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Physiological responses to alternative flooding and drought stress episodes in two willow (*Salix* spp.) clones.

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5 **Physiological responses to alternative flooding and drought stress episodes in two**
6 **willow (*Salix* spp.) clones.**

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26 **Abstract**

27 Climate change will increase the occurrence of flash floods as a consequence of
28 extreme rain events, creating alternate periods of drought and flooding during the growing
29 season. We analyzed the responses of two willow clones with contrasting responses to
30 flooding (clone B: *Salix matsudana* x *Salix alba* hybrid; clone Y: *Salix alba*) to different
31 combinations of stress treatments: continuous flooding or drought for six weeks, or cyclic
32 treatments of two weeks of stress separated by two weeks of watering at field capacity.
33 Drought reduced growth, stomatal conductance and total leaf area in both clones, but
34 flooding did not. Flooding reduced the root/shoot ratio in both clones. The hydraulic
35 conductivity of the main stem was significantly reduced by drought only in clone Y. The area
36 of the vessels was decreased by both drought and flooding, but the number was increased
37 only by drought. The occurrence of drought before flooding reduced the vessel area, but the
38 opposite treatment did not. An episode of drought after one of flooding is more stressful than
39 the opposite situation, especially for clone Y that could not adjust its water transport capacity
40 during the drought period.

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43 **Key words:** water stress, hydraulic conductivity, vessels, stomatal conductance, root to
44 shoot ratio

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46 Introduction

47 Climate change will increase the occurrence of flooding episodes in several areas of
48 the world (Kreuswieser and Rennenberg 2014, Cavalcanti et al. 2015). The riparian zones
49 are particularly susceptible to an increased risk of flooding and drought under climate
50 change, causing modifications in plant community composition and species richness
51 (Garssen et al. 2014, Garssen et al. 2015). Willows (*Salix* spp.) can colonize and grow
52 successfully on areas periodically disturbed by flooding, like floodplains (Karrenberg et al.
53 2002) and tidal wetlands (Markus-Michalczyk et al. 2016 a). In consequence, willows are
54 natural candidates for developing forest plantations in flood prone areas that are marginal for
55 agriculture. There is an increased demand for forest-derived products for different uses,
56 including the supply of biomass for energy production. To fulfill this demand, willow
57 plantations are being developed in areas that can experience alternate short periods of
58 drought and flash flooding during the growing season. To improve the success of plantations
59 in disturbed areas, it is necessary to gain a better understanding of the physiological
60 responses of willows growing under these particular combinations of environmental stresses.

61 Willow responses to drought and flooding have been previously analyzed separately,
62 and the responses are different according to the genotype and the duration of the stress
63 episode (Li et al 2004, Wikberg and Ögren 2004). It has been shown that willow responses to
64 continuous drought are different from those under cyclic drought (Bonosi et al. 2010).
65 Meanwhile, the responses of different tree species to a flooding event may vary if they
66 suffered from a previous episode of flooding. In poplars, the occurrence of a pre-conditioning
67 flooding period improved waterlogging tolerance (Bejaoui et al. 2012). In willows, the
68 combination of drought and flooding has been addressed to a lesser extent than both
69 stresses separately (Nakai et al. 2010, Nakai and Kisanuki 2011). Some morphological
70 responses to drought are the opposite of those to flooding. For instance, drought increases
71 the root/shoot ratio while flooding reduces it (Kozłowzki 1997, Markus-Michalczyk et al. 2016
72 b). In this context, a previous episode of flooding that reduced root biomass could be
73 detrimental for a plant experiencing drought later. It has been shown that drought

74 susceptibility in willows is related to their vulnerability to cavitation (Wikberg and Ögren 2007,
75 Savage and Cavender-Bares 2011, Ogasa et al. 2013). Flooding can alter water transport in
76 tropical trees (Herrera et al. 2008), and reduce vessel size and hydraulic conductivity in
77 *Quercus robur* (Copini et al. 2016). But nothing is known about the effects of a combination
78 of both stresses on the hydraulic conductivity of willows.

79 The aim of this work is to analyze the responses of two willow clones to drought,
80 flooding and the alternation of these stresses. In a previous work, we identified willow
81 genotypes with contrasting tolerance to flooding (Cerrillo et al. 2013). We expect that these
82 clones will modify their physiology in different ways to acclimate to drought, flooding and the
83 alternative occurrence of these stresses.

84 We hypothesize that: 1 – The clones will differ in their tolerance to the alternation of
85 drought and flooding stress; and 2 – The order of occurrence of drought and flooding
86 episodes will modify the growth, water transport capacity, xylem anatomy, gas exchange and
87 leaf area dynamics of willows in different ways.

88

89

90 **Materials and Methods**

91 *Plant material and growth conditions*

92 The clones used in this work were obtained in a breeding program from INTA
93 (Instituto Nacional de Tecnología Agropecuaria, Argentina), they were a hybrid *Salix*
94 *matsudana* x *Salix alba* “Barrett 13-44 INTA” (clone B); and an open pollinated *S. alba* clone,
95 “Yaguareté INTA CIEF” (clone Y). Clone B showed susceptibility to long periods of flooding
96 in the field (Cerrillo et al. 2013), while clone Y was more tolerant to flooding in such
97 conditions (T. Cerrillo, personal communication). Both clones have a similar sprouting date,
98 making growth comparisons straightforward.

99 One-year-old cuttings of 20 cm length were planted in 4.5 L pots filled with a 1:1
100 sand/soil mixture in a greenhouse in the city of La Plata, Argentina (34° 54' S). The

101 maximum irradiance at midday was $1600 \mu\text{moles photons m}^{-2} \text{ s}^{-1}$. There was no
102 supplemental artificial light added to increase the irradiance or alter the natural photoperiod.

103 The planting date was August 13th, 2012, and one cutting per pot was planted. The
104 pots were placed in a randomized design with 6 replicates for each clone and treatment
105 level. The plants were surrounded by a border row that was not used for measurements.
106 Plants were watered to keep the soil at field capacity (FC) until the beginning of the stress
107 treatment. Bud flush occurred in both clones between August 30th and September 4th. After
108 sprouting and before starting the treatment, 50 ml of complete Hoagland solution was added
109 weekly to the pots to ensure an adequate nutrient availability. To avoid pests, the trees were
110 treated every two weeks with an insecticide (o,s dimethyl-acethyl phosphoroamydotiathe).
111 Before the beginning of the treatment, cuttings were pruned and only one shoot was kept, in
112 order to minimize the variability induced by different number of shoots per tree. Flooding was
113 induced by placing the pots with the trees into a sealed 7 L pot filled with tap water up to
114 approximately 10 cm above soil level; water was added when necessary to keep this level.
115 Drought was induced by watering the plants with 50 ml of water every other day.

116 The stress treatment started on October 31th, 2012, and the different combinations
117 were applied as follows: 1 - watered to field capacity (FC); 2 - six weeks of continuous
118 drought (Drought); 3 - two weeks of drought, two weeks watered to field capacity, two weeks
119 of drought (D-FC-D); 4 - two weeks of drought, two weeks watered to field capacity, two
120 weeks of flooding (D-FC-F); 5 - two weeks of flooding, two weeks watered to field capacity,
121 two weeks of drought (F-FC-D); 6 - two weeks of flooding, two weeks watered to field
122 capacity, two weeks of flooding (F-FC-F) ; 7 - six weeks of continuous flooding (Flooding).
123 The period of watering at field capacity was included because it is possible to go from
124 drought to flooding immediately, but not the other way around, because after flooding the soil
125 will be saturated for several days, even without any watering.

126

127 *Growth, water consumption and gas exchange measurements*

128 Total shoot height (cm) was measured once a week with a graduate stick. At the
129 beginning of the experiment, the last completely expanded leaf was tagged with a color wire,
130 and all leaves below this mark were counted every week, providing a measurement of the
131 abscission of basal leaves in each treatment.

132 Stomatal conductance was measured on seven different dates with a Decagon SC1
133 porometer on the abaxial side of the latest fully expanded leaf. The measurements were
134 carried out on cloudless days without any artificial light supplements, between 11.30 am and
135 01.30 pm, and the average irradiance during those measurements was $1150 \mu\text{moles m}^{-2} \text{s}^{-1}$.
136 Six repetitions were measured for each clone and treatment.

137 The dry weight of leaves, stems and roots was determined at the end of the
138 experiment, after drying them at 65°C to constant weight. The total leaf area (cm^2) was
139 measured at the end of the experiment by scanning the leaves and determining their area
140 using the software IMAGE J (<http://rsbweb.nih.gov/ij/>, Schneider et al. 2012).

141 The water consumption (WC) of the whole plant was estimated as follows: Two days
142 before the end of the experiment, the pots were sealed with a double plastic bag, and
143 weighted. The last day, they were weighted again and the difference in weight gave an
144 estimation of the water consumed by the plant.

145

146 *Hydraulic conductivity measurements*

147 Hydraulic conductivity was measured in four plants of each clone and treatment at the
148 end of the experiment. Measurements were taken on the stem segment immediately above
149 the latest expanded leaf at the beginning of the experiment. This part of the stem was
150 selected because it was still growing, and would eventually reflect changes due to the
151 stresses imposed. In the early morning, shoots were cut under water and taken to the
152 laboratory in water buckets and kept in water until measurements were performed that same
153 day. The stem segment to be measured was re-cut under water, and connected to the
154 hydraulic head. Degassed and deionized water was perfused through one of the ends of the
155 segments. The pressure gradient was 0.011 MPa, and in this situation, the embolisms (if

156 present) were not removed. When the flux achieved a steady state, the water extruding from
157 the segments was collected in a pre-weighted tube. The time spent on water collection was
158 also measured (minimum 13 minutes, maximum 58 minutes). The water flux was estimated
159 by weighing the tubes on a digital balance. For the measurements, the segments were
160 decorticated. The total length of the segments and the diameter of both extremes were
161 measured with a digital caliper, and the xylem area was calculated with the mean of both
162 diameters; the pith area was not subtracted because the whole decorticated area was
163 conductive. The values of the hydraulic conductivity per unit stem length (k_h), the specific
164 hydraulic conductivity per unit of xylem area (k_s) and the specific hydraulic conductivity per
165 unit leaf area (k_l) were calculated according to the modified Poiseuille's law (Cruziat et al.
166 2002).

167

168 *Anatomical analysis*

169 The anatomical analysis was carried out on the same stem segment used to measure
170 the hydraulic conductivity. To determine wood anatomy, the entire cross-sections (20 μm) of
171 stem segments were cut using a sliding microtome, then stained in safranin (1%),
172 dehydrated, and mounted in Entellan® for microscopic analysis. Images were captured with
173 a digital camera (Olympus DP71) mounted on a research microscope (Olympus BX50,
174 Japan). The captured images were analyzed for the following parameters, using the image
175 analysis software to count/size and measure objects ImagePro Plus v. 6.3, Media
176 Cybernetics USA: vessel lumen diameter (μm), vessel area ($AV, \mu\text{m}^2$) and vessel number
177 ($NV, n^\circ/\text{mm}^2$).

178

179 *Statistical Analysis*

180 Most data did not meet the ANOVA assumptions of normality and equality of
181 variance, and this could not be improved by data transformation. In consequence, the
182 Kruskal-Wallis rank sum test was used for the analysis. The statistical analysis was carried

183 out with the R software 3.2.2 (R Core Team, 2015) using the package *agricolae* version 1.2-3
184 for the Kruskal-Wallis test.

185 For the Principal Components Analysis (PCA), the data were centered and
186 standardized. The variables included in the PCA were: RSR (root to shoot ratio); NV (number
187 of vessels); AV (area of vessels); kh (hydraulic conductivity per unit stem length), ks
188 (hydraulic conductivity per unit of xylem area); kl (hydraulic conductivity per unit leaf area),
189 NBL (number of basal leaves); WC (water consumption); TLA (total leaf area); TDW (total dry
190 weight); Hf (final height, day 41); gs (stomatal conductance measured on day 41).

191

192

193 **Results**

194 *Gas exchange measurements*

195 During the flooding periods, there were no significant differences in stomatal
196 conductance (gs, Fig.1) between control and flooded plants of clone Y. In some dates, gs
197 was significantly reduced in flooded plants of clone B compared to control treatment.
198 However, gs was reduced during the drought periods in all treatments including drought in
199 both clones. The reduction was more pronounced in the permanent drought treatment,
200 whereas in the cyclic drought treatments, gs recovered to the levels of controls when plants
201 were watered to field capacity. These results show that the treatments were effective at
202 inducing drought stress in the willow clones. Growth in height showed the same pattern,
203 being reduced only in the drought treatments (Supplementary Fig.1).

204

205 *Growth measurements*

206 The number of basal leaves indicate the occurrence of leaf area adjustment through
207 leaf abscission (Fig. 2). Leaf shedding was scarce on control and flooded plants, while
208 abscission was enhanced by drought in both clones, causing the reduction in total leaf area
209 at the end of the experiment (Supplementary Fig.2). In the F-FC-D and D-FC-F treatment,
210 leaf shedding occurred mainly during the drought periods. The reduction in the number of

211 basal leaves for the cyclic drought treatments was more pronounced in clone Y than in clone
212 B.

213 Total dry weight (Fig.3) was significantly reduced compared to controls under
214 continuous drought, D-FC-D, and F-FC-D. In D-FC-F treatment, the reduction was significant
215 only for clone Y. Continuous flooding and F-FC-F did not reduce the total dry weight in either
216 of the clones.

217 In spite of the similar value of total dry weight in control plants, the dry matter
218 partitioning was different in both clones. Clone B invested more in roots than clone Y, and
219 consequently had a significantly higher RSR (Fig. 3). Both clones significantly reduced RSR
220 under flooding, applied either in the continuous (Flooding) or cyclic form (F-FC-F). In the
221 other treatments, there were no statistically significant differences in root/shoot ratio
222 compared to control plants.

223

224 *Hydraulic conductivity and xylem anatomy*

225 The hydraulic conductivity measured as k_h , k_s or k_l (Fig. 4) was similar in the control
226 plants of clone B and Y. All three parameters were reduced in the D-FC-D and F-FC-D
227 treatments, but these differences were statistically significant only in clone Y.

228 The g_s / k_s ratio (Table 1) was calculated using the values of g_s at the end of the
229 experiment. This ratio gives an insight of the capacity to maintain water balance under
230 drought stress (Wikberg and Ögren 2007). Clone B significantly reduced this ratio for
231 drought, F-FC-D and D-FC-D treatments, while in clone Y the reduction was only significant
232 in D-FC-D.

233 The area (AV) and number of vessels per mm^2 (NV) were measured in the same
234 stem segment used to determine hydraulic conductivity (Fig. 5). The response of the
235 diameter and area of the vessels to the different treatments was similar, in consequence only
236 the area data is shown. In field capacity plants, clone B had a higher NV and of a smaller
237 size than those of clone Y, but the differences were significant only for the number. In clone
238 B, NV increased significantly only under continuous drought, while there were no differences

239 in the other treatments. In clone Y, NV increased significantly in drought, D-FC-D, D-FC-F
240 and F-FC-F compared to controls, while there were no changes in F-FC-D and flooding
241 treatments (Fig. 5, and S.Fig.3). AV decreased significantly in treatments drought, D-FC-D,
242 D-FC-F and flooding in clone B, while there were no differences in F-FC-D and F-FC-F. In
243 clone Y, AV was significantly reduced in the drought, D-FC-D, D-FC-F, F-FC-F and flooding
244 treatments, and did not change in F-FC-D (Fig. 5).

245 We carried out a PCA analysis to explore the relationship between the variables
246 measured in the different combinations of treatments (Fig. 6). For variables measured
247 several times (gs, height and number of leaves), only the last measurement of the
248 experiment was included in the analysis because they could be compared with the other
249 variables measured at the end of the experiment. The first and second components together
250 explained 78 % of the total variation. The variables WC, gs, NBL and TLA superposed with
251 each other, lying within the first component. The root to shoot ratio (RSR) had a negative
252 correlation with height (Hf) and total dry weight (TDW). The area and number of vessels (AV
253 and NV) had the opposite tendency: one increased while the other decreased.

254

255

256 Discussion

257 *Drought and flooding caused different degree of stress in willows.*

258 From our results, it is clear that flooding is a less stressful situation than drought for
259 willows, because the flooded plants differ less from the field capacity treatment. This is
260 clearly reflected in the PCA analysis, where the treatments are divided in two main groups
261 along the first component. One group includes the drought treatments (drought, D-FC-D and
262 F-FC-D); and the other group includes the FC, Flooding, F-FC-F and D-FC-F treatments. The
263 reason is that several growth and physiological variables were reduced by drought compared
264 to the field capacity treatment (gs, WC, TLA, TDW, NBL), but this did not happen with
265 flooding. The treatments including both drought and flooding (D-FC-F and F-FC-D) grouped
266 closer to the last treatment than to the first one. The second component of the PCA analysis

267 mainly reflected differences at clone level. This axis separated the clones because several of
268 the variables shared were different from the beginning, or because they had different
269 responses to the stress episodes (NV, AV, height, RSR, kl, ks).

270

271 *Flooding and drought caused different effects on willows growth.*

272 The main differences between treatments occurred in biomass accumulation and
273 partition between plant organs. Flooding did not reduce TDW in any of the clones, while
274 drought –continuous or cyclic– decreased it. In spite of having a similar total dry weight under
275 well-watered conditions, the dry matter partitioning was different in both clones. Clone B
276 allocated more biomass to the root system, whereas clone Y did so in the stem, resulting in
277 differences in RSR. Under F and FCF treatments RSR was reduced in both clones compared
278 to control plants, these results are similar to those of *Salix alba* where repeated flooding
279 reduced root biomass (Markus-Michalczyk et al. 2016 b).

280 In D, D-FC-D, F-FC-D and D-FC-F treatments, the RSR did not differ significantly
281 compared to field capacity (Fig.3). This result was similar to the one described previously for
282 *Salix gracilistyla*, where RSR was similar in control and drought-stressed plants (Nakai et al.
283 2010). The lack of a significant increase in RSR could be a factor increasing drought
284 susceptibility of willows compared with other forest trees. An increased allocation of biomass
285 to roots allows the exploration of a higher volume of soil, improving water extraction under
286 drought conditions, but this response did not occur in our clones. In addition, it has been
287 suggested that in *Salix gracilistyla*, a period of flooding sensitized plants to subsequent
288 drought because of root damage (Nakai and Kisanuki 2011).

289 The growth measurements closely correlate with total leaf area. Leaf area reduction is
290 a mechanism to diminish the water consumption of the whole plant when the water supply is
291 limited (Savage et al. 2009, Bonosi et al. 2010). *Salix* species tend to experience extensive
292 defoliation under drought, and it has been proposed that it is a mechanism to confine
293 embolism to petioles and leaves (Savage and Cavender-Bares 2011). The D and FCD
294 treatments showed the stronger reduction in leaf area due to leaf abscission. The reduction

295 in leaf area is not the only mechanism to reduce transpiration; stomatal closure is clearly
296 relevant to the control of water loss in plants under drought, as Fig.1 shows. This fact is
297 highlighted by the coincidence of TLA, g_s and WC in the first component of the PCA
298 analysis. This can explain why plants of the F-FC-D treatment experienced a sharper decline
299 in g_s and leaf abscission (NBL) in the drought period compared with the D-FC-F treatment
300 (Fig. 1 and 2).

301

302 *The order of occurrence of drought and flooding episodes caused different acclimation*
303 *responses of water transport capacity in willows.*

304 Drought, flooding and drought followed by flooding caused a significant reduction in
305 vessel size compared to non-stressed plants, but flooding followed by drought did not.

306 The reduction in xylem vessel size and the increase in vessel number are well-
307 documented drought responses in several species, including *Populus* (Fichot et al. 2009).
308 Smaller vessels in general have higher resistance to embolism, increasing drought tolerance.
309 This development is not surprising, since drought resistance correlates with sensitivity to
310 xylem cavitation in willows (Wikberg and Ögren 2004, Ogasa et al. 2013). We found a
311 reduction in AV and an increase in NV in drought and D-FC-D treatments, indicating that
312 both clones can acclimate to drought stress. Vessel size was reduced in the flooding
313 treatment, but without a significant increase in NV (Fig. 5). This fact could explain the lower
314 hydraulic conductivity observed in the same treatment, albeit it is not statistically significant
315 (Fig.4). The smaller vessel area in flooded plants may indicate an adaptation to a reduction
316 in water uptake caused by flooding, as found in *Quercus robur* (Copini et al. 2016). In
317 *Campsiandra laurifolia*, there was a reduction in hydraulic conductivity in the first stages of
318 seasonal flooding, but this fact was reversed later in the season (Herrera et al. 2008).

319 The D-FC-F treatment caused a reduction in vessel size in both clones, but in the F-
320 FC-D treatment the vessel area was not reduced. Since both drought and flooding alone
321 caused a reduction in vessel area, the results in F-FC-D are surprising. Clearly, the order of
322 occurrence of the stresses caused different responses in this trait. In F-FC-D treatment,

323 clone B experienced a 50 % drop in k_l compared to field capacity treatment but in clone Y, k_l
324 was decreased to 10 % compared with the same treatment. The reduction in hydraulic
325 conductivity can be due to loss of vessel functionality through embolism or the reduction in
326 vessel size or number in the xylem formed during the stress. Clone B demonstrated a
327 reduced g_s / k_s ratio under the F-FD-D treatment, while clone Y was unaffected (Table 1).
328 This effect occurs only in F-FC-D treatment, while the other drought treatments clone Y can
329 reduce the g_s / k_s ratio, as other willows species do to acclimate to moderate drought
330 (Wikberg and Ögren 2007). This difference in response may be due to clone Y having an
331 increased sensitivity to embolism. The cause for the greater sensibility to embolism in clone
332 Y is probably the lack of reduction of vessel size, although other reasons cannot be
333 discarded. It has been suggested that the vulnerability to cavitation in poplars is related to
334 the porosity of the vessel pit membrane (Fichot et al. 2015); accordingly this or other xylem
335 traits are responsible for the higher susceptibility of clone Y to embolism.

336

337 *The clones had contrasting responses to flooding and drought.*

338 In a revision evaluating stress tolerance in 806 tree and shrubs species, a negative
339 correlation has been found between drought and waterlogging tolerance, implying a trade-off
340 between tolerance to these stresses (Niinemeets and Valladares 2006). Our results seem to
341 be in line with the hypothesis of a trade – off between drought and flooding tolerance. The
342 flood tolerant clone Y was more drought sensitive, experiencing extensive defoliation and a
343 steep reduction of its water transport capacity under water shortage. On the other hand, flood
344 sensitive clone B (Cerrillo et al. 2013) retained a greater water transport capacity under
345 drought stress. It has been proposed that there are two possible strategies to cope with
346 drought in tree saplings: a low resistance to cavitation combined with lack of osmotic
347 adjustment and high abscission rate (desiccation avoidance), and a higher resistance to
348 cavitation combined with osmotic adjustment and leaf area retention (desiccation tolerance,
349 Yazaki et al. 2010). Willows seem to fit in the first strategy, because they are more sensitive
350 to cavitation than other tree species (Savage and Cavender-Bares 2011, Ogasa et al. 2013)

351 and experience extensive defoliation under drought (Savage et al. 2009, Bonosi et al. 2010).
352 Both clones can reduce leaf area and g_s under drought, but this is not enough to prevent
353 embolism in clone Y. In several temperate species, including *Salix*, a low cavitation
354 resistance is compensated by a higher recovery capacity through vessel refilling (Ogasa et
355 al. 2013). It is possible that clone Y has a reduced capacity to repair embolism, while clone B
356 is more efficient refilling the vessels and maintaining its water transport capacity under
357 drought. The architecture of clone B, with more biomass allocated to roots than shoot also
358 helps this clone to cope with water shortage. On the other hand, clone Y has a lower root to
359 shoot ratio, and this could explain the higher growth in height of this clone under field
360 capacity or waterlogged conditions.

361 .

362 *Conclusion*

363 Our first hypothesis stated that the clones will differ in their tolerance to the alternation
364 of drought and flooding stress. It proved correct for the F-FC-D treatment, since clone B was
365 more tolerant than clone Y under this treatment, while there was no difference between the
366 clones in the D-FC-F treatment. Regarding the second hypothesis, water transport capacity,
367 xylem anatomy and leaf area dynamics were affected in different ways in F-FC-D and D-FC-
368 F treatments. For willows, the occurrence of a drought episode after one of flooding is more
369 stressful than the opposite situation, especially for clone Y that is not able to adjust its water
370 transport capacity during the drought period.

371

372

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Table 1 Ratio between stomatal conductance (g_s , $\text{mmoles m}^{-2} \text{s}^{-1}$) and hydraulic conductivity per unit xylem area (k_s , $\text{g H}_2\text{O m MPa}^{-1} \text{s}^{-1} \text{m}^{-2}$) under Field Capacity, Drought, D-FC-D, D-FC-F, F-FC-D, F-FC-F and Flooding, at the end of the experiment. Mean values: plus minus one standard error of the mean. Treatments followed by the same letter did not differ according to the Kruskal-Wallis test ($p \leq 0.05$).

Treatment	Clone B	Clone Y
<i>Field Capacity(FC)</i>	0.31 \pm 0.08 bcd	0.49 \pm 0.18 abc
<i>Drought (D)</i>	0.07 \pm 0.03 ef	0.22 \pm 0.05 cdef
<i>D-FC-D</i>	0.06 \pm 0.02 ef	0.14 \pm 0.07 def
<i>D-FC-F</i>	0.52 \pm 0.12 ab	0.61 \pm 0.06 a
<i>F-FC-D</i>	0.07 \pm 0.05 f	0.43 \pm 0.16 abc
<i>F-FC-F</i>	0.27 \pm 0.08 bcd	0.66 \pm 0.02 a
<i>Flooding(F)</i>	0.24 \pm 0.02 bcde	0.84 \pm 0.32 a

Legends to the figures

Fig. 1 Stomatal conductance (gs) of plants of two willow clones growing under different water regimes and their combinations. FC: field capacity. Vertical bars: standard error of the mean. Means marked with an asterisk differ significantly from the field capacity treatment on the same date according to the Kruskal-Wallis test ($p \leq 0.05$).

Fig. 2 Number of basal leaves (leaves completely expanded at the beginning of the experiment) of plants of two willow clones growing under different water regimes and their combinations. FC: field capacity. Vertical bars: standard error of the mean. Means marked with an asterisk differ significantly from the field capacity treatment on the same date according to the Kruskal-Wallis test ($p \leq 0.05$).

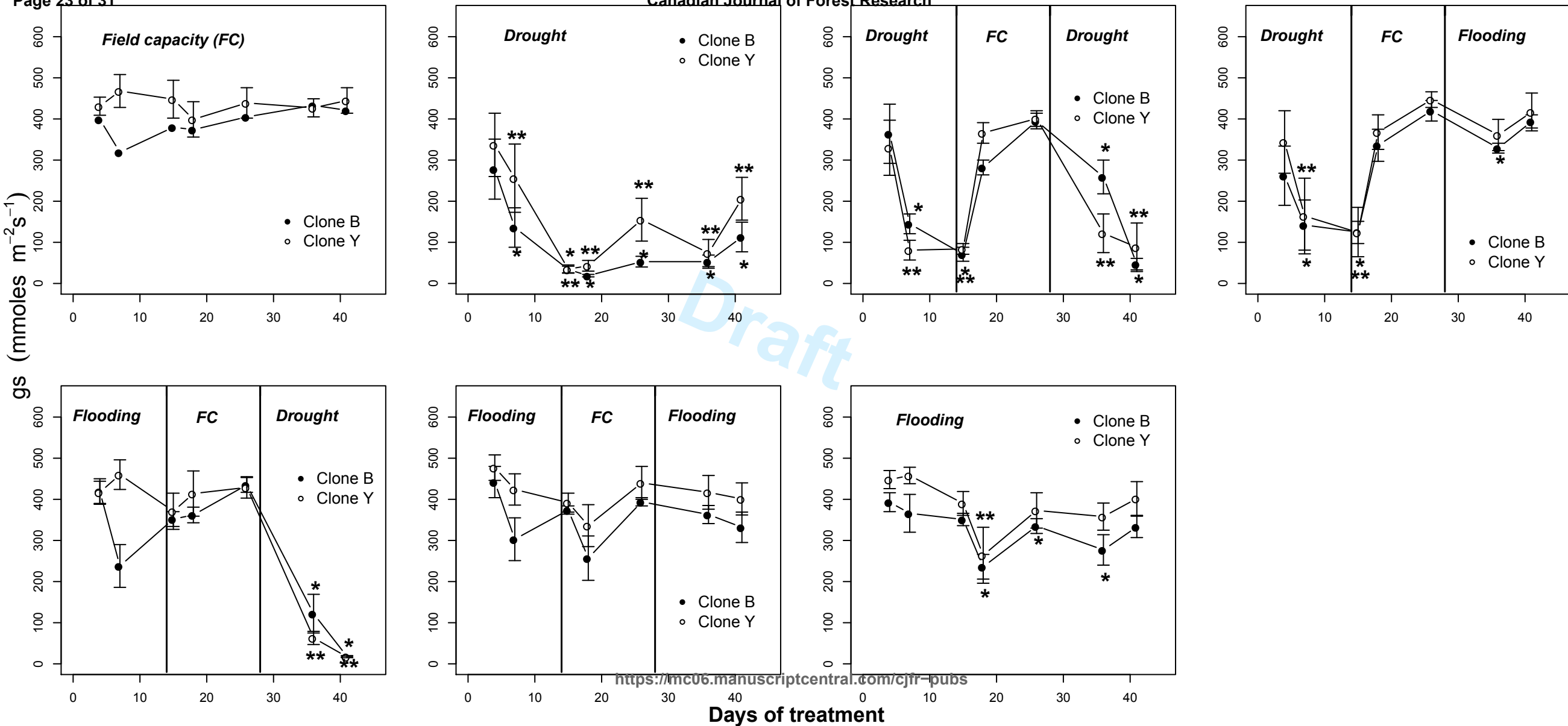
Fig. 3 Dry matter partitioning in plants of two willow clones growing under different water regimes and their combinations: FC (field capacity), F (flooding) and D (drought). Treatments followed by the same letter did not differ according to the Kruskal-Wallis test ($p \leq 0.05$). The significance indicated is that of each compartment (root, shoot, leaves, and total dry weight). In italics: root/shoot ratios. Those values that differ significantly from controls according to the Kruskal-Wallis test ($p \leq 0.05$) are marked with asterisks. B: clone B. Y: clone Y.

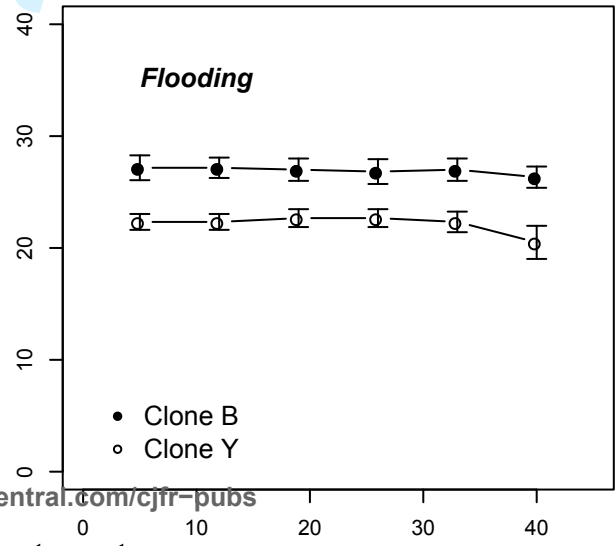
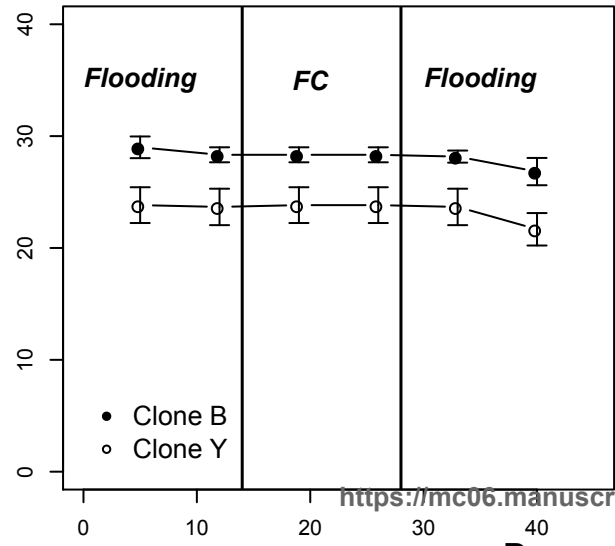
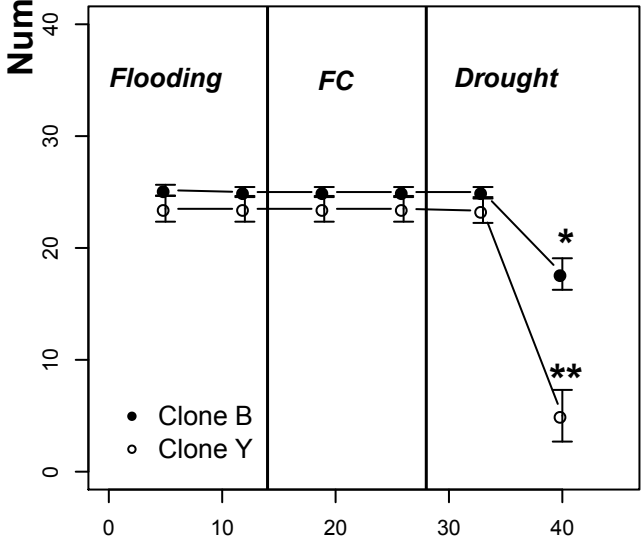
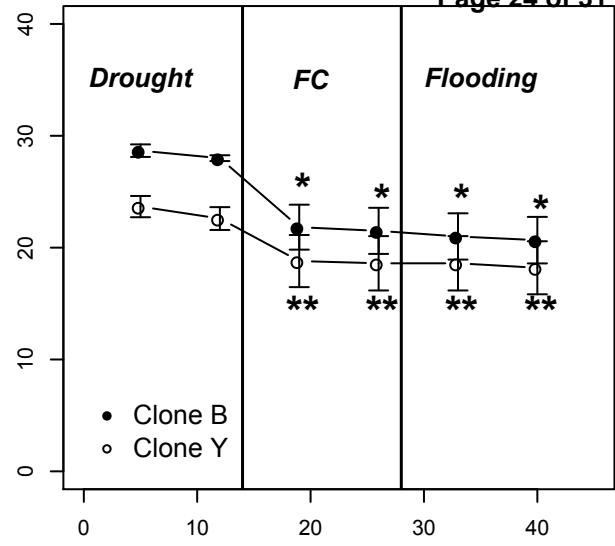
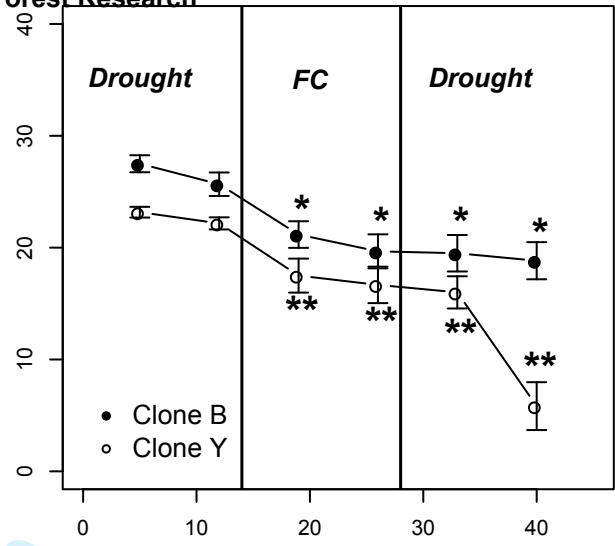
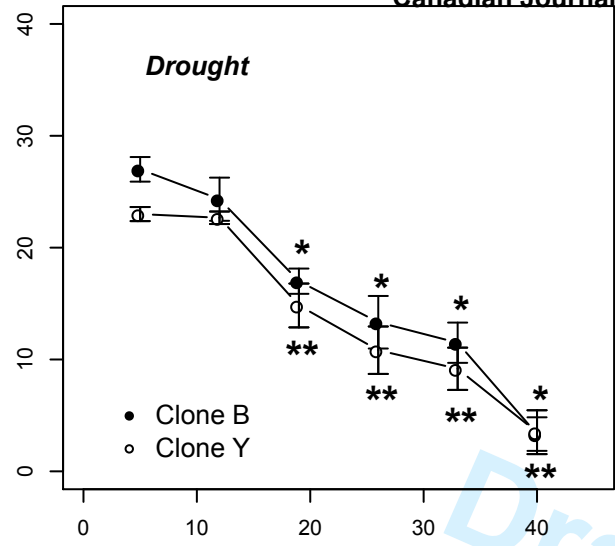
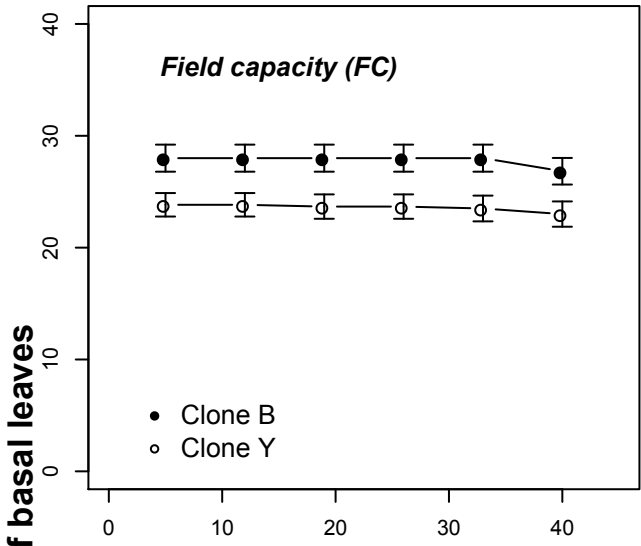
Fig. 4 Hydraulic conductivity per unit stem length (kl), hydraulic conductivity per unit xylem area (ks) and hydraulic conductivity per unit leaf area (kl) of two willow clones growing under different water regimes: FC (field capacity); F (flooding) and D (drought). Treatments followed by the same letter did not differ according to the Kruskal-Wallis test ($p \leq 0.05$).

Fig. 5 Number and area of vessels in plants of clone B and clone Y under different water regimes: FC (field capacity); F (flooding) and D (drought). Treatments followed by the same letter did not differ according to the Kruskal-Wallis test ($p \leq 0.05$).

Fig. 6 Principal Components Analysis (PCA) of the different variables measured in the experiment. The variables included in the PCA were: RSR (root to shoot ratio); NV (number of vessels); AV (area of vessels); kh (hydraulic conductivity per unit stem length), ks (hydraulic conductivity per unit of xylem area); kl (hydraulic conductivity per unit leaf area), NBL (number of basal leaves); WC (water consumption); TLA (total leaf area); TDW (total dry weight); Hf (final height, day 41); gs (stomatal conductance measured on day 41).

Draft





Days of treatment

FIG.3

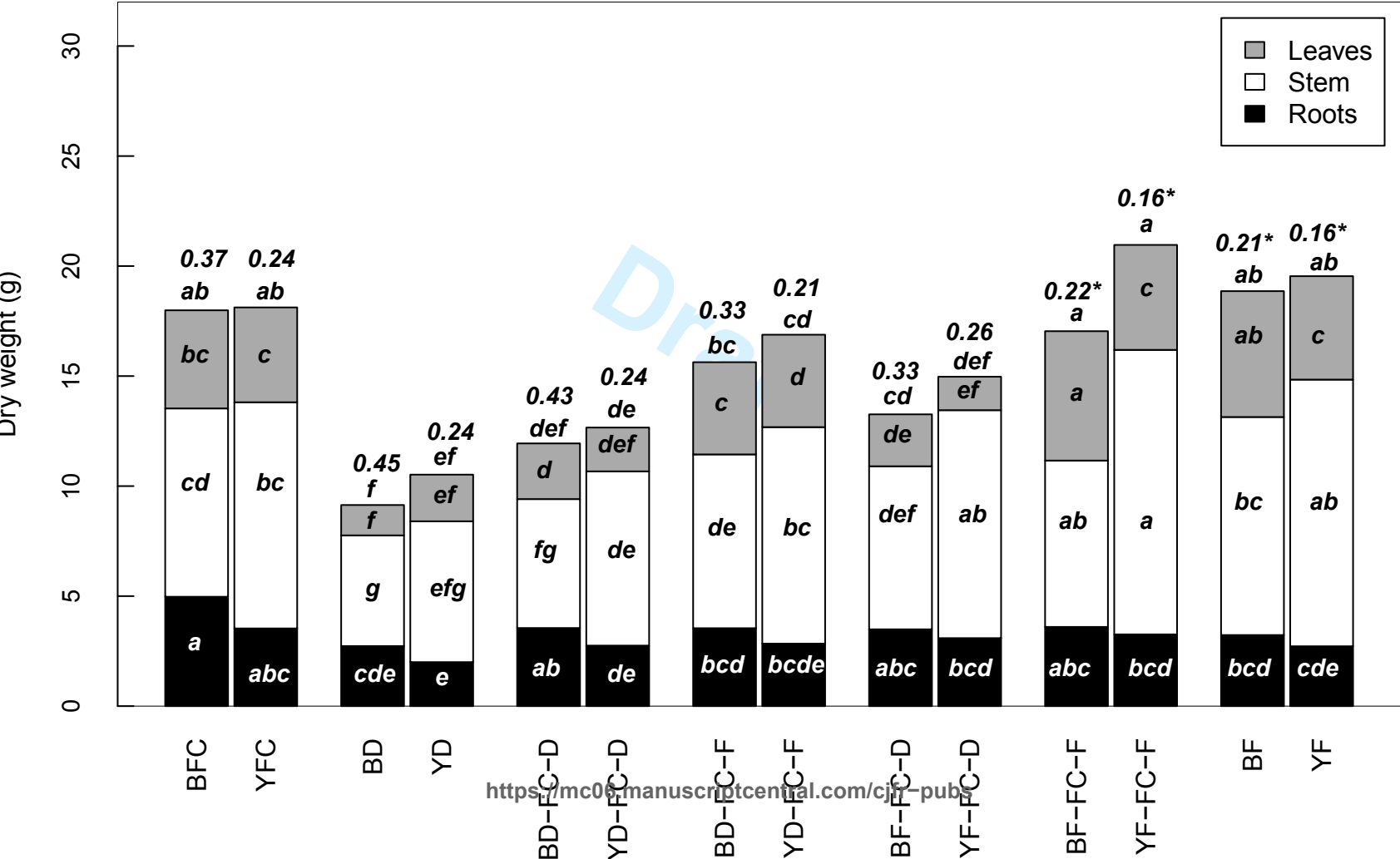


FIG. 4

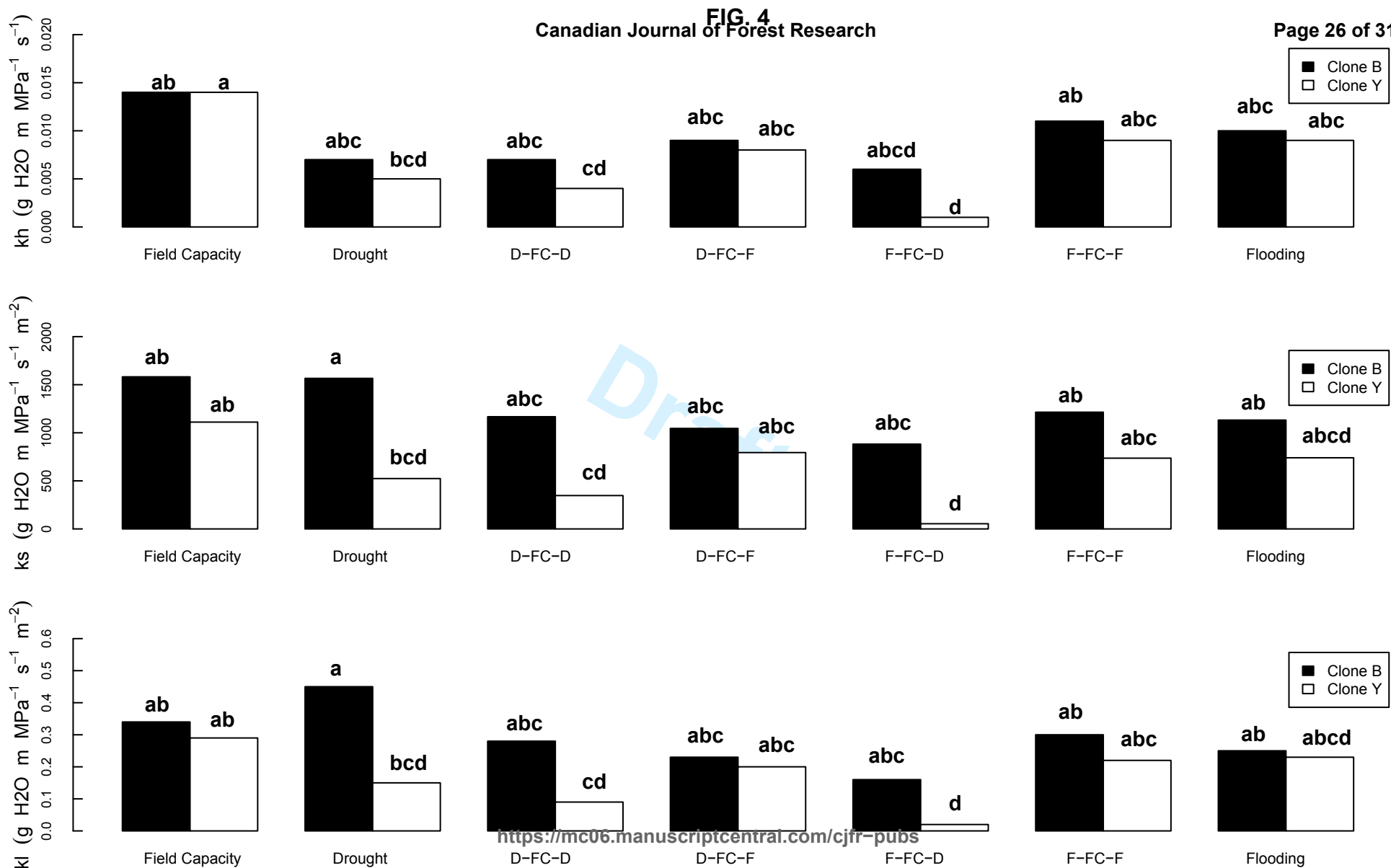


FIG 5

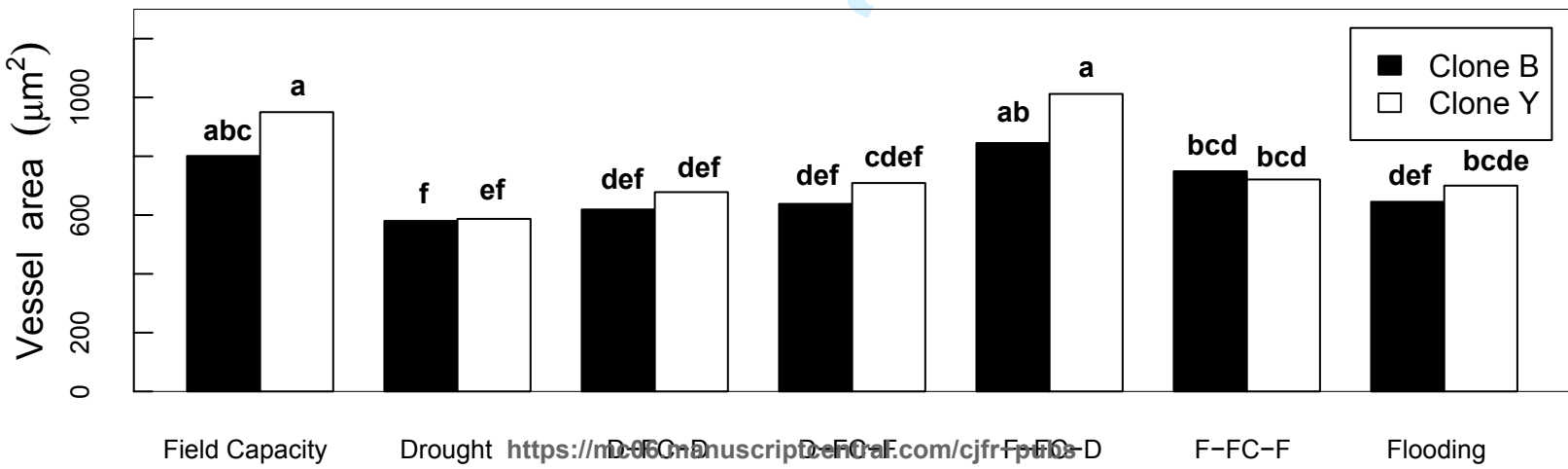
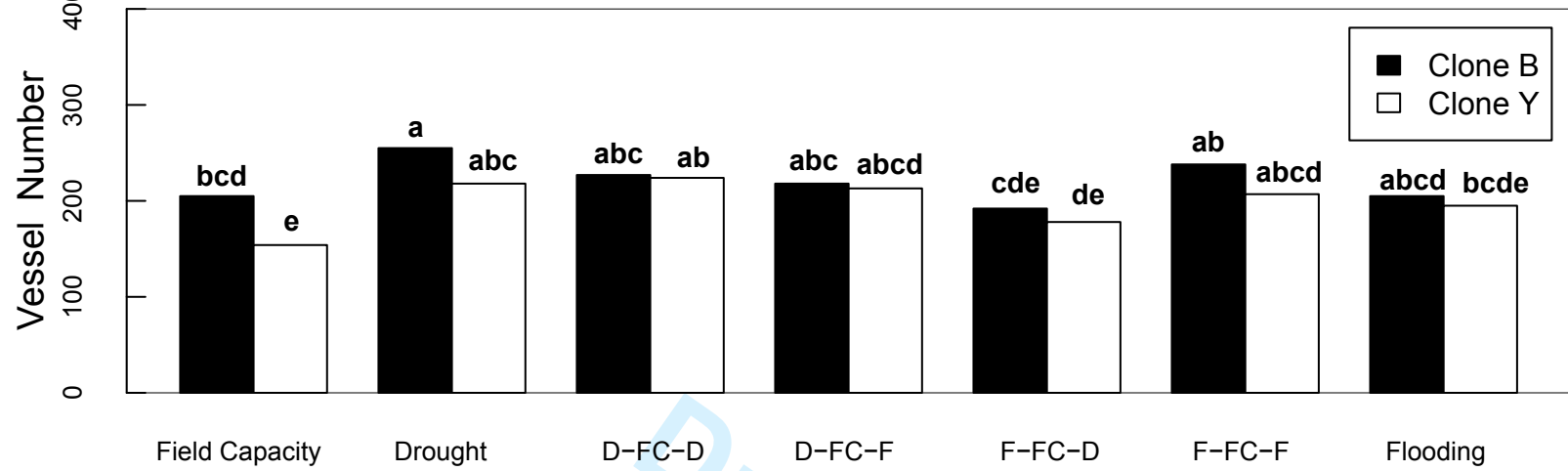
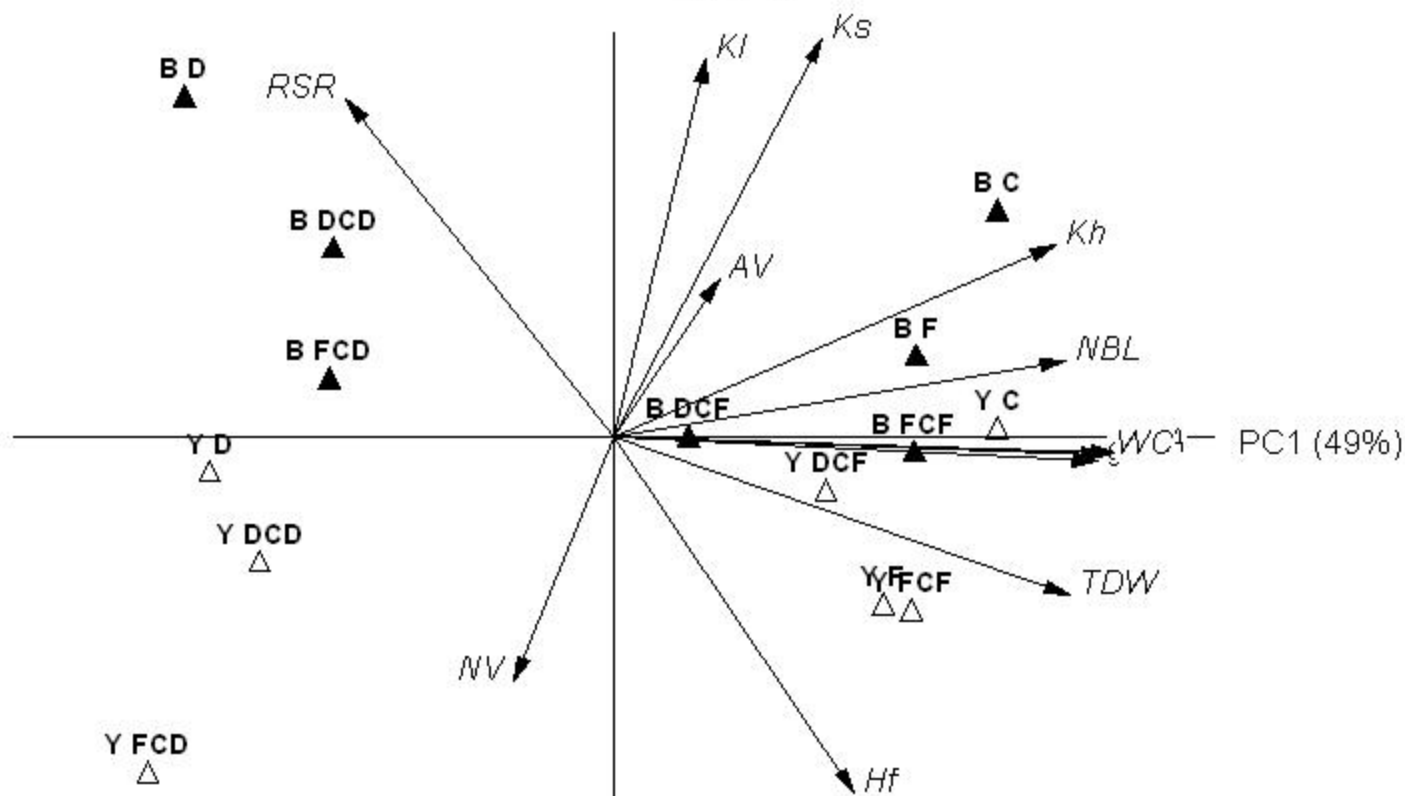
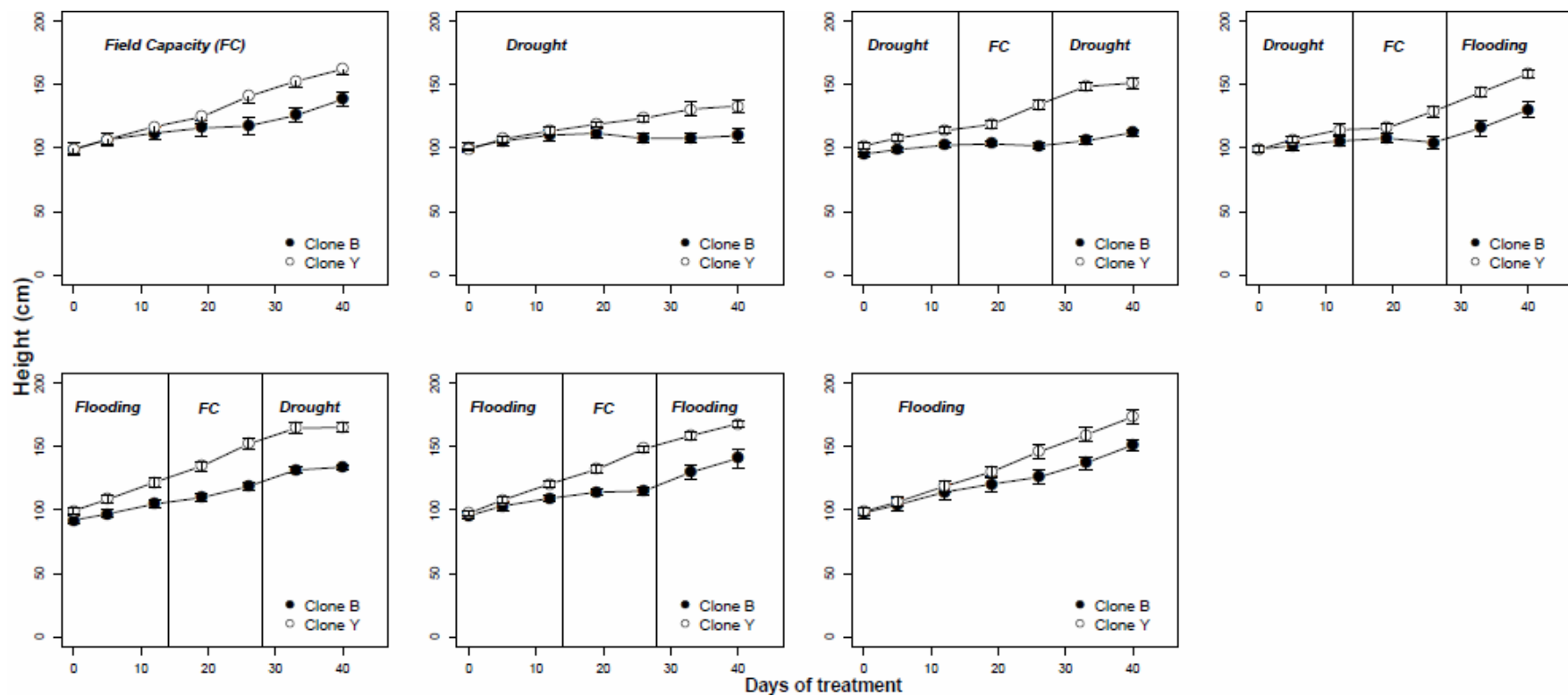


FIG. 6

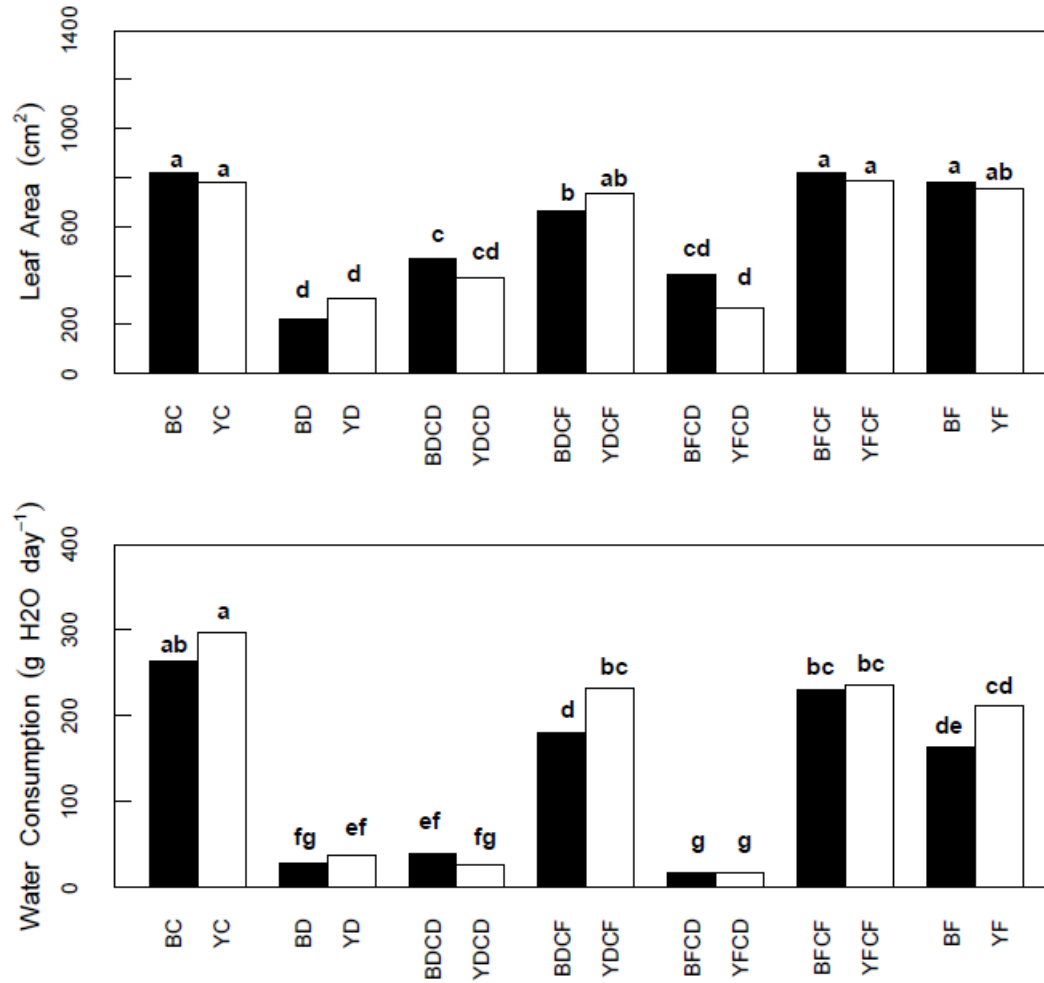


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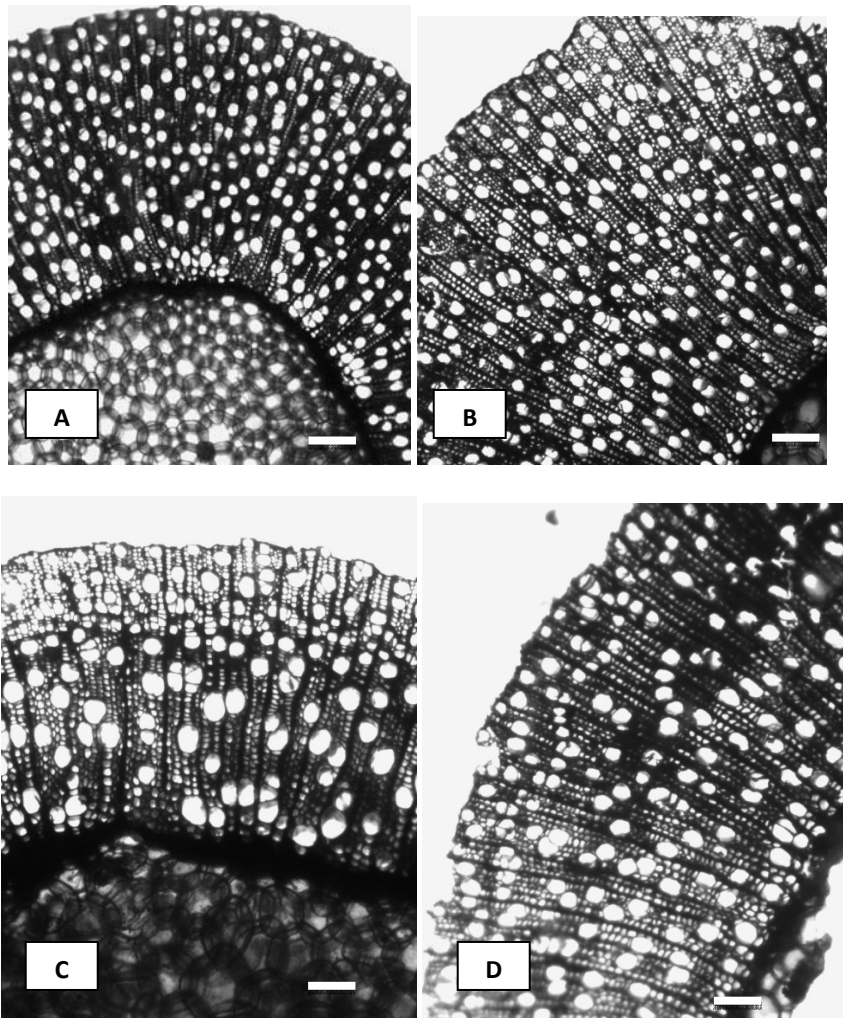
PC2 (29%)



S. Fig. 1 – Height of plants of two willow clones under different water regimes and their combinations: FC (field capacity); F (flooding) and D (drought). Vertical bars: standard error of the mean.



S. FIG.2 – Leaf area and water consumption in two willow clones under different water regimes: C (field capacity); F (flooding) and D (drought). Black: clone B, White: clone Y. Treatments followed by the same letter did not differ according to the Kruskal-Wallis test ($p \leq 0.05$).



Supplementary Figure 3 – Images of the vessels in the control treatment (Panel A clone B, panel B clone Y) and in the continuous drought (D, panel C clone B, panel D clone Y). Scale bar: 50 μm .