Simulated seawater flooding reduces oilseed rape growth, yield, and progeny performance

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Running Head: *Seawater flooding reduces oilseed rape yield and seedling performance*

1 • **Background and Aims** Of the many threats to global food security, one of the most

2 pressing is the increased incidence of extreme weather events. In addition to extreme

3 rainfall, a combination of global sea level rise and storm surge is likely to result in

4 frequent episodes of seawater flooding in arable systems along low-lying coasts. Our

5 aim was to elucidate the effects of simulated seawater and freshwater flooding on the

6 survival, growth, and reproductive potential of four cultivars of the important seed

7 crop, *Brassica napus* (canola, or oilseed rape [OSR]).

8 • **Methods** Established plants were exposed to 24- or 96-hour freshwater or seawater

9 root zone immersion (with a no immersion 'control'). Initial post-treatment

10 performance over 7-weeks was quantified using dry weight biomass. A second group

11 of plants, cultivated until maturity, were used to quantify reproductive yield (siliqua

12 and seed number, and seed size) and subsequent progeny performance (germination

13 and seedling growth).

14 • **Results** OSR growth and reproductive responses were unaffected by freshwater, but

15 seawater negatively affected growth and siliqua number for all cultivars, and seed mass

16 for two (Agatha & Cubic). In addition to impacts on crop yield, the growth of seedlings

17 cultivated from seed collected from maternal plants subjected to seawater immersion

18 was also reduced.

19 • **Conclusions** Our results demonstrate the potential impact of seawater inundation on

20 coastal cropping systems; although OSR may survive acute saline flooding, there are

21 longer-term impacts on growth and yield for some cultivars. The threat may necessitate

22 changes in land-use practice and/or the development of salt-tolerant cultivars to

23 maintain economically viable yields. In addition, by evidencing a hitherto unknown

24 effect on reproductive performance (i.e. reduced seed yield) and subsequent seedling

25   growth our study highlights an important potential impact of coastal flooding on plant

26   community dynamics for (semi) natural habitats.

27   **Key Words –** Flooding, Food security, Osmotic Stress; Salinity, Sea-level rise; Storm

28   surge

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32   INTRODUCTION

33   Food security and climate change are two of the most pressing environmental issues facing

34   the planet (Godfray *et al*., 2010; IPCC, 2014). When combined however, the increased

35   food demand imposed by a growing human population coupled with global-scale shifts in

36   temperatures, precipitation, and sea-level rise will inevitably impose significant demands

37   on agriculture (Parry *et al*., 2005; Lobell *et al*., 2008). Much of the environmental threat

38   from climate change stems from acute extreme events rather than simply longer-term

39   chronic change alone (Rahmstorf & Coumou, 2011; Vasseur *et al*., 2014; Parmesan &

40   Hanley, 2015), and of the former, flooding represents one of the greatest challenges. Future

41   climate scenarios consistently predict major regional shifts in the intensity of extreme

42   precipitation episodes across the globe and concomitant increases in large-scale regional

43   flooding along river catchments (Li *et al*., 2013; Singh *et al.,* 2013; IPCC, 2014). In

44   addition to fluvial flooding however, a combination of changes in sea-surface

45   temperatures, weather patterns, and sea-level rise are predicted to increase the frequency

46   and severity of oceanic storm surges (Vousdoukas *et al*., 2016; Vitousek *et al*., 2017). The

47   result will be that many low-lying coastal areas face an increased risk of seawater

48   inundation over coming decades (Nicholls & Cazenave, 2010; Hoggart *et al.,* 2014). When

49   taken together, extreme precipitation, storm surge, and sea-level rise are likely to cause

50   significant flood risk to global agriculture and as a result understanding crop response to,

51   and tolerance of, coastal flooding has become a research priority (Jackson & Ismail, 2015).

52   Although flooding can cause significant losses at any time of the crop production cycle,

53   yields are most likely to suffer if flooding occurs during critical development stages

54   (Rosenzweig *et al.,* 2001; Parry *et al.,* 2005). For the United Kingdom for example,

55 extreme winter and spring precipitation is projected to increase (Fowler *et al*., 2010) at a

56 time when many arable crops are establishing ahead of summer harvest. This is one reason

57 why the severe, but localised, 2014 winter floods that affected only a small portion of

58 southern England (14,000 Ha), caused economic losses estimated at £6.9 million in arable

59 systems alone (ADAS, 2014). Globally, severe freshwater and seawater flooding has long

60 been identified as a significant economic problem for agriculture, and one that poses an

61 additional regional threat to human nutrition (Page & Williams, 1926; Mirza, 2002; Chau

62 *et al*., 2015).

63 The impact of flooding on crop plants is well known, although research has almost

64 exclusively focussed on the effects of freshwater inundation. Flooding typically causes soil

65 anoxia, as O_2 is consumed without replacement, and the accumulation of various ions

66 (Mn^{2+}, Fe^{2+}, S^{2+}) and compounds detrimental to plant growth, while submergence also

67 denies plant tissues access to O_2 and CO_2 (Flowers & Colmer, 2008; Perata *et al*., 2011).

68 All of these factors can limit significantly crop growth and productivity (Malik *et al*., 2001;

69 Perata *et al*., 2011; Ren *et al*., 2014; Mutava *et al*., 2015). Due its high salinity (typically

70 about 35 gL^{-1} salt of which chloride and sodium contribute 19 gL^{-1} and 11 gL^{-1}

71 respectively), seawater flooding imposes additional osmotic and ionic stresses; the former

72 limits the plant's ability to absorb water and the latter can result in toxicity through the

73 accumulation of Na^+ and Cl^- in tissues (Munns & Tester, 2008). As a response, plants have

74 adapted to avoid or lessen the impact of salt ions via the synthesis and accumulation of

75 stress metabolites and the regulation of other tissue ions (i.e. K^+) to exclude or

76 compartmentalize Na^+ and Cl^- and re-establish homeostatic function (Maathuis &

77 Amtmann, 1999; Munns & Tester, 2008).

78   From the perspective of agricultural production, even if crop plants survive freshwater or

79   seawater flooding, any impact on growth or reproductive potential is of concern. Our

80   understanding of the effects of salinity on crop performance is, however, focussed largely

81   on soil salinization in arid cropping systems (Pitman & Läuchli, 2002; Albacete *et al*.,

82   2008; Flowers *et al*., 2010), and only a handful of studies have examined how

83   agriculturally important species respond to short-duration immersion in seawater. White *et*

84   *al.* (2014) report how immersion in seawater for only 24-hr resulted in the accumulation

85   of Na^+ and Cl^- in leaves of the pasture crop white clover (*Trifolium repens*). A concomitant

86   increase in two key organic solutes (proline and sucrose) to re-establish osmotic balance

87   was also observed, but nonetheless flooded plants showed reduced growth and flowering

88   potential. Interestingly White *et al*. (2014) also found variation between the responses of

89   three different ecotypes to seawater immersion, suggesting that the relative tolerance of

90   one ecotype could be of value in producing flood-tolerant cultivars for cultivation in low-

91   lying coastal pastures. Sun *et al*. (2015) is the only study to look at effects of seawater

92   immersion on arable crops, their experiment focussing on the responses of 1 month old

93   plants of ten crop plants (six of which were Brassicaceae) to 24-hr seawater immersion.

94   All crops were negatively affected by immersion, with two (cucumber and Chinese greens)

95   suffering complete mortality and the remainder showing reduced growth. Since plants

96   were harvested only 2 weeks after immersion treatments were imposed, it is unclear

97   however, what potential any of the surviving crops had for long-term recovery or whether

98   other key aspects of the crop production cycle (flowering & fruit/seed production) were

99   affected by flooding.

100   The aim of this study was to determine how exposure to simulated seawater and freshwater

101   flooding affected both immediate growth and longer-term reproductive performance of the

102   widely cultivated crop canola, or oilseed rape (OSR - *Brassica napus* L.). Beyond gaining

103   an understanding of the short-term impact of flooding on mortality and growth of

104   established plants, we also test the hypothesis that impacts on reproductive potential and

105   subsequent seedling performance vary across four different cultivars. Elucidation of these

106   responses is important for several reasons; OSR is grown primarily for seed yield and thus

107   any impact of prolonged fluvial or seawater flooding on reproductive output has potential

108   economic repercussions. In addition, establishing variation in growth and yield responses

109   may help identify established genetic materials from which to develop flood/salinity

110   tolerant cultivars. A more general understanding of plant growth and reproductive

111   responses to acute seawater flooding is also lacking in the literature (see Hanley *et al*.,

112   Submitted), and no study to date has tested the hypothesis that progeny performance is

113   affected by the exposure of parent plants to short-duration seawater immersion.

114   Consequently, our study offers an important insight into the longer-term impacts that this

115   increasing environmental issue may have on natural, as well as agricultural, plant species

116   and communities.

117   MATERIALS AND METHODS

118   *Study species*

119   Oilseed rape is a hybrid of *B. rapa* and *B. oleracea* (Chalhoub *et al*., 2014), wild forms of

120   the latter being a coastal species with some natural salt tolerance (Snogerup *et al*., 1990).

121   OSR is grown globally and its seeds are harvested to produce food and bioenergy oils, and

122   for use as an animal feedstock. It accounts for 14% (by area) of agricultural land use in the

123   UK (Garthwaite *et al*., 2018) and major producers globally include the EU, Canada, India

124   and China (USDA, 2015), regions likely to experience significant flooding events over

125   coming decades (IPCC, 2014). The OSR cultivars used in these experiments were Cracker

126   (LS Plant Breeding, Impington, UK), Agatha, Astrid, and Cubic (Grainseed Ltd., Eye,

127   Suffolk, UK). All are commonly cultivated in the UK.

128   *Plant cultivation*

129   One hundred seeds of each cultivar were germinated in 90 mm-diameter Petri dishes

130   containing two layers of Whatman No. 1 filter paper moistened with 5 ml of distilled water,

131   maintained in an incubator at 16.5 ℃ on a 12:12 light:dark cycle. On germination (radicle

132   emergence), seventy seedlings per cultivar were transplanted individually into 50mm

133   diameter, 50mm deep plastic pots containing John Innes No.2 potting compost and grown

134   on in a naturally lit greenhouse with weekly watering with tap water, until early November

135   2014. At this time seedlings possessing at least the first true leaf (equivalent to OSR

136   growth-stage 1.1; Sylvester-Bradley, 1985), were re-potted into larger 110 x 110 x 120

137   mm plastic pots containing John Innes No. 2 potting compost. Greenhouse temperatures

138   during this cultivation period were: mean daily minimum = 10.4 ℃ (±0.4 ℃ SE); mean

139   daily maximum = 25.7 ℃ (±1.0 ℃).

140   *Effect of immersion on growth*

141   When the plants were 67-d-old (early December 2014), twelve individuals from each

142   cultivar (OSR growth stage 1.3-1.6; i.e. emergence of third-sixth true leaf) were allocated

143   at random to one of five treatment groups. These were 24-, or 96-hrs immersion in seawater

144   (*Sw*) (collected from Plymouth Sound - electrical conductivity = 45.51 mS cm^{-1} at 16.2

145   ℃); 24-, or 96-hrs immersion in deionised water (*Dw*); or a no-immersion control

146   treatment. Although river floodplains can experience much longer periods of immersion,

147   sometimes extending to several months (Van Eck *et al*., 2004; Muchan *et al*., 2015), in this

148 way we simulated the average 1-d long seawater flooding event reported for low-lying UK

149 coastline habitats and extended the period to the maximum reported flood duration of 4-d

150 (Environment Agency, 2014).

151 We recognise that inundation following coastal storm-surge or fluvial flooding events

152 would likely result in full shoot submergence, but by immersing to pot-level (in large

153 plastic tubs) our approach allowed us to separate the effect of ionic imbalance in the root-

154 zone rather than the impact of oxygen deficiency caused by full immersion that both

155 treatments would impose. Immediately after immersion, the pots were allowed to drain

156 fully before being arranged randomly on a wire mesh-topped bench inside the greenhouse;

157 the wire mesh allowed free drainage and prevented cross-contamination between treatment

158 groups. The pots were watered to capacity (with tap water) 48-hr after seawater immersion.

159 Eight plants per treatment/cultivar combination were retained inside the greenhouse; the

160 remainder were positioned outside, on adjacent elevated mesh. For both sets of plants, each

161 individual was positioned at random, 20cm apart from its nearest neighbour in 1m long

162 rows separated by 30cm to simulate the recommended field density for OSR cropping

163 systems in the UK (HGCA, 2014).

164 Greenhouse plants were watered twice weekly for a further 56-d; temperatures during this

165 phase of the experiment were 4.9 °C ($\pm$ 0.5) min and 18.0 °C ($\pm$ 0.6) max. Surviving plants

166 (growth stages 2.0-2.3; i.e. plant has a rosette growth form and extended stem with

167 internodes) from each treatment/cultivar group were harvested at 113-d-old (late January

168 2015), cleaned of any adhering compost and oven-dried at 50 °C for 24-hr. A Levene's test

169 for homogeneity of variance across treatment and cultivar levels was negative ($F_{19,189} =$

170 2.896, $P < 0.001$) and biomass data were $\log_{10}$ transformed, resulting in a positive Levene's

171   test ($F_{19,189} = 1.335$, $P = 0.165$). Univariate GLM analyses were used to compare the results

172   of experimental flooding on transformed biomass. Rather than classifying plants in

173   different groups into two separate factors (water: *Dw vs. Sw*, and time: 24h *vs*. 96h), which

174   would decrease the power of the tests, contrasts were employed to evaluate specific

175   differences between treatment levels (control, *Dw*24, *Dw*96, *Sw*24 and *Sw*96). In addition

176   to the *F* statistic and its probability, we report effect sizes (η_p^2) and power at P<0.05

177   (power$_{0.05}$).

178   *Effect of immersion on crop yield and progeny performance*

179   The four individual plants from each treatment/cultivar group positioned on elevated

180   outdoor standing were grown to reproductive maturity in order to assess the effects of

181   immersion on seed yield. Plants were exposed to natural weather conditions and watered

182   (to capacity with tap water) only following prolonged dry periods. Since individuals were

183   randomly arranged with respect to treatment and cultivar, they were able to cross-

184   pollinate and so resulting progeny were most likely hybrids between cultivars. Three

185   plants (Agatha 24-hr *Dw*; Cracker 96-hr *Dw*; Agatha 96-hr *Sw*) died during this period.

186   The main stems of plants were harvested in mid-June when most siliqua were fully

187   ripened (growth stage 9.9). We quantified the total number of siliqua per plant, and from

188   six (randomly selected) siliqua per plant, quantified seed number and mean individual

189   seed mass per pod (averaging across all 6 replicate siliqua for each plant).

190   All seeds form each of the six sampled siliqua per plant were pooled. From these, 40

191   seeds were set to germinate in 90 mm-diameter Petri dishes containing two layers of

192   Whatman No. 1 filter paper moistened with 5 ml of deionised water, maintained in an

193   incubator at 18ºC on a 12:12 light:dark cycle. In addition to the three plants that died

194 before harvest, a further two parent plants - Cracker 96-hr *Dw* & Agatha 96-hr *Sw* –

195 failed to yield sufficient seeds for the germination/seedling growth trials. Petri dishes

196 were checked daily for 14 days; on germination (appearance of the radicle), seedlings

197 were counted and removed. Six seedlings from each Petri dish were retained, and planted

198 into a 50-mm diameter pot containing John Innes No.2 potting compost. These seedlings

199 were grown in controlled conditions (15ºC on a 12:12 light:dark cycle with daily

200 watering to capacity) until 14-d-old when harvested and oven dried (at 50 °C for 24-hr)

201 to determine dry weight biomass.

202 Levene's tests of homogeneity of variances were significant for siliqua number ($F_{19,57} =$

203 2.621, $P = 0.003$), seed number ($F_{19,56} = 2.962$, $P < 0.001$) and seedling biomass ($F_{19,498}$

204 $= 2.291$, $P = 0.002$), but did not show departure from homogeneity for seed mass ($F_{19,56} =$

205 1.264, $P=0.244$). Logarithmic transformation of the former three homogenised the

206 variance for siliqua number ($F_{19,57} = 1.304$, $P=0.217$), but not for seed number ($F_{19,56} =$

207 10.625, $P < 0.001$) and seedling mass ($F_{19,497} = 3.289$, $P < 0.001$). Consequently, we

208 present the results of GLM's hypothesis testing for; (i) seed mass where the

209 untransformed variable did not depart from homogeneous; (ii) seed number and seedling

210 mass where logarithmic transformation did not homogenise the variance; (iii) log siliqua

211 number where logarithmic transformation resulted in variance homogeneity.

212 <div align="center">RESULTS</div>

213 *Effect of immersion on plant growth*

214 'Treatment' had a significant effect on adult plant biomass with a moderate effect size

215 ($F_{4,189} = 17.71$, $P < 0.001$; $\eta_p^2 = 0.273$; power$_{0.05}$=1; Fig 1), and with the contrasts between

216 control and each of the other four treatment levels significant only for *Sw*96 (Contrast = -

217 0.339, *SE* = 0.066, *P* < 0.001). More specific comparison showed: (i) a negative contrast

218 between both *Sw* treatments *vs*. Control (Contrast = -0.219, *SE* = 0.058, *P* < 0.001); (ii) a

219 small, but significant, positive contrast between *Dw* treatments *vs*. Control (Contrast =

220 0.114, *SE* = 0.057, *P* = 0.048); and (iii) a then obvious positive contrast between *Dw* and

221 *Sw* (Contrast = 0.333, *SE* = 0.045, *P* < 0.001), highlighting the smaller size of plants

222 immersed in seawater. Plant biomass did not vary between cultivars but the cultivar effect

223 size was small ($F_{3,189}$ = 1.85, *P* = 0.140; η_p^2 = 0.029; power$_{0.05}$=0.475). A significant

224 'Treatment × Cultivar' interaction with moderate effect size ($F_{12,189}$ = 2.95, *P* = 0.001; η_p^2 =

225 0.158; power$_{0.05}$=0.989) emphasised several treatment- and cultivar-specific departures

226 from the general trends described above (Astrid and Agatha for example, were both

227 tolerant of 24-hr seawater immersion). No plants died during this first part of the

228 experiment.

229 *Effect of immersion on crop yield and progeny performance*

230 The number of siliqua (Table 1) varied with 'Treatment' with a small effect size ($F_{4,57}$ =

231 5.974, *P* < 0.001; η_p^2 = 0.295; power$_{0.05}$=0.978) but not by 'Cultivar' ($F_{3,57}$ = 2.004, *P* =

232 0.124; η_p^2 = 0.095; power$_{0.05}$=0.489) and there was no interaction ($F_{12,57}$ = 1.283, *P* = 0.254;

233 η_p^2 = 0.213; power$_{0.05}$=0.643). The contrast analyses revealed a significant negative effect

234 of *Dw* and *Sw* treatments compared to the Control (Contrast = -0.103, *SE* = 0.050, *P* =

235 0.046); specifically highlighting lower siliqua numbers in *Sw* vs. Control (Contrast = -

236 0.141, *SE* = 0.055, *P* = 0.014). There was no variation between *Dw* and *Sw* (Contrast =

237 0.76, *SE* = 0.046, *P* = 0.105) or *Dw* vs. Control (Contrast = -0.065, *SE* = 0.056, *P* = 0.250).

238 Seed number per pod (Table 1) was not influenced by 'Treatment' ($F_{4,57} = 2.206$, $P =$

239 0.080; $\eta_p^2 = 0.134$; power$_{0.05}$=0.612), and while varying between cultivars ($F_{3,57} = 20.265$,

240 $P < 0.001$; $\eta_p^2 = 0.516$; power$_{0.05}$=1), there was no interaction with 'Treatment' ($F_{12,57} =$

241 1.31, $P = 0.239$; $\eta_p^2 = 0.216$; power$_{0.05}$=0.655). Mean individual seed mass (Table 1) did

242 however, vary according to 'Treatment' ($F_{4,57} = 5.456$, $P = 0.001$; $\eta_p^2 = 0.277$;

243 power$_{0.05}$=0.965) and 'Cultivar' ($F_{3,57} = 19.658$, $P < 0.001$; $\eta_p^2 = 0.509$; power$_{0.05}$=1),

244 although there was no significant interaction ($F_{12,57} = 1.799$, $P = 0.070$; $\eta_p^2 = 0.275$;

245 power$_{0.05}$=0.822). While contrasts highlighted that *Dw* and *Sw* had an overall difference

246 with Control (Contrast = -0.009, *SE* = 0.004, *P* = 0.036), this was driven primarily by

247 variation between *Sw* and Control (Contrast = -0.010, *SE* = 0.004, *P* = 0.023), and not by

248 the difference between *Dw* and Control plants (Contrast = -0.007, *SE* = 0.005, *P* = 0.122).

249 Interestingly, however, there was no variation between *Dw* and *Sw* (Contrast = 0.003, *SE*

250 = 0.004, *P* = 0.373). These results emphasise the different intensity of effects on different

251 cultivars; i.e., Agatha and Cubic showed the most marked negative responses in the 96-

252 hour *Sw* treatment (Table 1).

253 Germination was unaffected by any of the immersion treatments imposed on parent plants

254 (data not shown); the lowest germination for any one cultivar/treatment group being the

255 76% recorded for seeds produced by cv Cracker 96-hr *Sw* (with 3 of 4 Petri dishes for this

256 group nonetheless attaining >85% germination).

257 Finally, seedling mass (Figure 2) differed between treatments with a small effect size

258 ($F_{4,498} = 6.192$ $P < 0.001$; $\eta_p^2 = 0.047$; power$_{0.05}$=0.988), but not among cultivars ($F_{3,498} =$

259 1.874 $P = 0.133$; $\eta_p^2 = 0.011$; power$_{0.05}$=0.486), and there was a significant 'Treatment $\times$

260 Cultivar' interaction also with a small effect size ($F_{12,498} = 2.295$ $P = 0.008$; $\eta_p^2 = 0.052$;

261   power$_{0.05}$=0.959). Contrasts analysis revealed significant differences between Control and

262   *Dw* & *Sw* together (Contrast = -0.004, *SE* = 0.001, *P* < 0.001), between Control and *Dw*

263   (Contrast = -0.003, *SE* = 0.001, *P* = 0.004), and between Control and *Sw* (Contrast = -

264   0.005, *SE* = 0.001, *P* < 0.001), but not between *Dw* and *Sw* (Contrast = 0.002, *SE* = 0.001,

265   *P* = 0.089). The significant interaction between the two main factors highlights differences

266   in the intensity and direction of cultivar responses. The most affected, Cubic, displayed

267   reduced seedling growth in both the 24-hr and 96-hr treatments, while progeny collected

268   from cv Cracker showed no response to *Sw* immersion (Fig 2). Seedlings grown from

269   Astrid and Agatha parents exhibited reduced growth in the 96-hr *Sw* treatments only,

270   although given the low parental replication for Agatha (only two plants survived to

271   reproduction), the apparent negative response for seedlings collected from this cultivar

272   should be taken with caution.

273   DISCUSSION

274   Our results evidence substantial differences in OSR response to acute freshwater and

275   seawater inundation; while the former exerted some impact on some of the responses

276   examined here, the latter was more generally associated with reduced parent plant

277   growth, seed yield, and even progeny performance. As far as we are aware this is the first

278   time that the impacts of short-duration, acute, *Sw* flooding on several key growth and

279   reproductive responses have been demonstrated across stages of the life-cycle for any

280   plant species. Indeed the only previous study to examine this issue in arable crops, Sun *et*

281   *al*., (2015) was limited to the immediate (2-week) post-immersion response, and while

282   White *et al*., (2014) did look at growth and flowering in white clover over a 70-d post-

283   immersion period, they did not consider seed yield or progeny performance. While there

284   was some variation between cultivars, this study shows that even transitory immersion in

285   seawater has significant, consistent, and long-lasting impacts on OSR crop yield. Indeed,

286   the fact that reduced reproductive output (most notably siliqua number) was manifest 6

287   months after maternal plants were exposed to *Sw* immersion highlights the potential

288   long-term impacts of seawater flooding on arable cropping systems.

289   The consequences of reduced seed yields on agricultural crops like OSR are obvious, but

290   our results also raise the possibility that wild plant species could suffer reduced

291   reproductive success as a result of acute seawater flooding stress on seed development. It

292   is well established that maternal plant environment alters phenotypic expression in

293   progeny (Herman & Sultan, 2011) and as such the reduced growth of seedlings from

294   maternal plants subjected to *Sw* immersion reported here is unsurprising. Nonetheless,

295   and while there is evidence that salinity stress can induce similar shifts in maternal

296   provisioning and seedling performance (for example as shown in the grass *Cenchrus*

297   *ciliaris*; Ruiz & Taleisnik (2013)), the fact that parent immersion in seawater for only 4

298   days can negatively influence subsequent seedling growth, highlights more generally the

299   impact of coastal flooding on plant community dynamics. Put simply, even if parent

300   plants (of any native species) survive prolonged flooding, their later ability to contribute

301   to the recovering community could be compromised. We know of no study to have

302   demonstrated this response. For two of our maternal cultivars (Cubic and Agatha) where

303   96-hour *Sw* immersion reduced mean seed mass by 50% or more, the most plausible

304   explanation for the arrested growth of seedlings is that it resulted from reduced seed

305   provisioning by the parent (Zas *et al.,* 2013). We cannot however, discount the

306   possibility that other responses to salinity stress, including epigenetic changes, impact

307   progeny performance and highlight this as a key area for future research.

308   From the perspective of arable plant species, progeny performance may be of minor

309   importance however, since crops are routinely cultivated from commercially sourced

310   seeds. Nonetheless, we demonstrated a consistent and marked reduction in siliqua number

311   and seed mass for at least two cultivars (Cubic and Agatha) following 96-hr *Sw* immersion,

312   highlighting the significant economic impact that seawater flooding might have on coastal

313   OSR crop yields. While a number of studies have shown that prolonged (i.e. one to several

314   weeks) waterlogging reduces both OSR growth and yield (Cannell & Belford, 1980; Zhou

315   & Lin, 1995; Xu *et al*., 2015), these studies looked at freshwater effects only. Although

316   the relatively short-term *Dw* immersion treatments imposed here did not yield similar

317   responses (but noting that we did not consider seed oil yield or content (Cannell & Belford,

318   1980; Xu *et al*., 2015)), all four cultivars exhibited much reduced plant growth, and later

319   siliqua number in the longer duration *Sw* treatment (see also Hanley *et al*., 2013). This

320   highlights the challenge posed by salinity; even if a plant survives acute seawater

321   immersion it must prevent or alleviate damage caused by the accumulation of salt ions

322   (Na^+ and Cl^-) in tissues. It is interesting that even the most prolonged immersion times (4

323   days) imposed here failed to induce morality; a response that perhaps reflects an innate

324   salinity tolerance due to OSR's heritage in *B. oleracea* and this parent species' natural

325   affinity for maritime conditions (Snogerup *et al*., 1990). Salt tolerance is however, often

326   accomplished by the accumulation of stress metabolites and the regulation of tissue ions

327   to exclude or compartmentalize the potentially damaging Na^+ and Cl^-. Nonetheless even if

328   successfully achieved, as seems to be the case here, there are costs on subsequent plant

329   growth and reproductive performance (Munns & Tester, 2008; White *et al.,* 2014).

330   At a time of human population growth and economic development there are increasing

331   demands on food supplies, but when coupled with unpredictable and likely ever more

332 extreme climate events, global food security is far from assured (Godfray *et al.,* 2010;

333 IPCC, 2014). Flooding is widely recognised as one of the key threats to arable crops, but

334 most research emphasis is placed on pluvial, freshwater flooding where the major negative

335 impact comes from soil anoxia. In the UK the threshold for crop viability under this

336 scenario is 15 days (ADAS, 2014), but as we show here, by virtue of the added effect of

337 salinity, seawater flooding of only 4 days duration can impact OSR yield (while the same

338 duration under freshwater does not). Historically much of the global agricultural

339 salinization problem stems from poor irrigation coupled with excessive evaporation and/or

340 deforestation in hot, dry climates (Vinod *et al*., 2015). Nonetheless, sea-level rise and the

341 expected increase in frequency and severity of storm surges (Vousdoukas *et al*., 2016;

342 Vitousek *et al*., 2017) are likely to increase the risk of seawater inundation to temperate

343 coastal arable systems (Nicholls & Cazenave, 2010). Under these conditions, farmers face

344 a choice between changing land-use practise, or cultivation of flood-tolerant crops. There

345 is a rich literature documenting salt-tolerance in crops grown in regions where the

346 problems of soil salinization are long established; indeed for one of the most important,

347 rice, crops grown near to coasts are frequently subjected to seawater intrusions and a

348 genetic capacity for salt-tolerance has been identified (Ganie *et al.,* 2014). The impact of

349 seawater immersion demonstrated here for OSR, coupled with the increasing risk of

350 seawater flooding for coastal agriculture globally, underscores a new impetus for research

351 into salt tolerance in a wider range of arable crop species (Jackson & Ismail, 2015).

Acknowledgements

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496

497 Table 1: The effect of root-zone immersion in deionised (*Dw*) and seawater (*Sw*) on plant reproductive potential (crop yield) of four

498 oilseed rape (*Brassica napus*) cultivars (Cracker, Cubic, Agatha & Astrid) 7 months after plants (growth stage 1.3-1.6) were subject

499 to transient immersion (24, or 96 hours with a zero hour control). N=4 for all treatment/cultivar combinations except 'Cracker' 96h

500 Dw, and 'Agatha' 24h Dw & 96h Sw where N=3.

501

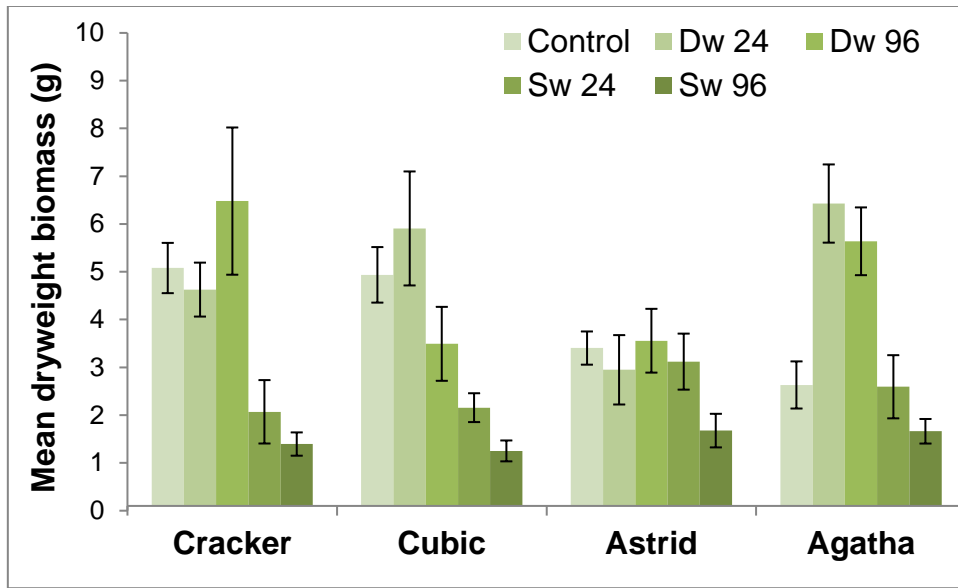| Treatment		Number of siliqua per plant				Seeds per siliqua				Seed mass (mg)			
		Cracker	Cubic	Astrid	Agatha	Cracker	Cubic	Astrid	Agatha	Cracker	Cubic	Astrid	Agatha
Control	Mean	35.5	29.8	22.0	35.8	13.2	22.4	22.3	20.3	0.04	0.08	0.07	0.06
	SE	8.6	2.8	2.4	4.8	2.7	1.2	1.0	1.2	0.009	0.009	0.004	0.005
24h *Dw*	Mean	30.3	18.3	22.8	21.7	16.5	21.6	22.5	17.7	0.04	0.06	0.07	0.04
	SE	3.4	2.7	6.2	3.3	0.6	1.0	2.0	1.0	0.005	0.003	0.01	0.004
96h *Dw*	Mean	40.7	28.0	28.8	27.5	11.4	25.0	20.9	19.9	0.03	0.07	0.06	0.05
	SE	13.2	3.6	2.7	5.0	4.9	0.5	1.1	1.4	0.02	0.004	0.005	0.004
24h *Sw*	Mean	25.8	38.5	25.5	43.8	13.8	22.8	23.0	20.2	0.04	0.08	0.07	0.07
	SE	6.0	11.1	0.9	5.9	1.6	0.8	0.7	1.5	0.004	0.006	0.008	0.006
96h *Sw*	Mean	24.3	11.5	17.5	14.0	13.6	19.4	22.5	9.2	0.03	0.04	0.07	0.02
	SE	6.2	3.2	3.0	5.1	3.2	2.0	1.2	4.7	0.01	0.006	0.006	0.01

502

503

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505

506   Figure Legends

507   Figure 1: The effect of root-zone immersion in deionised (Dw) and seawater (Sw) on

508   mean (± SE) total above-ground dry mass of four oilseed rape (*Brassica napus*)

509   cultivars (Cracker, Cubic, Agatha & Astrid) 2 months after plants (67-d-old; growth

510   stage 1.3-1.6) were subject to transient immersion (24, or 96 hours with a zero hour

511   control). N=8 for all treatment/cultivar combinations.

512

513   Figure 2: The effect of root-zone immersion in deionised (Dw) and seawater (Sw) on

514   mean (± SE) seedling dry mass of four oilseed rape (*Brassica napus*) cultivars

515   (Cracker, Cubic, Agatha & Astrid) of plants grown from seeds collected from parent

516   plants subject to transient immersion (24, or 96 hours with a zero hour control, at

517   growth stage 1.3-1.6). N=4 for all treatment/cultivar combinations except 'Agatha'

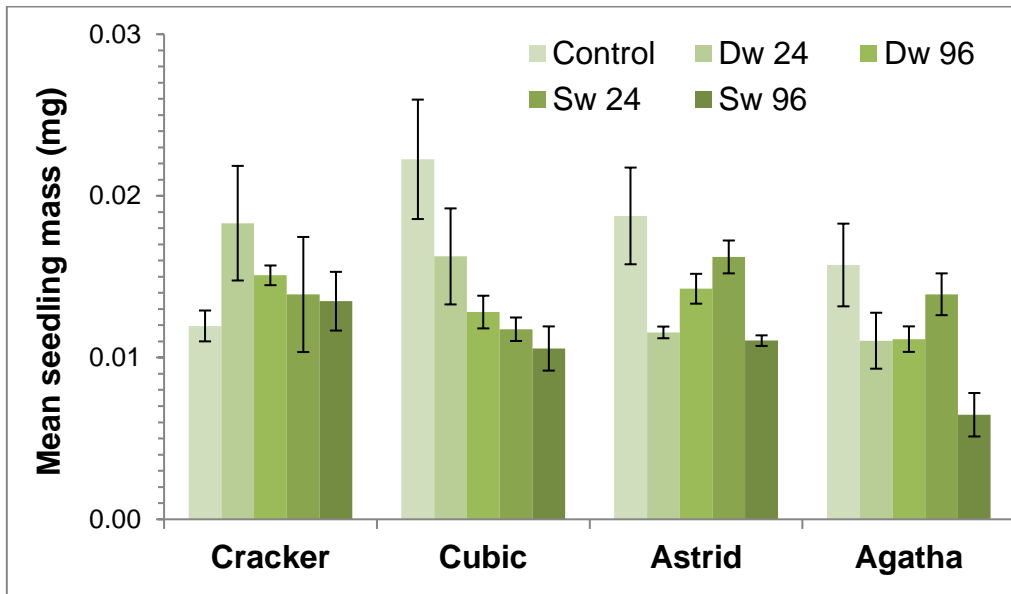518   24h Dw where N =3 and 'Cracker' 96h Dw, & 'Agatha' 96h Sw where N=2.

519

520   Fig 1

521

522

523   Fig 2

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525