients were not tested but groundwater

144 pH values were very similar at both sites throughout (median 6.5, $n = 35$) and there were no significant

145 differences (t tests) in nitrate (mean 0.76 and 0.74 mg l^{-1}, $n = 14$) or phosphate (mean 0.31 and 0.46 mg l^{-1}, $n = $

146 14) between the riparian and interior sites, respectively. Both sites were subjected to the same low-intensity

147 grazing pressure during the three growing seasons; livestock could not be excluded from the experimental sites

148 because the grasslands provide valuable early-season production.

149

150 Experiment 1: Immediate impacts of contrasting hydrologies

151 Three grassland plant species were selected to represent a variety of functional traits and replicate plants were

152 introduced into the two field sites as well as grown under controlled hydrological conditions to act as a

153 reference. All three species were absent from both field sites at the beginning of the experiment. *Primula veris*

154 is a spring-flowering, rhizomatous perennial most commonly found on species-rich, low nutrient, moist-dry,

155 neutral and calcareous grassland (Table 1; Brys and Jacquemyn 2009). It is considered a drought-tolerant

156 species (Whale 1984), and does not perform as well in waterlogged conditions as its roots cannot penetrate

157 anoxic soils (Brys and Jacquemyn 2009). Compared to the other two species selected for this experiment, *P.*

158 *veris* is more tolerant of stress (Table 1). *Lathyrus pratensis* is a rhizomatous perennial and the only legume in

159 this experiment. It prefers damper and more fertile grasslands than the other species selected (Table 1).

160 *Rhinanthus minor* is a hemi-parasitic annual found most commonly in open grasslands (Westbury, 2004). It

161 extracts water and nutrients from a host plant through haustoria (Westbury 2004). Although *R. minor* can

162 photosynthesise, its growth and flower production are reduced without the exploitation of host plants (Seel and

163 Jeschke 1999). This species has the most ruderal strategy of those used in this experiment (Table 1).

164

165 Seedlings of the three experimental species were sourced as 'plugs' from commercial suppliers (British Wild

166 Flower Plants (www.wildflowers.co.uk) for *P. veris* and *L. pratensis,* and Naturescape (www.naturescape.co.uk)

167 for *R. minor*), ensuring that each plug of each species was of similar physiognomy. Plugs are young plants each

168 in an individual cell containing a growth medium so that they can be planted without disturbance to the roots.

169   Twenty-four plugs of *P. veris* and *L. pratensis* were planted into each of the riparian and interior sites on 4th

170   April 2016. *R. minor* plugs were supplied with a *Festuca* sp. as a host plant and were planted with the hosts on

171   9th May because they were not available earlier in the spring. Initial survival of *R. minor* was incomplete so

172   only 20 plugs per site were planted. Plugs were planted in three transects across each site and georeferenced for

173   ease of relocation. The plug plants were all 3-4 cm tall growing in cells each with 0.05 l of compost. Soil cores

174   of the same volume were removed in the field and replaced with the plugs. The plants were well-watered in the

175   morning before being planted into the field sites, but were not subsequently watered in the field.

176

177   Control conditions were established within an unshaded compound at the University of Brighton Moulsecoomb

178   campus (50^0 50' 41.209", 0^0 7' 11.514") that could be regularly watered. Here, 24 plugs of both *P. veris* and *L.*

179   *pratensis,* and 20 of *R. minor,* were planted in compost in 10 cm diameter plastic pots on the same day as their

180   respective species were planted at the field sites. Nutrients in the compost were not tested. Each pot was

181   watered when necessary with tap water to maintain a damp soil surface and provide favourable hydrological

182   conditions for survival and growth.

183

184   All control and field treatment plants were monitored weekly for plant survival, which was a visual assessment

185   of whether each plant was alive at the time of survey, and flowering, with all open flowers counted. Monitoring

186   ceased, and plants were removed from the field sites and control pots for production analyses, once flowering

187   had finished. This was 25th May for *P. veris*, and 6th July for *L. pratensis* and *R. minor*.

188

189   Biomass analysis on *P. veris* was performed by rinsing soil from roots before plants were oven-dried at 60 ºC

190   for 72 hours. The roots were separated from the rest of the plant and above and belowground material were

191   weighed separately. In contrast to the other two species, aboveground biomass of *L. pratensis* was affected by

192   grazing in the field, and root nodules prevented soil from being removed sufficiently from belowground

193   biomass. Therefore, root lengths were measured to evaluate belowground performance for this species. For *R.*

194   *minor*, there were too few survivors from the field sites in suitable condition to perform reliable biomass

195   analysis. However, they did produce abundant seeds so 20 of these were sampled at random from each

196   treatment and weighed.

197

198   Experiment 2: Lag effects of altered hydrology

199   *Caltha palustris* was selected to investigate any lag effects of altered hydrology because it is a conspicuous,

200   characteristic floodplain species that is abundant at the riparian field site so could be transplanted into the

201   interior site where it was absent. *C. palustris* is a spring flowering herbaceous perennial common to many

202   wetlands in temperate regions of the northern hemisphere (Table 1; Hill et al. 2004). It is tolerant of

203   waterlogged conditions (Table 1), preferring water tables between +15 cm to -35 cm of the soil surface

204   (Newbold and Mountford 1997). *C. palustris* exhibits several adaptive mechanisms for saturated soil and

205   inundation, including well developed aerenchyma (Smirnoff and Crawford 1983) and hyponastic growth

206   (Colmer and Voesenek 2009).

207

208   There were three experimental treatments encompassing a total of 96 *C. palustris* plants. Thirty-two plants were

209   removed from the riparian site and transplanted into the interior site (the transplant treatment). Individuals for

210   transplanting were sourced from the floodplain immediately surrounding the riparian study site to minimize

211   disturbance and genetic difference. Plants were selected of a similar size and developmental stage, each with

212   four leaves. Each plant was removed using a spade with a soil core of approximately 3 l containing its roots.

213   Plants were then inserted into cavities of the same size excavated on the interior site, arranged into four

214   randomly distributed arrays. Each array comprised eight individuals formed into a circle approximately 3 m in

215   diameter so that *C. palustris* plants would not mutually interact. To ascertain whether any differences in plant

216   survival and performance were due to the disturbance of being removed and replanted, 32 *C. palustris* plants

217   were lifted from the floodplain and replaced within the riparian study site using exactly the same methods as the

218   transplants (the lift treatment). Thirty-two plants were also monitored *in-situ* on the riparian site without

219   experimental disturbance (the control treatment), with four randomly distributed arrays of eight plants each on

220   average over 1 m apart to minimise interaction. All arrays in all three treatments were georeferenced so that the

221   same individual plants could be relocated. No removal of the matrix vegetation was undertaken around any of

222   the plants during the experiment.

223

224   Plants for the transplant and lift treatments were selected and moved on 20th March 2014. All *C. palustris*

225   individuals were monitored weekly but only within the growing season until flowering plants had produced

226   seeds, over three successive years, ending on 25th May 2016. Performance monitoring comprised survival,

227   growth and flowering. Survival was a visual assessment of whether each plant was alive at the time of survey.

228   Plant growth was recorded at each survey by counting the number of leaves and measuring maximum leaf width

229   and length. Flowering was recorded by counting the number of open flowers on each plant at each survey. An

230   additional field visit was undertaken on 11th April 2018 simply to provide an insight into survival and flowering

231   after five years; the outcomes are noted in the Discussion as they extend beyond the main experimental period

232   when monitoring took place.

233

234   Data analysis

235   Data sets were examined where applicable for normal distribution of continuous variables using the Anderson-

236   Darling test and for homogeny of variance with the Levene's test.

237

238   For experiment 1, survival times were examined using Kaplan-Meier analysis with log rank chi-square tests

239   (Rich et al. 2010). Flower counts were analysed between treatments using the Generalised Estimating Equation

240   (GEE) test, above and belowground biomass was investigated using MANOVA with Tukey post-hoc testing,

241   root lengths were examined with Kruskal-Wallis and post-hoc Dunn's tests of median values, and seed weights

242   were compared using a *t* test.

243

244   For experiment 2, leaf and flower counts were analysed between treatments with Generalized Linear Models

245   (GZLM) (O'Hara and Kotze 2010). A Poisson log-linear model for main effects testing was used, with post-hoc

246   pairwise comparisons of estimated marginal means using Least Significant Difference (LSD). Maximum step-

247   halving was five except for the comparison of leaf abundance for the first season, where it was necessary to

248   increase this to 20. Leaf size measurements were examined with ANOVA and Tukey post-hoc testing, except

249   where unequal sample numbers were present due to plant losses in the second and third years. In these cases,

250   the more conservative post-hoc Tamhane's T2 test was used (Primpas and Karydis 2010).

251

252   Statistical analysis was conducted using Minitab 17 and SPSS 24. For both experiments, differences are

253   considered significant at $P < 0.05$.

254

255 **Results**

256 Experiment 1: Immediate impacts of contrasting hydrologies

257 This experiment lasted 7-13 weeks, dependent upon the completion of flowering of *Primula veris, Lathyrus*

258 *pratensis* and *Rhinanthus minor*. Survival between the species differed across the control and the two field sites

259 (Table 2). *P. veris* had the highest survival in all treatments compared to the other species, and there was no

260 significant difference in survival time between treatments (chi-square = 0.831, $P = 0.362$). *R. minor* had the

261 lowest survival of the three species and there was insufficient data from the field for survival time analysis;

262 survival was limited on the interior site and it was lost from the riparian site after four weeks. Survival of *L.*

263 *pratensis* was intermediate between the other two species and was better on the interior site than the riparian

264 site. There was a significant difference in survival time between treatments (chi-square = 58.793, $P < 0.001$)

265 and the median survival time for *L. pratensis* plants at the interior site was 12 weeks compared to 9 weeks at the

266 riparian site.

267

268 All of the species flowered in the control conditions and interior site, however only *P. veris* flowered at the

269 riparian site (Table 2). Both *P. veris* and *R. minor* had a greater percentage of surviving plants flowering under

270 the field treatments compared to the control condition plants, while only two *R. minor* plants survived in the

271 field, both on the interior site. Cattle are suspected of grazing much of the aboveground biomass of *L. pratensis*

272 at the field sites, so only one flowering individual was counted, at the interior site.

273

274 *P. veris* was the most successful of the three species in terms of flowering across all three treatments. Its total

275 production of flowers was greatest in the riparian site with a maximum count of 319 compared to 295 at the

276 interior site and 276 in control conditions. However, there was no significant difference between treatments in

277 the number of flowers per flowering plant (GEE = 0.241, $P = 0.886$).

278

279 There was a significant difference in *P. veris* biomass between the three treatments when above and

280 belowground data were combined (MANOVA F (4, 138) = 14.848, $P < 0.001$). However, within-subject effects

281 indicated that only the aboveground biomass was significantly different, with control plants showing

282 significantly more biomass than riparian or interior plants (Fig. 2). There was no significant difference between

283 the treatments for belowground biomass (F (2, 70) = 2.011, $P = 0.142$; Fig. 2).

284

285 Roots of *L. pratensis* were significantly longer in control conditions than in either of the field treatments, which

286 were not significantly different from each other (Fig. 3).

287

288 There was a significant difference in the weight of seeds of *R. minor* between the control plants and those from

289 the interior site (*t* test = 6.76, *P* < 0.001). Seeds from control plants were significantly heavier (mean 0.00342

290 mg, *n* = 20) than those from the interior site (mean 0.00276 mg, *n* = 20). No *R. minor* plants survived to flower

291 and seed on the riparian site.

292

293 Experiment 2: Lag effects of altered hydrology

294 Comparisons of *Caltha palustris* leaf metrics at the outset of the experiment indicated that there were no

295 significant differences in leaf width (ANOVA F (2, 32) = 1.42, *P* = 0.246), nor abundance (GZLM chi-square

296 (2, 32) = 2.817, *P* = 0.245), between the three treatments. However, leaves were significantly longer for control

297 plants compared to plants in the lift and transplant treatments (ANOVA F (2, 32) = 12.33, *P* < 0.001; Tukey).

298

299 All 32 control plants in the riparian site survived all three growing seasons of the experiment. The 32 plants that

300 were lifted and replaced in the riparian site also survived the first season, but survival fell to 87.5 % in the

301 second year and was similar in the third year at 84.4 %. Thirty-two plants were transplanted from the riparian to

302 the interior site and all of these survived the first season but then survival declined to 78.1 % in the second year

303 and 59.4 % in the third year.

304

305 Leaf abundance for *C. palustris* was analysed for late April or early May across all years and treatments. There

306 were annual variations in the number of leaves, as shown by the control plants, which fluctuated from a mean of

307 11 leaves per plant in the first year to 34 in the second year and then 29 in the third year (Fig. 4a). Significantly

308 more leaves were produced by the control plants than by plants in the other two treatments in the first two

309 seasons (Fig. 4a). In the third season, plants in the lift treatment produced on average more leaves than in

310 previous years, resulting in significant differences between all three treatments (Fig. 4a). Transplanted plants

311 had consistently relatively low numbers of leaves throughout the three seasons.

The length and width of all leaves were measured weekly throughout the three growing seasons. Both variables were highly positively correlated (Pearson product-moment r = 0.826 $P < 0.001$, $n = 259$) so only maximum leaf width is shown here (Fig. 4b). Leaves on control plants were significantly larger than on plants in the two other treatments in the first season (Fig. 4b). In the second and third years, leaves on transplanted plants were significantly smaller than those produced by plants in the control and lifted treatments (Fig. 4b).

In the first year of the experiment, 78 % of *C. palustris* plants in control conditions flowered, followed by 97 % in the second year and 100 % in the third year. This compared to 47 % of lifted and transplanted plants in the first year. Plants in the lift treatment then recovered well with 86 % and 100 % of surviving plants flowering in the second and third years, respectively. Surviving transplanted plants failed to sustain improvement, with 76 % flowering in the second year and 58 % in the third year.

Counts of the total number of flowers produced, irrespective of the number of plants flowering, show that peak flower production for control plants was greater and earlier in the first season (42 on 11th April) than for lifted (32 on 25th April) or transplanted (34 on 18th April) plants (Fig. 5). A similar pattern was observed in the second year, but with a much higher maximum total flower count (234) later in the season (21st April) for the control plants (Fig. 5). In the third year, control plants produced a larger maximum total number of flowers (252) than lifted (132) or transplanted (34) plants, although for the lifted plants this represents a substantial increase on previous years (Fig. 5). The peak flowering period for transplanted plants was later than for plants in the other two treatments.

Average peak flower abundance per plant in the first season was not significantly different between treatments (Fig. 6). The following season, production increased substantially for control plants and was significantly greater than the other two treatments, although a more modest increase was shown by lifted plants (Fig. 6). A further increase in the number of flowers produced by lifted plants was recorded in the third season, while transplanted plants continued to produce relatively few flowers, resulting in significantly lower production in the transplant treatment compared to the other two treatments (Fig. 6).

341 **Discussion**

342 Immediate impacts of contrasting hydrologies

343 Survival, flowering and other performance metrics for *Primula veris, Lathyrus pratensis* and *Rhinanthus minor*

344 within one growing season were better in unsaturated control conditions than at the two wet grassland field sites.

345 Survival and flowering were variable at the interior field site with its seasonally wet hydrology, and except for

346 *P. veris* poor at the riparian site with sustained high groundwater levels. This indicates immediate hydrological

347 impacts on plant performance related to their ability to adapt or tolerate water regimes (Bailey-Serres and

348 Voesenek 2008). Effects may have been mediated through plant competition as control plants avoided this,

349 unlike plants in the field. In addition, the combination of compost and tap water in control conditions may have

350 provided a more nutrient-rich medium than the field sites, enhancing biomass, flowering and seed production

351 (Kirkham and Wilkins 1994).

352

353 There was a differential impact of contrasting hydrologies on species performance related to their traits. Of the

354 three species, *P. veris* survived and flowered best under the different water regimes and, although aboveground

355 biomass was much lower at the field sites, belowground mass was not significantly affected. This may be

356 because the species can adapt to saturated soil by producing lateral roots close to the surface (Brys and

357 Jacquemyn 2009), rather than the deeper taproots that are developed in drier conditions. *P. veris* is also more

358 tolerant of stress than the other species in this experiment (Table 1), which may not only have allowed it to

359 persist in the field environment but could also account for the greater proportion of plants flowering in the

360 waterlogged riparian site than the interior site (Tito et al. 2016). In addition, this species flowers early in spring

361 and therefore may have escaped grazing and intensifying competition from the growing vegetation (Grime

362 1977).

363

364 The worst performing species in contrasting water regimes was *R. minor*, which was rapidly lost from the

365 riparian site and produced significantly lighter seeds at the interior site compared to the control plants. There is

366 limited research about seed weight and hydrology, although studies of crops found that waterlogging does not

367 reduce seed weight (Ahmed et al. 2002; Xu et al. 2015). It is surprising that this species failed at the field sites

368 because it is tolerant of saturated soils, and found in flood-meadows, spring flushes and mires (Westbury 2004).

369 There was no evidence of grazing of this species but it is possible that the sward was too dense for the plants to

370 establish and thrive (Westbury 2004) when it was introduced into the floodplain later in the season.

371

L. pratensis performed rather poorly overall in the field in terms of survival and root production, although root

length could have been affected by the more difficult removal of plants in the field than from control conditions.

L. pratensis was impacted by preferential grazing in the field, likely because it is a leguminous species able to

fix atmospheric nitrogen and provide nutritious forage (Lee 2018). Wright et al. (2016) concluded that the

growth of legumes is negatively affected by flooding irrespective of their plant community, which may explain

the similarity in root lengths between the interior and riparian field sites. Evidence from a range of other

legumes suggests that waterlogging negatively affects tap root length, although lateral roots may compensate

(Gibberd et al. 2001). In this study, however, survival was much lower on the riparian site with sustained high

groundwater levels than the interior site where water levels receded.

Lag effects of altered hydrology

There was evidence of two types of lag effects due to altered hydrology after *Caltha palustris* was transplanted

from the wetter riparian zone to the drier interior floodplain: a decline in survival and flowering over time, and

constrained leaf and flower production. The interior site represents sub-optimal hydrology for this species,

which probably contributes to its natural absence from the interior grassland. Grootjans et al. (1996) suggest

that *C. palustris* may survive in less favourable environmental conditions for up to 14 years, but the initial

population will not increase. In this three-year experiment, no new *C. palustris* plants appeared at the interior

site. Instead, there was a sustained decline in plant performance, such that survival of plants transplanted into

the interior site decreased annually. An additional field survey, in April five years after the experiment was

established, confirmed this trend, with survival amongst the transplanted plants falling to 31 % (from 59 % two

years earlier) compared to 78 % (from 84 %) for lifted plants and 100 % survival continuing for the control

plants. Only 20 % of the transplanted plants were in bud and/or flowering, compared to 92 % for lifted and 94

% for control plants. The second hydrological effect manifested as a lack of recovery following transplantation

to the interior site, shown by significantly reduced leaf size and abundance compared to other plants, and the

suppression of flowering frequency and abundance. Sherry et al. (2011) also report lagged flowering effects

following climate events, with delayed flowering in a number of grassland forb species the year after higher

temperatures and rainfall.

400 Plant performance was affected by the disturbance of transplantation, as indicated by plants that had been lifted

401 and replaced within the riparian zone. These showed significantly reduced leaf abundance and size, and

402 flowering, then recovery in later years. Survival of lifted and replaced plants decreased in the second year but

403 barely so in the third year. Transplant experiments examining differences between ecological and geographical

404 distance from source populations suggest that, in general, ecological distance has a more significant effect on

405 plant performance (Raabová et al. 2007; Bowman et al. 2008). Results from Joshi et al. (2001) and Ågren and

406 Schemske (2012) using a range of forb species suggest that transplanted plants have a higher fitness level when

407 planted back into their original source location than within an ecologically different space. Thus, whilst

408 geographical distance was negligible and direct disturbance impacts were mostly short-lived here, the ecological

409 distance created by different hydrological regimes was substantial enough to induce lasting effects on plant

410 performance. It is also possible that the interactive effects of transplantation and hydrological alteration

411 combined to produce detrimental performance by plants.

412

413 There were annual fluctuations in plant performance, highlighted by the control plants that remained

414 undisturbed by experimental treatments throughout. Leaf and flower abundance increased markedly from the

415 first to second year, with little change in the third year. Such variations are likely due to the prevailing weather

416 conditions at the field sites. The first year was exceptionally warm and wet, with high groundwater levels until

417 early summer, so plants may have been stressed, prompting earlier but subdued flowering. The second and third

418 years were more benign and production increased. There is evidence to suggest increased spring temperatures

419 that induce earlier flowering (Jentsch et al. 2009) also reduce flower abundance (Saavedra et al. 2003;

420 Semenchuk et al. 2013), and increased temperatures may shorten the flowering period (Sherry et al. 2011) in a

421 range of ecosystems (Jolly et al. 2005). Unsuitable weather for plant growth in the first season of the

422 experiment may also have exacerbated any detrimental impacts of experimental disturbance.

423

424 **Conclusions**

425 This field research has shown that floodplain grassland plants may be susceptible to the type of rapid and

426 substantial hydrological change facilitated by climate events. The study has demonstrated that plant functional

427 metrics provide a valuable means of assessing the consequences of hydrological change. Sensitive species or

428 those unable to tolerate stressful conditions may succumb rapidly to hydrological change, within one season,

429   while others could show detrimental effects for years by declining or through constrained plant performance.

430   Reduced flower availability could impact pollinator populations. Differential responses by plant species, as a

431   consequence of their functional traits, will change community composition. This is likely to be detrimental for

432   associated wetland biodiversity such as invertebrates and birds, particularly as vegetation structure plays a key

433   role in nest site selection for wet grassland birds (Durant et al. 2008). Other ecosystem services affected by

434   vegetation change include carbon and nutrient cycling if more stress-tolerant species prevail at the expense of

435   ruderal or nitrogen-fixing species and agricultural production if legumes are lost. The lag effects of

436   hydrological events, indicated over several years in this study, highlight the need to consider the resilience,

437   longer-term monitoring and management of floodplain and other wetland ecosystems in the face of climate

438   change.

439

440   **Conflict of interest**

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

442

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571

572 Table 1. Summary of traits for the plant species used in the field experiments.

Species	Life strategy[1]	Ellenberg[2]			Clonal growth	Life History
		F	R	N		
Primula veris	S/C-S-R	4	7	3	Rhizome	Perennial
Lathyrus pratensis	C-S-R	6	6	5	Rhizome	Perennial

Rhinanthus minor	R/C-S-R	4	7	3	N/A	Annual
Caltha palustris	C-S-R	9	6	4	Secondary	Perennial

573 [1]Life strategy: Competitor - Stress tolerator – Ruderal classification (Grime, 1977)

574 [2]Ellenberg Indicator Values for moisture (F), Reaction or pH (R), nutrient level (N) (Hill et al. 1999)

575

576

577 Table 2. Plant survival as % of those planted, with % of the surviving plants that flowered in parentheses. Twenty

578 four plants of each species were originally planted for each treatment, except for *Rhinanthus minor* of which 20

579 were planted.

	Control	Interior	Riparian
Primula veris	96 (70)	100 (83)	96 (100)
Lathyrus pratensis	88 (81)	75 (6)*	25 (0)*
Rhinanthus minor	70 (50)	10 (100)	0 (0)

580 * evidence of grazing

581

582 Fig. 1. Groundwater levels at the riparian and interior sites at the Amberley Wildbrooks study area. Mean (±1

583 SE) water levels for three years combined (2014-2016) are shown. Early water levels are measured from the1st-

584 14th of the month. Late water levels are from the 16th-31st of the month.

585

586 Fig. 2. Mean (±1 SE) above and belowground biomass of *Primula veris* under three treatments with contrasting

587 hydrologies. Treatments that do not share the same letter annotation are significantly different for aboveground

588 biomass (MANOVA F (2, 68) = 30.143, $P < 0.001$; Tukey at $P < 0.05$). $n = 23$ for the control and riparian

589 treatments, $n = 24$ for the interior treatment.

590

591 Fig. 3. Root length for *Lathyrus pratensis* under three treatments with contrasting hydrologies. Horizontal lines

592 represent medians, boxes quartiles, whiskers the range, and the asterisk an outlier. Treatments that do not share

593 the same letter annotation are significantly different (Kruskal-Wallis H (2, 43) = 25.44, $P < 0.001$; Dunn's test at

594 $P < 0.05$).

595

596   Fig. 4. Mean (±1 SE) leaf a) abundance and b) maximum width for *Caltha palustris* in three growing seasons in

597   three treatments investigating altered hydrology. Treatments that do not share the same letter annotation each

598   year are significantly different (For a) GZLM chi-square = 17.438 for 2014, 72.413 for 2015, 53.429 for 2016, *P*

599   < 0.001 each year; LSD at *P* < 0.05. For b) ANOVA F = 8.426 for 2014, 13.458 for 2015, 15.154 for 2016, *P* <

600   0.001 each year; Tukey for 2014 and Tamhane's T2 for 2015 and 2016 at *P* < 0.05). *n* = 32, except 2015 lift *n* =

601   28 and transplant *n* = 25, 2016 lift *n* = 27 and transplant *n* = 19.

602

603   Fig. 5. Total number of open *Caltha palustris* flowers at each survey date in three growing seasons for three

604   treatments investigating altered hydrology.

605

606   Fig. 6. Mean maximum number of flowers per flowering plant in three growing seasons for three treatments

607   investigating altered hydrology. Treatments that do not share the same letter annotation each year are

608   significantly different (GZLM chi-square = 0.354 *P* = 0.838 for 2014, 32.602 *P* < 0.001 for 2015, 8.554 *P* =

609   0.014 for 2016; LSD at *P* < 0.05). For control, lift and transplant treatments respectively, *n* = 25, 15 and 15 in

610   2014, 31, 24 and 19 in 2015, and 32, 27 and 11 in 2016.
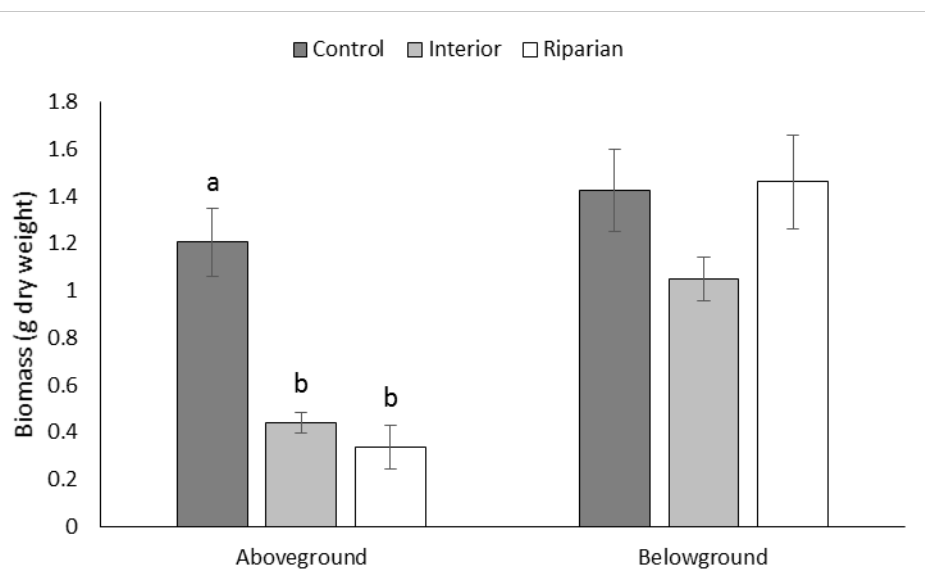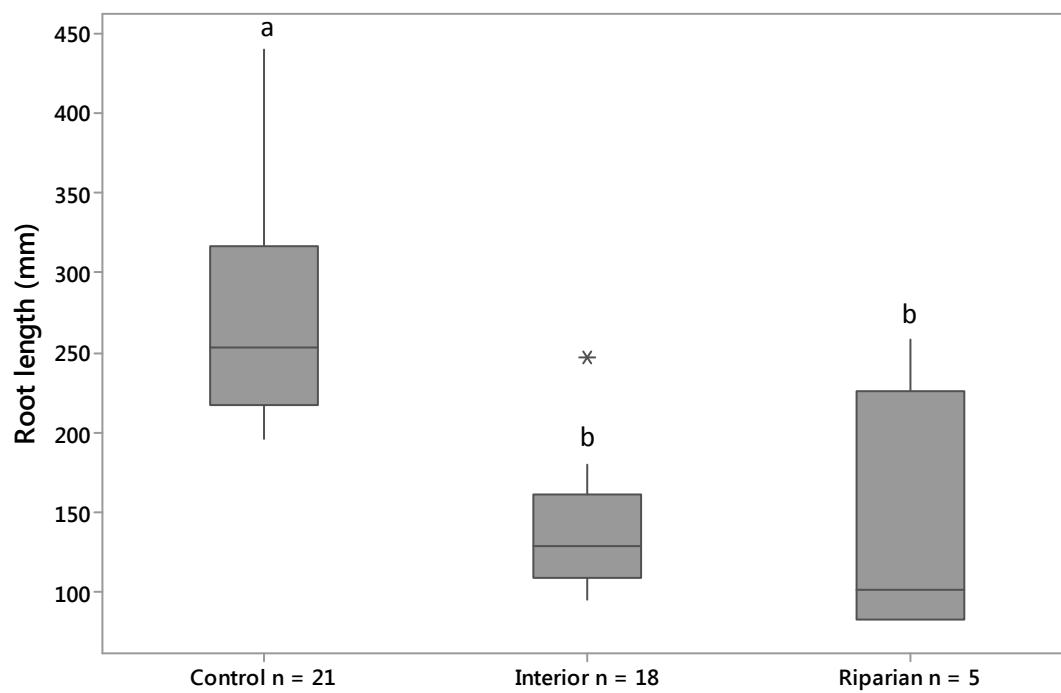
611
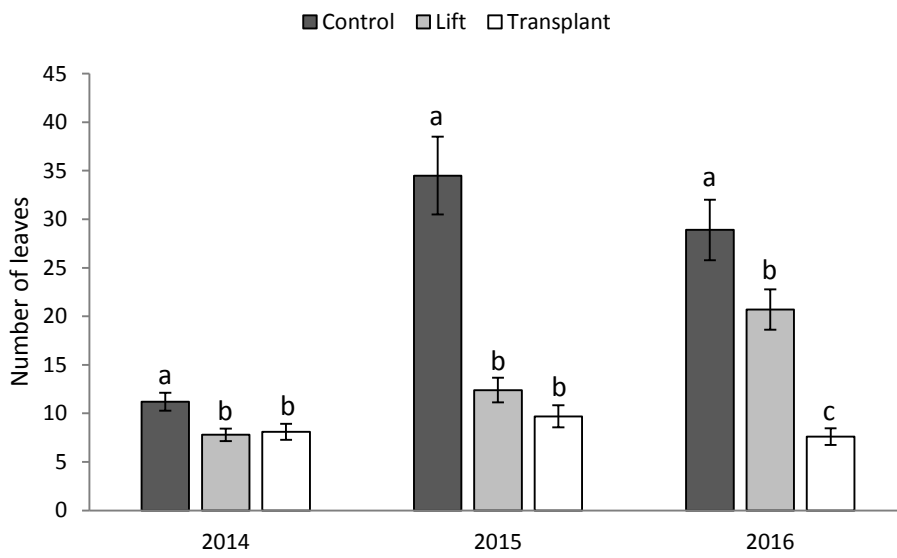
612 Fig. 1
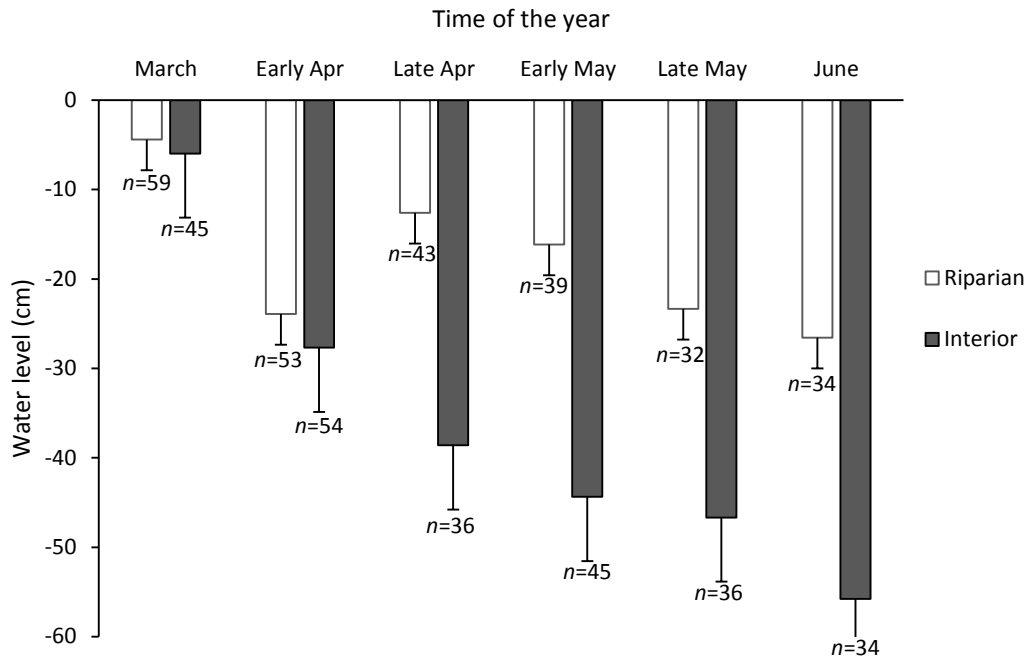
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615   Fig. 2

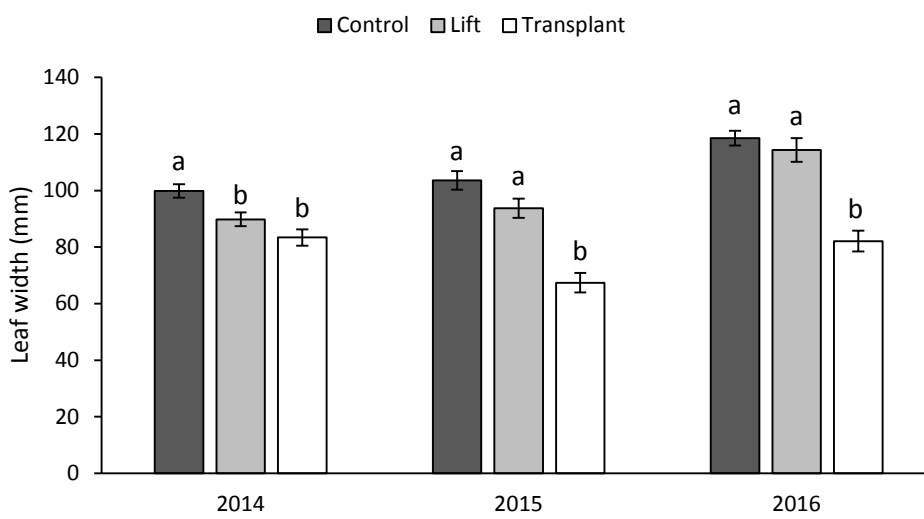

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618   Fig. 3

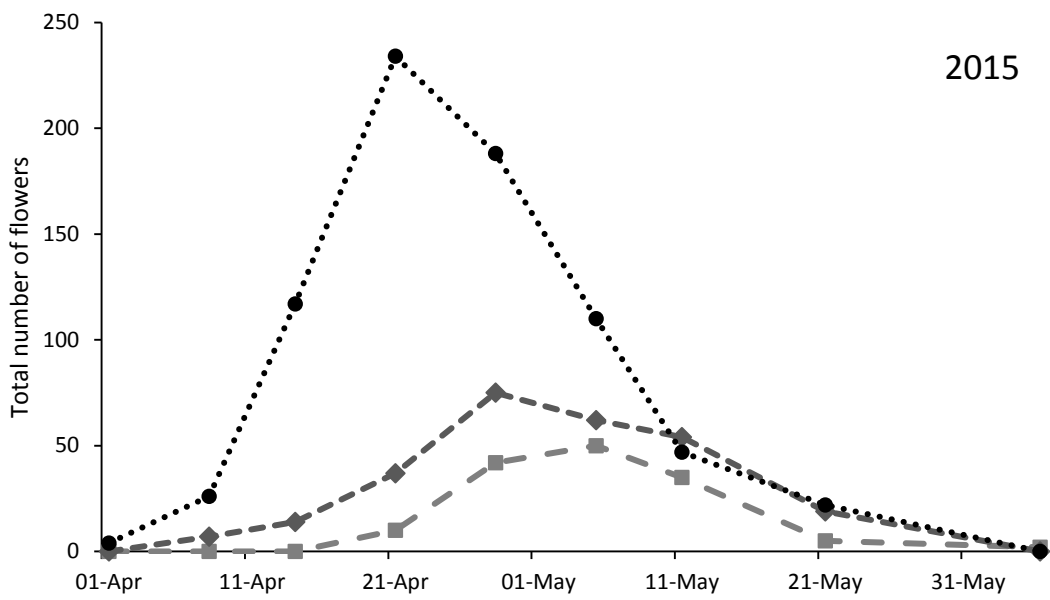
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622   Fig. 4
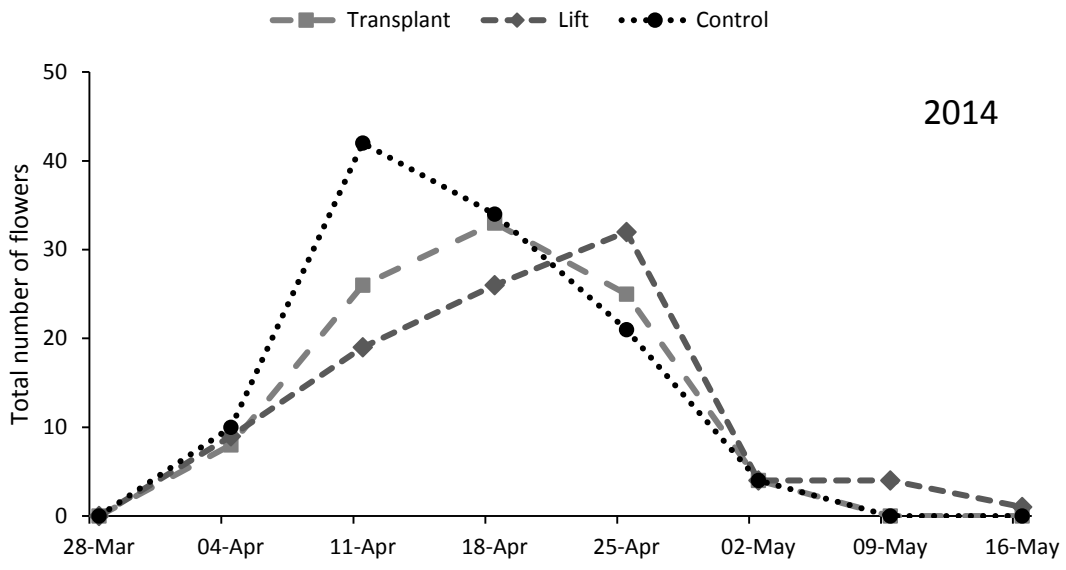
623   a)

624

625   b)

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627

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632

633   Fig. 6

Number of flowers — Control, Lift, Transplant — 2014, 2015, 2016

634

635