Achene traits involved in the water dispersal of the invasive Fallopia x bohemica complex: Variability and dispersal strategies

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Abstract

Secondary dispersal of terrestrial plant species through watercourses increases the dispersal range of floating seeds but their exposure to water may also challenge their viability and colonisation potential. Many terrestrial invasive species benefit from watercourses to extend their invasive range because water exposure can favour seed germination and early survival of seedlings for species sensitive to dry conditions and water flow enhances their dispersal distance downstream. Floatability, germination rate and survival after water exposure are seed traits involved in water dispersal and their variability may lead to different dispersal strategies. To address the question of the variability of achene traits involved in dispersal, we experimentally measured these three traits for *Fallopia* × *bohemica* achenes collected from 10 different stands. The floatability of achenes varied between stands from 2.5 to 5 days and their germination in water extended this period to 20 days through the emergence of floating seedlings. Germination proportions also differed between stands from 0.3 to 0.95 and were negatively related to the median germination time. Seedling survival after planting in soil was affected by their exposure time. This effect differed between stands, but a short germination time did not explain a better survival of some stands. These results show that achenes may differ in their dispersal potential in terms of floatability, as well as tolerance of seedlings to water exposure during dispersal. This study also highlights the fact that achenes of some stands present a combination of traits particularly adapted to long distance dispersal through watercourses, which can contribute to the extension of the invaded area.

1. Introduction

One of the factors influencing the spread of an invasive plant species through the landscape is its aptitude for dispersal (Theoharides and Dukes, 2007). A successful dispersal may be related to the propagule traits that increase the transport distance from the parental plant (Lloret et al., 2005; Theoharides and Dukes, 2007) and to the environmental characteristics of the recipient habitat. Many terrestrial invasive species benefit from watercourses to extend their invasive range (Catford and Jansson, 2014). For instance, water exposure can favour seed germination and early survival of seedlings for species sensitive to dry conditions (Funkenberg et al., 2012) and water flow enhances their dispersal distance downstream (Boedeltje et al., 2003). This is particularly true for buoyant propagules, such as seeds, which can disperse freely on the water surface, limiting their interaction with submerged obstacles, such as vegetation (Chang et al., 2008).

Buoyant seeds are also easily shed over higher zones of the riverbank (Coops and Velde, 1995). These zones are less subject to disturbances generated by floods and to continuous water exposure, which could challenge the colonisation of a terrestrial plant (Mahoney and Rood, 1998). The water dispersal of a terrestrial species may also positively affect seed germination and seedling establishment after deposition on the riverbank, as, for some species (e.g. *Populus* sp. and *Salix* sp.), this may accelerate seed imbibition and reduce the risk of drought stress on seedlings (Niiyama, 1990; Mahoney and Rood, 1998). However, water can also negatively impact viability, if seeds experience anoxic conditions or mechanical stress caused by water flow, which are frequently encountered by non-floating seeds (Casanova and Brock, 2000; Boedeltje et al., 2002). Young seedlings are generally more sensitive to water exposure, leading to reduced plant biomass that affects their survival (Niiyama, 1990; Casanova and Brock, 2000; Parolin, 2002).

63 The success of terrestrial species, in terms of dispersal distance and the effective number of
64 dispersing seeds, may therefore depend on the interaction between the seed traits (e.g.
65 floatability), survival and water exposure (Fumanal et al., 2007). Successful dispersal may be
66 achieved using different strategies. For many riparian species, long floating periods that
67 increase the dispersal distance (T_1, Fig. 1A) are accompanied by delayed germination (T_2, Fig.
68 1A), which diminishes the risks of seed loss if the seeds germinate during dispersal (S_1, Fig.
69 1A, Clarke et al., 2001; van der Broek et al., 2005). For other species that present short
70 floating periods, floating seedlings may extend dispersal time on the water surface (T_3, Fig.
71 1B) by a rapid germination in water (Sculthorpe, 1967). In this case, the tolerance of seedlings
72 to water exposure is critical (S_2, Fig. 1B).

73 Previous studies have demonstrated that some species can produce two types of seeds
74 presenting different dispersal strategies (e.g. short-floating vs. long-floating seeds, Pollux et al.
75 2009). This phenotypic variability constitutes an efficient mechanism for invasive species to
76 adapt to broader environmental conditions and therefore extend their invaded range (Lee,
77 2002). Intraspecific variability in seed traits may therefore increase the dispersal potential in
78 different habitats (Hantsch et al., 2013). For example, intraspecific seed heteromorphism
79 affecting flotation and germination times could be involved in the dispersal of the terrestrial
80 invasive species *Ambrosia artemissifolia* through watercourses (Fumanal et al., 2007).

81 The *Fallopia* complex, one of the most invasive terrestrial taxa in the world (Lowe et al.,
82 2000), presents a large phenotypic variability in achene morphology and flotation force
83 (Lamberti-Raverot et al., 2017). Some studies on the role of sexual spread in the *Fallopia*
84 complex have highlighted the potential role of watercourses in sexual dispersal, where this
85 phenomenon is regularly present (Mandák et al., 2005; Bailey et al., 2009; Lamberti-Raverot
86 et al., 2017). *F.* × *bohemica* produces a large number of fertile achenes each year (Engler et
87 al., 2011; Groeneveld et al., 2014), which have the ability to remain afloat for 2-3 days and

88 germinating seedlings may also extend the floatation period (Engler et al., 2011; Rouifed et
89 al., 2011). These results were seen only for one *Fallopia* stand, but given the large genetic
90 variability of the complex (Bzdega et al., 2016), the variation in dispersal traits of *Fallopia*
91 achenes between different stands and the combinations of traits leading to different dispersal
92 strategies remain to be tested. To address this question, we experimentally measured the
93 floatability, germination and survival rates after water exposure of *F.* × *bohemica* achenes and
94 seedlings from 10 different stands. First, we tested if achene dispersal traits (flotation, i.e. T1
95 and T3, and germination timing, i.e. T2, Fig. 1) and propagule pressure (germination
96 proportion and seedling survival after water exposure, i.e. S1 and S2, Fig. 1) differ between
97 stands. Second, to assess the dispersal strategy of *Fallopia*, we tested if germination in water
98 leads to an increased seedling dispersal time compared to that of the achenes (T1 < T3, Fig. 1),
99 and whether seedling survival in soil is reduced due to extended water exposure (S1 > S2, Fig.
100 1). Finally, we tested if delayed germination (T2, Fig. 1) leads to the conservation of
101 propagule pressure (S2, Fig. 1).

102

103 **2. Materials and Methods**

104 2.1 Species studied and sampling

105 The *Fallopia* complex includes *F. japonica, F. sachalinensis,* the complex of polyploid *F.* ×
106 *bohemica* hybrids (Chrtek & Chrtková) J.P. Bailey, originating from the hybridization of *F.*
107 *japonica* and *F. sachalinensis*, backcrosses and F2, and other crosses involving *F.*
108 *baldschuanica* (Bailey et al., 2009). Within the complex, *F.* x *bohemica* presents the highest
109 genetic and morphological variation (Mandák et al., 2005; Tiébré et al., 2007). Although the
110 spread of *F.* × *bohemica* has been mainly considered as clonal, a recent sexual spread has
111 been shown in Europe (Engler et al., 2011; Rouifed et al., 2011, Buhk et al., 2015). Sexual

propagules consist of a single-seeded achene that is surrounded by a winged fruiting perianth. For the sake of simplicity, we use the term achene to designate the whole structure, i.e. the achene and the fruiting perianth.

The stands were identified as *F.* × *bohemica* and *F. japonica*. The achenes sampled were probably *F.* × *bohemica,* due to the male sterility of *F. japonica*, the large presence of *F.* × *bohemica* stands and the rarity of *F. sachalinensis* and *F. baldshuanica* in the region studied. Achenes were collected in November 2012 from 10 geographically distant stands, growing along seven different watercourses (Rhône-Alpes, France, Fig. 2). The sampling date was chosen relatively late in the season to maximize the chance that all the achenes were mature enough. All the stands were located on the river bank, very close to the water level. The stands sampled were characterized by high levels of achene production, except for two stands (BER and ELE) for which the achenes quantity at the sampling date was lower. As achene size may be relevant in dispersal (Lamberti-Raverot et al., 2017), sampling also reflected the range of achene sizes observed in the region. Achenes were randomly collected from several shoots in the same stand and stored under dry conditions at 4°C until they were used in the experiments.

2.2 Floatability and germination timing

Experiments started in February 2014. Floatability tests were performed under controlled conditions, in a climate room, to measure the floating time of achenes from the ten sampled stands. For each stand, 100 achenes were placed in two mesocosms (50 achenes per stand and per mesocosm). The mesocosms consisted of 35.5 × 26 × 7 cm (length × width × height) aquaria filled with tap water. Water was slightly agitated using perforated plastic tubing fixed around the bottom of the aquaria walls and connected to an air pump (0.6 $L.h^{-1}$). Water

agitation was used to allow good conservation of achenes throughout the experiment and prevent the achenes adhering to the aquarium walls and to themselves. The aquaria were illuminated using artificial light (42 µmol m^{-2} s^{-1} surface light with a 12 h photoperiod) and the water temperature was maintained at 19.1 ± 0.4 °C. All the achenes were released simultaneously in the water, and the numbers of floating and sunken achenes were recorded twice a day for 11 days and then once a day until the end of the experiment (50 days). Germination of the achenes occurred in water, so the number of seedlings and their buoyancy (floating versus sunken) were also recorded.

2.3 Germination and survival after water exposure

To measure the effects of water exposure on seed germination and long-term seedling survival, 200 achenes per stand were placed in ten mesocosms (one per stand) placed in the same conditions as for measuring floatability and germination timing. Twenty achenes were collected at each of the following times: 0 (no exposure to water), 1, 2, 4, 8, 12, 20 and 28 days after achenes were placed in water. Immediately after collection, achenes were planted in four germination pots (five achenes per pot) filled with a mixture of potting compost (Favorit®, NPK: 14.16.18) and sand (80/20). Germination pots were illuminated by artificial light (42 µmol m^{-2} s^{-1} with a 12 h photoperiod) and were regularly watered throughout the experiment. After 4 to 12 days in water, most of the seeds had germinated so that the plants transferred to germination pots were mostly seedlings. The germination and survival rates of seedlings were recorded 3 times a week for 5 weeks.

2.4 Variable calculation and statistical analyses

After the first experiment, the count of floating achenes and seedlings, as well as the number of germinations, were used to estimate the following variables: median floatability of the achenes (T_1) and of the seedlings (T_3), as well as the median germination time in water (T_2) and the maximum germination proportion (G). These time-to-event data were described using four different four-parameter log-logistic models (drc R package, eq. 1):

$$f(x,(b,c,d,e)) = c + \frac{d - c}{1 + exp\{b(log(x) - log(e))\}} \quad \text{(eq. 1)}$$

We were specifically interested in three of the four parameters estimated by the model: *b*, *c* and *e*, where *e* represented the time producing a response half-way between the upper limit *d* and the lower limit *c*. T_1 was estimated as the parameter *e* of the first model. As all the floating seeds sank, the upper and lower limits of the model were fixed at 1 and 0, respectively. All ten stands were included in the model and T_1 was estimated for each stand. T_1 was compared between stands with the compParm function by means of ratios (the null hypothesis is that the ratio equals 1). T_2 and G were estimated as the parameters *e* and *d* of the second model, where the lower limit was fixed at 0. The ten stands were included in the model and T_1 and G estimated for each stand were both compared to each stand with the compParm function. T_3 was calculated as the difference between the estimated parameter *e* of seedlings floating again (third model) and the estimated parameter *e* of seedlings sinking (fourth model), which were independently estimated for each replicate (n=2) of each stand. Depending on the stand, either the upper or the upper and the lower limits of the models were fixed and the best model was chosen using the AIC criteria. The difference between stands was assessed by the Kruskal Wallis test as n=2. Student's t-test was used to compare both dispersal times, T_1 and T_3.

After the second experiment, we tested the effect of water exposure on the survival proportion of seedlings for each stand using generalized linear models, with a binomial error. We then

183 estimated the probability of the seedlings surviving after planting in soil for two different
184 cases of water exposure. In the first one, only achenes were exposed to water, corresponding
185 to S_1 (Fig. 1): the probability of seedling survival after planting in soil was estimated for the
186 longest time of achene exposure to water before they germinate. In the second one, both
187 achenes and seedlings (after germination in water) could be exposed to water, corresponding
188 to S_2 (Fig. 1): the probability of probability of seedling survival after planting in soil was
189 estimated for the longest water exposure (i.e. 28 days, corresponding to the experimental
190 duration). The probability of survival of S_1 and S_2 were compared to each other for each stand
191 by performing χ^2 tests. A Pearson correlation test was carried out to test the relationship
192 between T_2 and S_2 on the values estimated from the generalized linear models.

193

194 **3. Results**

195 The estimated median floatability of the achenes (T_1) differed significantly between the ten
196 stands and varied from 2.5 to 5 days of flotation (Fig. 3). The median floatability of seedlings
197 (T_3) was estimated for all the stands except one (BER) for which we could not fit a response
198 curve. Seedling floatability for the nine other stands varied from 15 to 25 days and differences
199 between stands were not significant (χ^2=13.2, df=8, p=0.1, Fig. 3). Average seedling
200 floatability was significantly longer than average achene floatability (t=-8.86, df=8.49,
201 p<0.001, Fig. 3).

202

203 The two germination traits (estimated median germination times and germination proportion)
204 differed between stands (Fig. 4). The proportion of germination in water (G) varied from 0.3
205 to 0.95 (Fig. 4A) and the median germination time (T_3) varied from 2 to 20 days (Fig. 4B).
206 Regarding the survival traits, the survival probability of achenes germinating in soil after

207 water exposure (S_1) was almost 100% for the ten stands. Seedling survival was reduced after
208 28 days of water dispersal (S_2), but the effect of water exposure differed between stands (Fig.
209 5). The correlation between median germination time (T_2) and seedling survival after a long
210 water exposure (S_2) was not significant (t=0.86, df=8, p=0.4).

211

212 **4. Discussion**

213 4.1 Trait variability

214 In accordance with our first hypothesis, some variability in achene traits involved in dispersal
215 was observed. Variability of the *Fallopia* complex, and particularly of the genetically diverse
216 *F.* × *bohemica* hybrid has been previously assessed for multiple sets of traits involved in
217 growth (Pyšek et al., 2003; Herpigny et al., 2012), salt tolerance (Walls, 2010), secondary
218 metabolite expression (Piola et al., 2013) or resistance to herbivory (Krebs et al., 2011).
219 Although riparian ecosystems are some of the main natural dispersal vectors for *F.* ×
220 *bohemica* (Pyšek and Prach, 1993; Mandák et al., 2004; Bailey et al., 2007), this is the first
221 study demonstrating the variability of achene traits that directly affect water dispersal.

222 Our analysis showed that most of the dispersal traits differed between stands, except median
223 seedling floatability and seedling survival from achenes germinating in soil after water
224 exposure. In the case of median seedling floatability, the floatability of seedlings ranged from
225 15 to 25 days and the lack of significant difference between stands may be partly due to a low
226 statistical power. The morphological traits determining achene floatability (Carthey et al.,
227 2016) seem to be more variable between stands than within, compared to those involved in
228 seedling floatability. This difference could be related to variations in anatomical structure,
229 related to the amount of air-filled tissues (Koch et al., 2010). Regarding S_1, the lack of
230 difference between stands suggests that for all the stands, the seeds equally protected the

231 embryos from water exposure. This is not surprising, as inhibition of germination has been
232 shown to be a mechanism to protect the viability of dispersing seeds of many species,
233 maximising the probability of seedling survival if germination occurs under favourable
234 conditions (van den Broek et al., 2005).

235 The other four dispersal traits were highly variable. Achene floatability varied from 2.5 to 5
236 days between stands (Fig. 3). Differences in achene floatability may be related to different
237 achene densities or surface tension (Carthey et al., 2016; Rendu et al., 2017). The proportion
238 of achenes germinating in water differed between stands (30 to 95%), as well as the time to
239 germination in water (from 2 to 20 days; Fig.4). Seedling survival between stands only
240 differed after long water exposure and ranged from 0.1 to 0.98 (Fig. 5). Larger seeds may
241 increase seedling survival (Moles and Westoby, 2004; Skarpaas et al., 2011; Hantsch et al.,
242 2013). However, in our study, germination-related traits and seedling survival after water
243 exposure were not correlated to achene mass (data not shown). Instead, variations in seedling
244 survival could be due to an intrinsic tolerance to low oxygen conditions of seedlings, as rapid
245 starch degradation or enhanced ethanol fermentation (Ismail et al., 2009). Further research
246 should focus on the determinants of seedling tolerance to water exposure and whether these
247 mechanisms are related to the adaptation to *Fallopia* riverbank colonisation. Furthermore, the
248 origin of the variability in achene germinability, traits and response to water exposure should
249 also be investigated to determine the relative role of genetic background and environmental
250 factors and phenology.

251 For the *Fallopia* complex, which exhibits a quick spread, hybridization may help to increase
252 the genotypic diversity in the invasive area, leading to high phenotypic variations (Parepa et
253 al. 2014), including those observed for seed traits. Phenotypic plasticity has been also
254 observed in the vegetative traits of *Fallopia* (e.g. lamina size, growth and biomass allocation),
255 which facilitate the colonisation of contrasting environments (Walls, 2010). In the case of

256 achenes, resource availability in the maternal environment may affect achene morphology and
257 its subsequent dispersal and/or survival (Vaughton and Ramsey, 1998). Both mechanisms
258 may therefore increase the variation of achene traits (Li and Feng, 2009; Hantsch et al., 2013),
259 resulting in phenotypes with different water dispersal abilities. Large variations in dispersal
260 traits may therefore favour the colonisation of different habitats, contributing to the
261 invasiveness of the species along the rivers, but also, more generally, in terrestrial
262 environments.

263 4.2 Dispersal strategies

264 Achene flotation times were relatively short (2.5 to 5 days) compared to the period after
265 which 50% of the seeds had sunk (t_{50}) of 23 days measured by Boedeltje et al. (2003) for
266 semi-aquatic and terrestrial plants. Our results are more consistent with the period after which
267 90% of the seeds had sunk (t_{90}) of 11.2 days measured for seeds defined as hydrochore or
268 anemochore (Carthey et al., 2016). Achene flotation times among stands differed up to 100%.
269 A 3-day difference in flotation may lead to greater dispersal distances in flowing water, and
270 limits the risk of seed loss if environmental conditions do not lead to a rapid deposition on the
271 riverbank (van den Broek et al., 2005). In addition, we have shown for all stands that the
272 flotation time of achenes was significantly extended by germination in water, as expected in
273 the second hypothesis and in accordance with Rouifed et al. (2011). Seedlings were able to
274 remain afloat for over a month, compensating for short achene dispersal periods. This
275 dispersal mechanism has been observed for several riparian species (Sculthorpe, 1967; Barrat-
276 Segretain, 1996). *Fallopia* achenes germinate under water and seedlings develop cotyledons
277 that increase the drag forces experienced (Koch et al., 2010), allowing them to be easily
278 transported to the surface and benefiting from a secondary dispersal phase when temperatures
279 start to rise.

In accordance with our second hypothesis, seedling survival was impacted by water exposure (Fig. 5). Before germination, seeds protect the embryo from mechanical damage and rotting due to the low-oxygen conditions of water to which seedlings are more exposed. A long germination period may participate in the conservation of achene viability during transport (van den Broek et al., 2005; Dacasa Rüdinger and Dounavi, 2008). Moreover, germination proportion is also negatively correlated to germination time, as previously demonstrated by Shipley and Parent (1991). If a long germination time helps to maintain survival levels, the propagule pressure of low germinating stands should be conserved after water exposure compared to high germinating stands. However, germination time and seedling survival were not significantly correlated as expected in our third hypothesis. This means that not only do achenes act to protect embryos from water exposure, but some mechanisms of seedling tolerance to water exposure may also support seedling survival. Moreover, seedling survival to long water exposure times differed greatly between stands. Indeed, half of the stands had a good tolerance to water exposure, and survival did not differ from that of achenes that germinated directly in soil. Tolerance to water exposure not only increases the dispersal potential, but also favours a rapid anchoring and colonisation at the deposition site (Scarano, 1998). For the other half, survival was significantly reduced, leading to low propagule pressure, despite the high intrinsic germination proportion of some stands. This means that water may act as an important selective force for individuals of terrestrial species, participating in long distance water dispersal.

Even if vegetative dispersal represents a significant part of the dispersal strategy of the *Fallopia* complex, sexual reproduction also has a major role, particularly in contributing to the increase in variability and therefore to the evolution of the taxon (Ellstrand and Schierenbeck, 2000; Parepa et al. 2014). Combining the measured achene traits did not lead to any contrasting dispersal strategies between stands, but some of the stands presented traits

that seem to be particularly adapted to dispersal along watercourses. The success of the dispersion also depends on the propagule pressure (i.e. the quantity and physiological condition of the propagules released, Lockwood et al., 2007), which increases the number of propagules released and effectively contributes to colonisation. Propagule pressure may differ between stands and also be affected by changes in propagule physiological conditions (germination, regeneration and survival aptitude), which alters the effective number of propagules able to establish (Lockwood et al., 2007). According to the spatial selection theory, better dispersers would benefit from reproductive isolation, leading to phenotypes exhibiting traits particularly adapted to long-distance dispersal (Shine et al., 2011), which was also observed for an invasive species colonisation process (Huang et al., 2015). This mechanism may contribute, in the case of *Fallopia*, to expanding the distribution range of the complex, through the evolution of its seed dispersal traits.

5. Conclusions

Our study demonstrated the variability of achene traits involved in dispersal and the differences in trait values observed between stands. This study also demonstrates that achenes of some stands present a combination of traits particularly adapted to long distance dispersal through watercourses, which can contribute to the extension of the invaded area. Sexual reproduction and hybridization may represent a permanent addition of highly invasive genotypes to the complex and contribute to the increase in variability and therefore to the evolution of the complex. The invasion potential of the complex is thus driven by a large phenotypic diversity leading to the emergence of new morphological or physiological traits Large variations in dispersal traits and combination of traits may favour adaptation and

colonisation of different habitats, contributing to the invasiveness of the species along the rivers and in terrestrial environments.

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482

483 **Figures**

484 **Figure 1**. Dispersal strategies observed in riparian ecosystems, in terms of time (T) during
485 which seeds disperse and germinate and the consequences for survival (S). A) Seeds may
486 show long floating periods (T_1) and long germination periods (T_2) that favour high seed
487 survival (S_1) or B) Short floating periods (T_1), rapid germination in water (T_2) and extended
488 dispersal time (T_3) through seedling flotation. In this case, the tolerance of seedlings to water
489 exposure is critical (S_2).

490 **Figure 2**. Study area and stands sampled. Each square represents a stand of *Fallopia* complex
491 sampled. The towns where the stands were located are Saint-Bernard (BER), Chavanay
492 (CHAV), Laveyron (ELE), Saint-Romain-en-Gier (ENT), Saint-Etienne-le-Molard (LIG),
493 Lozanne (LOZ), Montrond-les-Bains (MEY), Villieu-Loyes-Mollon (PCHA), Châtillon-la-
494 Palud (PGEV), Tencin (TEN).

495 **Figure 3.** Estimated median flotation values (± SE) of achenes (T_1, black bars) and seedlings
496 (T_3, grey bars) of 10 stands of *F.* x *bohemica.* The hached bar indicates the absence of the
497 median flotation time estimation for one stand. Different letters indicate significant
498 differences between estimated parameters ($p<0.05$).

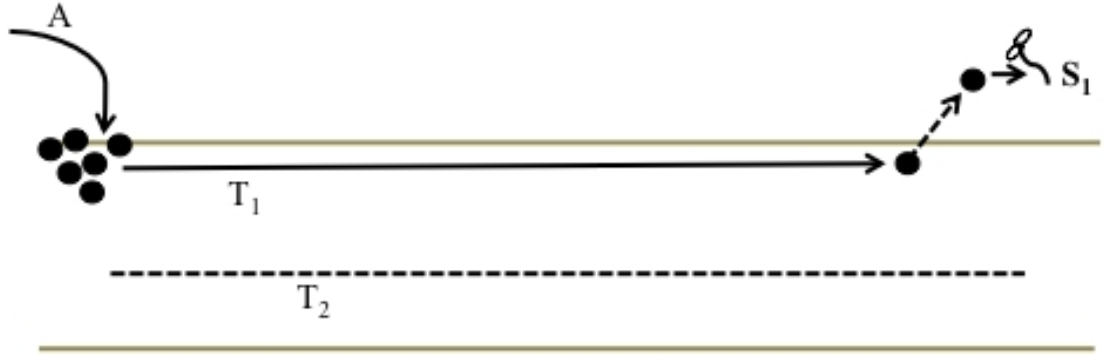
499 **Figure 4.** The right axis shows the estimated median germination times (±SE, bars) and the
500 left axis the estimated germination proportion (±SE, points) for 10 stands of *F.* x *bohemica*.
501 Different letters and numbers indicate significant differences between stands ($p<0.05$).

502 **Figure 5.** Probability of seedling survival (± SE) germinating in soil after water exposure (S_1,
503 grey bars) and after 28 days which was the maximum exposure duration (S2, black bars), for
504 10 stands of *F.* x *bohemica*. Different letters indicate significant differences between stands (χ

505 ^{2} tests) and stars show significant differences (ns, not significant; **p < 0.01; ***p < 0.001)

506 between S_1 and S_2 for each stand.

507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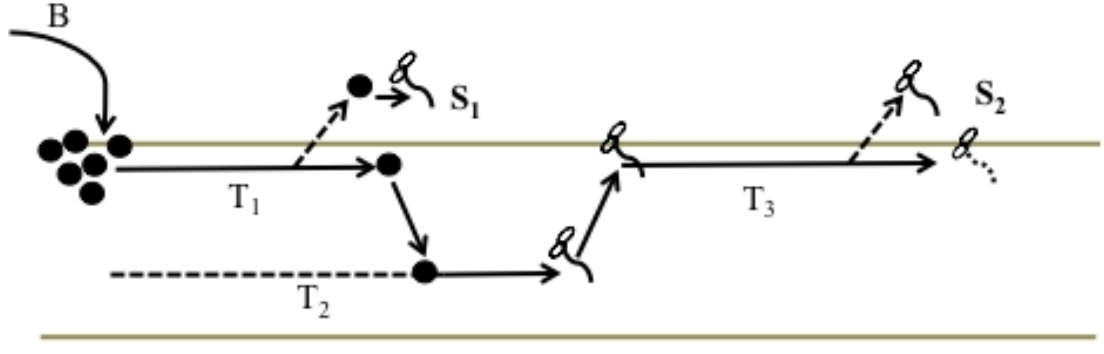
T2


B
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S2
T1
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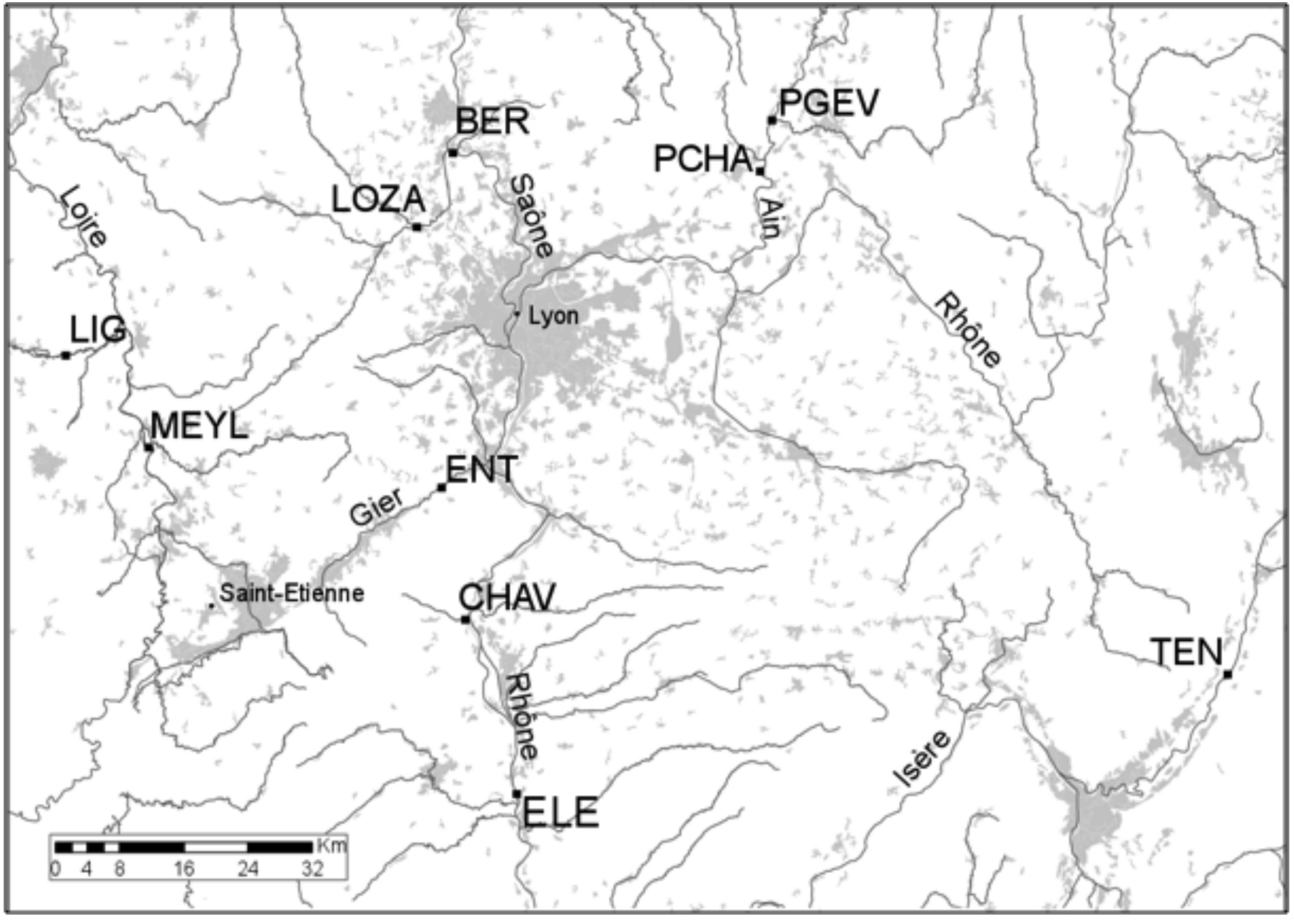
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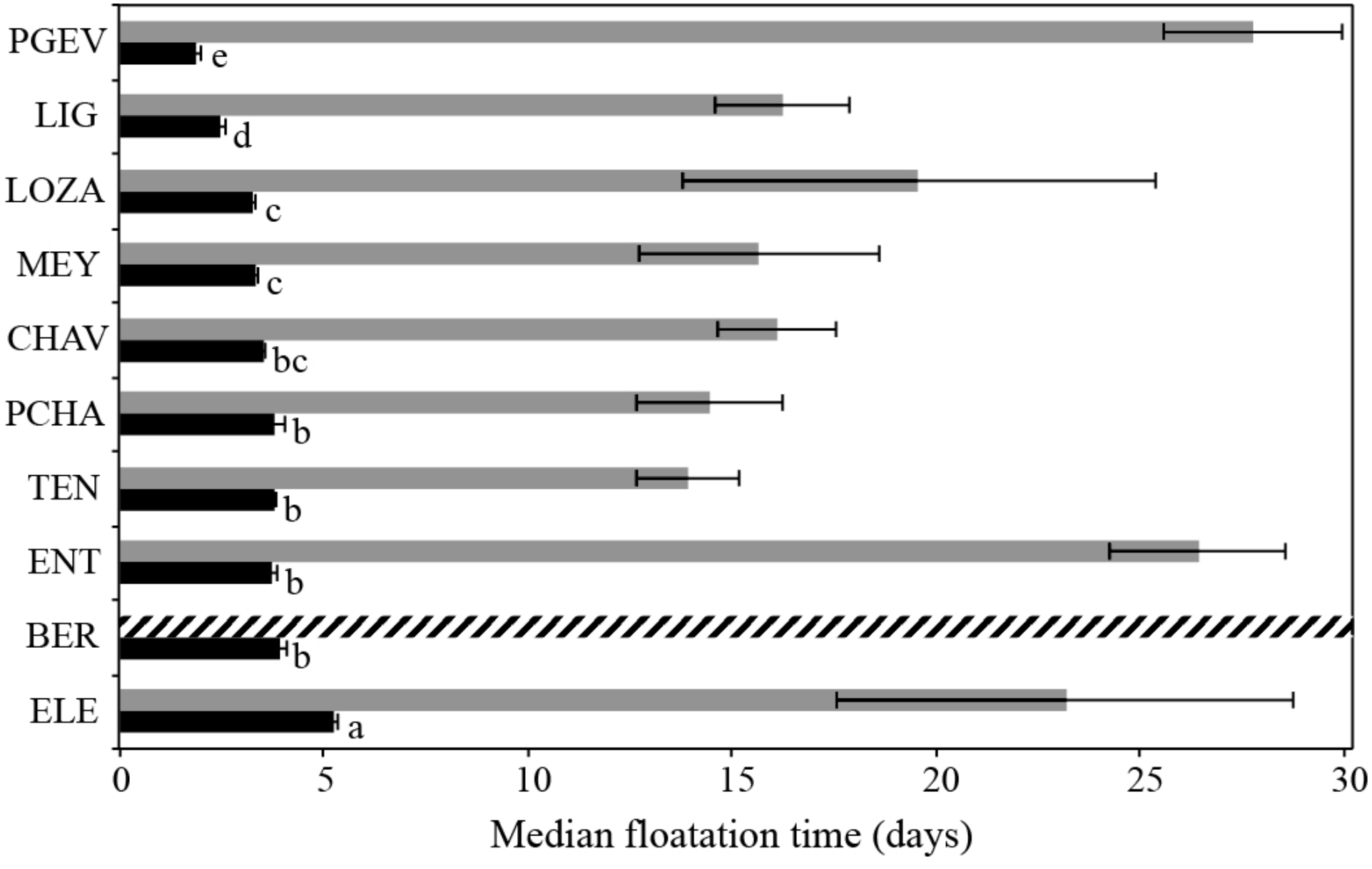
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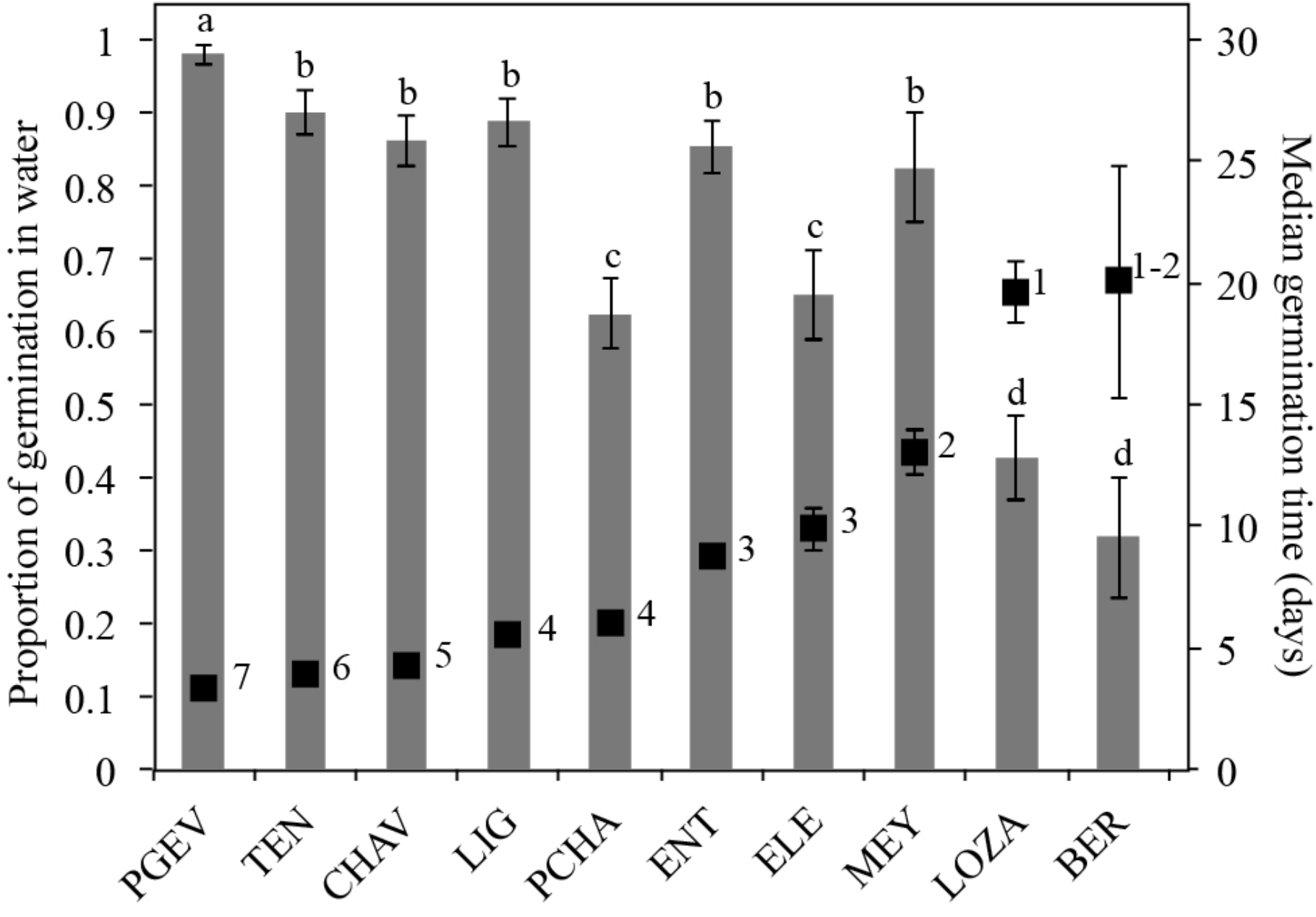
Median floatation time (days)


Proportion of germination in water
Median germination time (days)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
30
25
20
15
10
5
0
a
b
b
b
c
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b
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d
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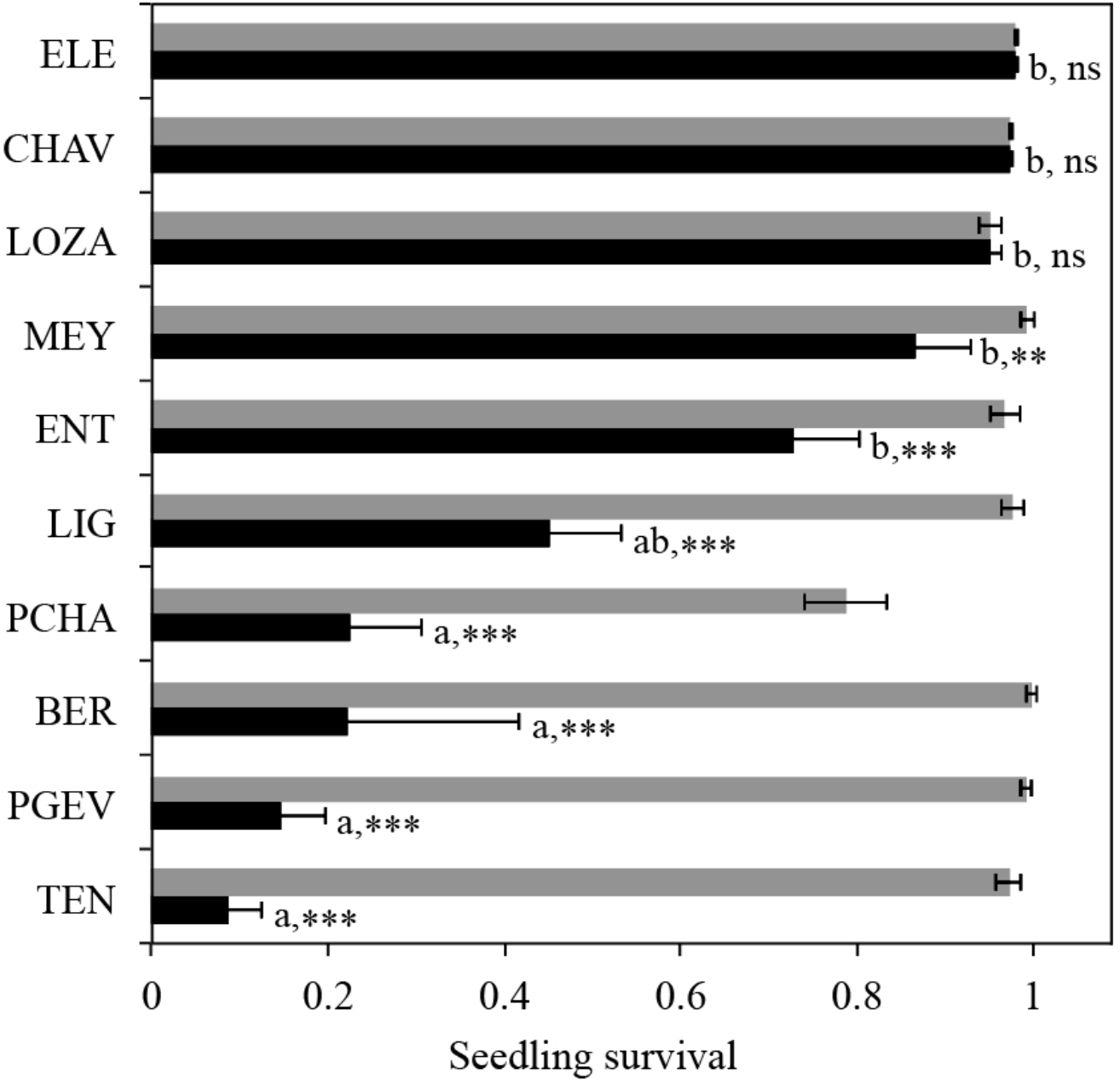
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a,***
a,***
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0.2
0.4
0.6
0.8
1
Seedling survival