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UNDERSTANDING SEED DORMANCY BREAKAGE and GERMINATION ECOLOGY of *Cynara cardunculus* (Asteraceae)

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Running head: Achenes dormancy and germination in *Cynara cardunculus*

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Summary

Cynara cardunculus is a troublesome weed in temperate grazing lands. *C. cardunculus* achenes are usually dormant at dispersal and require alternating temperatures to terminate dormancy and germinate. Laboratory and greenhouse experiments were conducted to determine: I) the treatments able to terminate dormancy and II) the effect of environmental factors and agronomic practices germination and emergence of non-dormant (dry afterripened) achenes. Scarification, hydrogen peroxide and sodium hypochlorite promoted germination of dormant achenes. Dry afterripening and cold stratification were tested in two different populations. Dormancy of both populations were released from dormancy by dry afterripening. In contrast, cold stratification allowed dormancy release in just one of the populations, while the other was induced into secondary dormancy. Germination of non-dormant (dry afterripened) achenes was maximum in a range of temperatures from 10 to 20°C and was inhibited at higher temperatures. Reduction of osmotic potential below -0.6 MPa. led to a decrease in final germination. These results explain synchronic emergence of *C. cardunculus* seedlings in autumn after dormancy release during summer. Maximum seedling emergence was close to 60% at soil depths of 1 cm and only decreased as depth increased over 6 cm. In contrast, seedling emergence was not reduced by the presence of cover residues, while a flooding duration of 21 d was required to suppress emergence significantly. These results suggest that the achenes burial and the uses of agronomic practices that take advantage of synchronic emergence of achenes could be useful tools leading to better long-term management of *C. cardunculus.*

Keywords: alternating temperatures, dormancy, germination, emergence, imbibition.

Introduction

Cynara cardunculus L. (var. sylvestris) (wild artichoke) is a cross-pollinated species belonging to the Asteraceae (Sonnante et al., 2007). It is a C3, perennial herbaceous plant native to the Mediterranean basin that has invaded thousands of hectares in temperate grasslands of Argentina (Busso et al., 2013), Australia (Parsons & Cuthbertson, 2001) and Uruguay (Marzocca, 1994). It is also present in the United States (Potts et al., 2008), Italy (Mauromicale et al., 2014), Spain and north Africa (Soumaya et al., 2013). In temperate grassland *C. cardunculus* is a highly competitive weed of perennial forages crops such as lucerne (*Medicago sativa* L.), orchardgrass (*Dactyllis glomerata* L.), tall fescue (*Festuca arundinacea* L.) and brome (*Bromus catharticus* L.), and also invades natural pastures (Marzocca, 1994). *Cynara cardunculus* is polycarpic and can produce several thousand wind-dispersed achenes each year that usually germinate throughout autumn (White & Holt, 2005).

Although it is an important weed in many cultivated and natural systems, there is scarce information about *C. cardunculus* achene biology (Raccuia et al., 2016). Reported data show that most *C. cardunculus* populations produce dormant achenes at dispersal. Dormancy breakage of these dormant achenes depend on exposure of alternating temperatures (Huarte, 2006; Raccuia et al., 2016), and in a lesser extent of light (Huarte & Benech-Arnold, 2010). Germination of these populations at constant temperatures is scarce (Huarte & Benech-Arnold, 2005; Ierna et al., 2004). Some of the physiological processes behind the response of *C. cardunculus* achenes to alternating temperatures have been recently investigated. Alternating temperatures enhance embryo growth potential by reducing the abscisic acid (ABA) to gibberellins (GA) ratio (Huarte & Benech-Arnold, 2010; Huarte et al., 2014). Abscisic acid and gibberellins are essential for the induction and maintenance of seed dormancy and the promotion of seed germination, respectively (Kucera et al., 2005). Despite of the progress made in relation to understanding the responses of *C. cardunculus*

achenes to alternating temperatures, there is almost no information about treatments that might improve germination of *C. cardunculus* dormant achenes when achenes are incubated at constant temperatures.

Batlla and Benech-Arnold (2010) pointed out that the stimulus of alternating temperatures, light, or both, are essential for germination of many species that have physiological dormancy. Finch-Savage and Leubner-Metzger (2006) stated that this type of dormancy is observed in many Asteraceae species and is exerted by the embryo covering layers, such as endosperm and/or pericarp, or by the embryo inability to growth. Physiological dormancy is gradually alleviated once achenes are dispersed from the mother plant by dry after-ripening or cold stratification (Nee et al., 2017). Seed dormancy alleviation is associated with a widening of the environmental conditions at which seeds can germinate. In many cases, when dormancy level is sufficiently low, seeds lose their requirement of alternating temperatures and/or light to germinate and can germinate under a wide range of constant temperatures (Benech-Arnold et al., 2000). Knowledge about the way in which the environment modulates dormancy alleviation in weed species is useful to predict germination and seedling emergence patterns in the field, and to develop rational strategies of weed management (Ramirez et al., 2014). Up to date, factors affecting dormancy alleviation of *C. cardunculus* achenes have not been assessed. Moreover, no information in relation to the range of temperatures under which non-dormant *C. cardunculus* achenes can germinate is available in the literature.

As previously commented, in situations where *C cardunculus* is a troublesome weed in cool season perennial forages and natural grasslands, achenes are usually accumulated on the soil surface and only buried during seed bed preparation for the new crop. To date, there has been no information about the way in which the presence of a close canopy and the depth of burial might affect emergence of *C. cardunculus* is absent. This knowledge may be useful to reduce *C. cardunculus* emergence and could be included as a component of integrated weed management systems. *Cynara cardunculus* is also found in some lowlands environments exposed to frequent flooding, as for example The Salado River basin (Buenos Aires Province, Argentina) (Busso et al.,

2013). Duration of flooding in this ecosystem is quite variable and might affect the fate of *C. cardunculus* soil seed-bank, as for example, its temporal germination pattern. However, no information is available about the capacity of *C cardunculus* achenes to survive and germinate after flooding events.

The general objective of this paper is to investigate different aspects of *C. cardunculus* seed biology that can be relevant for managing *C. cardunculus* natural seed-banks in the field. Some key questions are: I) what treatments can substitute alternating temperatures requirement for dormancy breakage and germination; II) what is the nature of *C. cardunculus* fruit dormancy (i.e. imposed by the embryo or the seed coats); III) how is dormancy alleviated (i.e. dry after-ripening or cold stratification); IV) what is the effect of temperature and osmotic stress on germination of non-dormant achenes and V) what is the effect of achenes burial depth, the presence of stubble of different heights and flooding duration on *C. cardunculus* seedling emergence.

Materials and Methods

Seed collection

Mature achenes of *Cynara cardunculus* (L.) were collected from plants growing at infested roadsides in two locations in Buenos Aires Province, Argentina. Achenes were considered mature when the pappus of the capitulum had fully expanded and achenes could be easily detached from the receptacle. The first collection site (1) was located in Olavarría (latitude 37° S, longitude 60° 35´ W) and achenes were collected in 2013, 2015 and 2016. The average annual precipitation is 730 mm and the mean temperature is 15.7°C. The second site (2) was in Abasto (latitude 35° 05S, longitude 58° 11´W) and achenes were collected in 2013. The average annual precipitation is 940 mm and the mean temperature is 17.2°C. Achenes were collected from randomly selected plants and bulked to obtain experimental samples. Achenes collected at site 1 and 2 in 2013 were used to evaluate achenes response to dry afterripening and cold stratification. The rest of the experiments were performed using achenes collected in Olavarría in 2015 and 2016. For this set of experiments achenes were cleaned and immediately stored at -18°C to maintain their initial dormancy level (Benech-Arnold *et al.,* 2006) until used in the experiments.

General procedures for germination tests

Achenes were placed in 9-cm-diam petri dishes over two pieces of filter paper wetted with 7 ml of distilled water or the corresponding treatment solution. Germination tests were performed in darkness. Previous experiments showed that the stimuli of alternating temperatures is enough to the breakage of achenes dormancy (e.g. Huarte 2006). Germination was scored daily for 14 days (otherwise stated). Achenes with visible radicle protrusion were considered as germinated and

133 removed. Dishes were wrapped in a double layer of aluminium foil to prevent achenes from
134 exposure to light.

135

136 *Imbibition*

137 To evaluate achene coat permeability to water, forty-five achenes collected at site 1 during 2015
138 were individually weighed with an electronic balance A200S (Sartorius, Gottingen, Germany) and
139 placed in a 2 cm plastic box on two filter paper discs moistened with 2 ml of distilled water.
140 Individually identified achenes were incubated throughout 10 d in darkness at 15 and 20/10°C. Each
141 individual achene was blotted dry and weighed once a day, so measures of moisture content over
142 time were made on the same individual achene. Water content (WC) was determined as the actual
143 increase based on achene initial air-dry mass (Baskin et al., 2002) according to the following
144 equation:

145 $$WC = (Wn - W1) / W1 \quad (1).$$

146 Where Wn is the weight after n days of imbibition and W1 is the weight before imbibition started.

147

148 *Effect of scarification on germination at constant temperatures.*

149 To determine the role of the pericarp on achene germination, achenes collected at site 1 during 2015
150 were scarified using a 98% (w/w) concentrated H_2SO_4 (Cicarelli, Argentina) and mechanical
151 abrasion. Achenes were acid scarified for 0, 5, 15 or 45 min in a H_2SO_4 solution. Mechanical
152 scarification was done by scrapping the pericarp using sandpaper Doble A No 200 (Abrasivos
153 Argentinos, Argentina) covering the cotyledon area (i.e. the pericarp at the opposite side of the
154 embryo) to avoid any damage to the embryonic axis. After the different treatments achenes were

155 incubated in water at constant 15°C. Germination was also tested at alternating temperatures
156 (20/10°C) and this value was considered as a positive control.

157

158 *Effect of oxidants and nitrogenous compounds on germination at constant temperatures.*

159 To determine the effect of (A) hydrogen peroxide (H_2O_2) (Cicarelli, Argentina), (B) Sodium
160 hypochlorite (i.e. commercial bleach, 36.8 gr NaClO per L) and (C) KNO_3 (Cicarelli, Argentina) on
161 germination, achenes collected at site 1 during 2015 were: (A) incubated at constant 15°C in H_2O_2
162 solutions at concentrations of 0, 0.4, 0.8 and 1.2M, (B) soaked for 0, 1, 5, 10, 15, and 30 min in a
163 sodium hypochlorite solution and then incubated at constant 15°C in water and (C) incubated at
164 constant 15°C in the presence of 0.2% KNO_3. Germination was also tested at alternating
165 temperatures (20/10°C) in distilled water and this value was considered as a positive control.

166

167 *Effect of time and storage temperature on achene dormancy alleviation.*

168 To determine the effect of time and temperature on achene dormancy alleviation (i.e. a reduction of
169 alternating temperatures requirement for germination), achenes collected in Olavarría and Abasto in
170 2013 were stored under moist conditions at 6°C (stratification) and under dry conditions at 6, 15 and
171 25°C (dry-afterripening) for 75 and 40 days, respectively. Before storage (0 days), and after 15, 30,
172 45, 60 and 75 days (Olavarría population), and 10, 20, 30 and 40 days (Abasto population) of
173 storage achenes were incubated in water at 15 and 20/10 °C in darkness for 20 d.

174

175 *Effect of temperature and osmotic stress on germination of non-dormant achenes.*

176 To determine the effect of temperature on *C. cardunculus* non-dormant achenes germination, dry
177 afterripened (75d at 15°C) achenes collected in Olavarría in 2015 were incubated at alternating

178 temperatures (12/12h) of 20/10, 25/15 and 20/30°C and at constant temperatures of 10, 15, 20, 25,
179 30 and 35°C (these temperatures reflect typical seasonal variation temperature regimes and the
180 average high and low temperatures in Buenos Aires Province, Argentina). The effect of moisture
181 stress on achenes germination were assessed by incubating achenes at 15°C in solutions with
182 osmotic potentials of 0, -0.2, -0.4, -0.6, -0.8, -1 and -1.2 MPa. Solutions with different osmotic
183 potentials were prepared by dissolving polyethylene glycol 8000 in distilled water according to
184 Michel (1983). Achenes incubated in water or PEG solutions were transferred to fresh solutions
185 after the first 24 h and then after 6 d, to maintain a constant osmotic potential during the
186 experiment.

187 *Effect of simulated agronomic practices and flooding duration on seedling emergence*

188 The effect of burial depth was tested by sowing achenes collected in Olavarría in 2016 at soil depths
189 of 0, 1, 2, 3, 4, 6 and 8 cm. The effect of crop residues on seedling emergence was tested sowing
190 achenes collected in Olavarría at 1 cm depth and spreading oat pasture stubble on soil surface at
191 rates equivalent to 0, 1, 2, 3, 4, and 6 t ha^{-1}. The treatments 1, 2, 3, 4, and 6 t ha correspond to
192 approximate oat pasture cover heights of 0.4, 0.6, 1, 1.4 and 1.80 cm, respectively. To determine
193 flooding tolerance, achenes collected in Olavarría in 2016 were sowed 1 cm deep in soil contained
194 in Styrofoam cups. Flooding durations were 0, 4, 7, 14, and 21 d. To simulate flooding, water was
195 maintained 2 cm above the soil surface for the mentioned periods. After exposure to a given period
196 of flooding, surface water was drained by poking holes at the side of the cups to drain the excess
197 water. The three experiments were performed in a greenhouse (17 ± 2 °C during the day and 10 ±
198 2°C at night) and ten dry afterripened achenes (after 75 days at 15 °C) were sown in each
199 experimental unit (9 cm diameter pots and 12 cm high and 9.5 cm diameter Styrofoam cups). Soil
200 (31% sand, 37% silt, 32% clay, pH 6.5, 5.4% organic matter) used for the experiments was
201 collected from an experimental plot at Facultad de Ciencias Agrarias (National University of Lomas
202 de Zamora, Buenos Aires Province, Argentina) free from *C. cardunculus* plants. During the
203 experiments pots were kept close to field capacity by regular watering and in the flooding

experiment soil was kept close to field capacity after the flooding treatments ended. Emerged seedlings were counted at two days intervals from 3 d to 45 d after sowing in all experiments. Seedling emergence was defined as the appearance of the cotyledons, and emerged seedlings were counted and then removed. Seedling emergence was expressed as a percentage of the achenes sown.

Data analysis.

All experiments were conducted in a completely randomized design. Each experiment was conducted twice, and treatments of each experiment were replicated three times. Germination was expressed as final percentage of total achenes except for the imbibition experiment where cumulative percentages are shown. Each germination value is the mean ± SE of three replicates of 25 achenes each. Because data analysis showed non-significant interaction between treatment effect and each experimental run, data of both experiments were combined for the analysis. The effect of scarification and KNO_3 and H_2O_2 on germination were subjected to analysis of variance, and means were separated by Tukey´s and Krustal-Wallis's test at P= 0.05 using InfoStat (Balzarini et al., 2008). The rest of treatments (i.e. immersion in H_2SO_4 and NaClO, osmotic potential, depth of burial, forage cover and flooding duration on germination and seedling emergence) were analysed by means of regression analysis using Graph Pad Prism 7.0 (GraphPad Software, La Jolla California USA) and CurveExpert Basic 2.1.0 (Hyams Development). The goodness of fit of the models was assessed by R^2 or R² and SE.

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224 **Results**

225

226 *Imbibition*

227 Water uptake of achenes incubated at alternating and constant temperatures increased in a similar
228 manner from the start of imbibition up to day 4 (Fig. 1A). Thereafter, achenes incubated at
229 alternating temperatures took up water because of the start of germination (Fig. 1B). In contrast,
230 achenes exposed to constant temperatures, under which very few achenes germinated (Fig. 1B), did
231 not show a subsequent increase in WC till the end of the experiment. Germination started by day 5
232 after imbibition at alternating temperatures, reaching its maximum value by day 9. Final
233 germination percentages were 81± 10% and 6 ± 1% for alternating and constant temperatures
234 treatments, respectively (Fig. 1B).

235

236 *Effect of scarification on germination at constant temperatures.*

237 Total germination at different times of soaking in H_2SO_4 was fitted by an exponential plus linear
238 model. An enhancement of germination until the extent to that observed at 20/10°C in water (right
239 panel) (i.e. the positive control) was scored for 5 and 15 min concentrated H_2SO_4-treated achenes.
240 However, germination of 45 min H_2SO_4-treated achenes was similar to that scored at 15°C in water.
241 On the other hand, germination of mechanical scarified-treated achenes reached an intermediate
242 value among control treatments ($P<0.05$) (Fig. 2B).

243

244 *Effect of oxidants and nitrogenous compounds on germination at constant temperatures***.**

245 The presence of H_2O_2 at doses higher than 0.4M increased achenes germination at constant
246 temperatures (Fig. 3A). Achenes incubated at 1.2 M H_2O_2 showed a threefold increase in

247 germination in relation to that observed at constant 15°C in water. Germination of achenes soaked
248 in NaClO from 1 to 15 min showed an increase in germination in relation to non-soaked achenes
249 incubated in water. However, this increase did not reach germination values of achenes incubated at
250 alternating temperatures in water (Fig. 3B). Finally, achene germination in the presence of 0.2%
251 KNO_3 reached an intermediate value to those scored in positive and negative control treatments
252 (Fig. 3C).

253

254 *Effect of time and temperature of storage on achene dormancy alleviation.*

255 Total germination of fresh achenes (i.e. recently dispersed) collected in Olavarría (site 1) was 11.6 ±
256 6.01% (mean ± SE) and 70 ± 2.89% for constant (15°C) and alternating temperatures (20/10°C),
257 respectively (Fig. 4). These results evidence both, a high level of dormancy of the fresh achenes,
258 and the efficacy of alternating temperatures to break *C. carduncullus* dormancy. Achene exposure to
259 cold stratification for 15 d reduced achene population dormancy level (Fig. 4). Indeed, after 15 days
260 of cold stratification germination at 15°C (61.6 ± 7.26 %) was similar to that observed for fresh
261 achenes incubated at alternating temperatures (71.6 ± 8.82%). However, extended cold stratification
262 (>30 d) induced achenes into secondary dormancy, showing a level of dormancy higher than that
263 observed at dispersal. In these cases, alternating temperatures were not able to promote germination,
264 showing similar values to that observed at constant temperatures. Dry storage was effective to
265 alleviate dormancy. This fact was proved by the progressive increase in germination at constant
266 temperature in darkness with storage time (i.e. loss of the alternating temperatures requirement for
267 germination). Likewise, dormancy alleviation was faster as storage temperatures increased from 6
268 to 25°C. Indeed, achenes stored at 6°C showed just a partial alleviation from dormancy, presenting
269 germination percentages at constant temperatures below 50% during the entire storage period. On
270 the other hand, achenes stored at 15°C incubated at constant temperatures showed similar values to
271 those observed for achenes incubated under alternating from 45d onwards, while achenes stored at

272 25°C showed no difference in germination between constant and alternating temperatures (i.e.
273 dormancy alleviation) just after 15 days of storage. A reduction in germination at constant
274 temperatures was observed after 75d of storage for achenes incubated at this temperature.

275 Germination of fresh achenes collected in Abasto (site 2) was 56.67 ± 7.26 % and 86.7 ± 4.41 % for
276 constant temperature (15°C) and alternating temperatures (20/10°C), respectively (Fig. 5). These
277 results indicate a less dormant achene population to that collected in Olavarría (Fig. 4). In the
278 Abasto population, both cold stratification (from 20d onwards) and 10 days of dry afterripening at
279 15 and 25°C were effective to reduce the initial level of dormancy (Fig. 5). On the contrary, dry
280 storage at 6°C did not allow achene dormancy release (defined here as the requirement of
281 alternating temperature to germinate).

282

283 *Effect of temperature and osmotic stress on germination of nondormant achenes*

284 Germination of achenes (i.e. storage at 15°C by 75d) was affected by tested incubation temperature
285 regimes ($P < 0.0001$) (Fig. 6A). Higher germination percentages were scored at constant 10, 15 and
286 20°C and at 20/10°C. An increase of temperatures till 25°C significantly reduced germination,
287 while germination was almost null at constant 30 and 35°C. In addition, alternating temperatures
288 regimes of 25/15°C and 20/30°C were not effective to stimulate germination, showing germination
289 values lower than 25%. To evaluate the effect of simulated water stress on germination, a sigmoidal
290 dose-response regression model was fitted (Fig. 6B). Germination was greatest at 0, -0.2 and -
291 0.4MPa, while a gradual decrease of total germination occurred from -0.6 MPa to -1.2 MPa.

292

293 *Effect of burial depth, crop residues and flooding duration on seedling emergence.*

294 A plateau followed by one phase decay regression model was fitted to seedling emergence from 0 to
295 8 cm burial depth. Burial depths ranging from 0 to 4 cm showed similar emergence values (Fig.

296 7A), while achenes buried at a depth of 6 cm showed a reduction in seedling emergence. This fact
297 was corroborated at a burial depth of 8 cm, where no seedling emergence was scored (Fig. 6A).
298 Seedling emergence of *C. cardunculus* was not significantly reduced by the addition of stubble on
299 the soil surface (Fig. 7B). In this case, a linear regression model was used where a non-significative
300 effect of forage cover on seedling emergence was observed. Total emergence ranged from 63.3 ±
301 6.15% (bare soil) to 53. 3 ± 12.28% beneath a dry forage cover equivalent to 6 t ha^{-1}. Seedling
302 emergence decreased with increasing flooding durations. A linear model was fitted to assess
303 germination of achenes exposed to different flooding durations. Achenes subjected to flooding
304 conditions for periods longer than 14 days showed a significant decrease in emergence percentage,
305 showing values close to 3% (Fig. 7C).

306

Discussion

C. cardunculus achenes are usually dormant at dispersal and this primary dormancy state can be broken by exposure of achenes to cycles of alternating temperatures (Huarte & Benech-Arnold, 2010; Raccuia et al., 2016). Seed dormancy can be imposed by different seed or fruit tissues. For example, physiological dormancy, which is the most frequent type of dormancy in temperate species, can be associated with properties of the embryo covering layers, such as endosperm and/or pericarp, and/or by the embryo itself (Finch-Savage & Leubner-Metzger, 2006). Results presented here, show that the covering layer seems to play a crucial role in *C. cardunculus* achene dormancy (Fig. 2). Nasreem et al. (2002) stated that covering layers may reduce germination by interfering with water and/or oxygen uptake or by exerting a mechanical resistance to embryo growth. Results obtained in the present work from the imbibition experiment pointed out that a reduction of water uptake is not the prevalent mechanism through which the covering layers block germination in *C. cardunculus.* Indeed, achenes exposed to constant or alternating temperatures exhibit a common dynamic of water uptake until the beginning of germination (Fig. 1A and B). To understand the role of covering layers in imposing *C. cardunculus* fruit dormancy we evaluated the efficacy of treatments described to modify covers characteristic (e.g. hardness) and to increase oxygen availability. Soaking achenes with H_2SO_4 by 5 and 15 min effectively promoted germination up to similar values to those scored at alternating temperatures in water (Fig. 2A). Sulphuric acid treatments are associated with a disruption of covering layers (Aliero, 2004) and are frequently used to increase germination of legumes seeds. On the other hand, only an intermediate increase in germination was scored for mechanical scarified-treated achenes (Fig. 2B). Kimura and Islam (2012) stated that the response to scarification treatments are based on an enhancement of water and/or oxygen uptake. Considering that results showed that alternating temperatures did not affect water uptake (Fig. 1A), it can be proposed that scarification may increase germination by an enhancement of oxygen diffusion to the embryo. Moreover, this is further supported by the fact that

the presence of H_2O_2 (1.2M), KNO_3 and the immersion of achenes in NaClO were also effective to enhance germination (Fig. 3A-C). Hydrogen peroxide and NaClO are oxidants compounds known to increase oxygen availability. These results show that those treatments able to increase oxygen availability, such as oxidants compounds, and mechanical scarification, allowed a higher dormancy breakage, suggesting that a reduction in the oxygen diffusion to the embryo could be one of the mechanisms involved in the dormancy exerted by the covering layers in *C. cardunculus*.

Dry after-ripening was effective in alleviating dormancy of *C. cardunculus* achenes (Fig. 4 and 5). These results agree with that observed in some other Asteraceae species (Schütz et al., 2002) and winter annual weeds (Iglesias-Fernández & Matilla, 2009). As dry afterripening progressed, *C. cardunculus* achenes lost their requirement of alternating temperatures to germinate and were progressively able to germinate at constant temperatures. This is consistent with Finch-Savage and Leubner-Metzger (2006), who stated that dry after-ripening involves a widening of the environmental conditions allowing seed germination. However, the different level of primary dormancy exhibited by both populations determined an apparent different response to dry afterripening temperature. For the most dormant population (i.e. Olavarría, site 1) the increase of storage temperature hastens the rate of dormancy release. This is consistent with the behavior observed in *Lollium rigidum* (Steadman *et al*., 2003) and some other winter annual weed species (Baskin & Baskin, 1986). Indeed, achenes stored at 25 °C required 15 days to lost the requirement of alternating temperatures for germination, while those stored at 15°C required longer storage (45 days). In contrast, primary dormancy level of the Abasto population, (site 2) was lower than that observed in the Olavarría population (this fact was evidenced by the extent of fresh achenes able to germinate at constant temperatures). This lower primary dormancy level determined an almost entire dormancy loss after just 10 days of storage at 15 and 25°C, precluding the possibility of detecting differences in the dormancy release rate between these storage temperatures. In addition, both populations did not show significant dormancy release when stored dry at 6°C. This result agrees with data reported by Bazin et al. (2011), who determined that storage temperatures below

359 8.17°C prevent dormancy loss due to dry afterripening in another Asteraceae, sunflower
360 (*Helianthus annus*, L.). Differences among tested populations were also evidenced by their response
361 to cold stratification (humid 6°C) (Fig 4 and 5). Indeed, in the most dormant population cold
362 stratification for longer than 15 days provoked an induction into secondary dormancy in such an
363 extent that exposure to alternating temperatures was totally ineffective to promote achene
364 germination. At the opposite, a similar treatment duration allowed for the lesser dormant population
365 a full exit from dormancy, without induction into secondary dormancy with extended storage.
366 Differences among *Cynara cardunculus* populations were already described for many attributes
367 (Ben Ammar *et al*., 2014). For instance, tolerances to abiotic stress (Raccuia *et al*., 2004) and
368 chemical profiles (Portis *et al*., 2005). Here, differences between populations were evidenced for the
369 first-time on their response to temperature-dependent dormancy release.

370 Temperature experiments performed on dry afterripened (i.e. nondormant) achenes allowed to
371 characterize the thermal range permissive for *C. cardunculus* germination. Achenes had high
372 germination percentages at constant temperatures from 10 to 20°C and at an alternating temperature
373 regime of 20/10°C, while germination was progressively inhibited by constant 25°C onwards.
374 Likewise, alternating temperatures of 20/30 °C and 25/15°C did not stimulate germination (Fig.
375 6A). These results show that *C. cardunculus* nondormant achenes exhibit a narrow thermal range
376 permissive for germination, in which germination is prevented at temperatures higher than 20°C.
377 Altogether these results indicate that *C. cardunculus* behaves as a typical winter annual weed, in
378 which achenes come out from dormancy through dry-afterripening when exposed to the "high"
379 summer temperatures and go to into secondary dormancy when exposed to low winter temperatures
380 (although this can differ between populations). Under field conditions, this temperature-dependent
381 regulation of achene dormancy level may establish a non-dormant seed-bank population from mid-
382 summer to late autumn. However, the impossibility of *C. cardunculus* non-dormant achenes to
383 germinate at "high" temperatures prevent emergence during summer and sets the emergence
384 window throughout autumn months, in which lower temperatures allow achenes germination (10-20

385 °C). This information can be used to design alternative practices to manage *C. cardunculus*
386 populations. For instance, by a shift to an earlier sowing date of cool-season forages crop. Thus,
387 crop seedling emergence may occur before a reduction of soil temperatures allowing germination
388 and seedling emergence of *C. cardunculus*. This may be useful in forage crops such as lucerne, tall
389 fescue, wheatgrass due to germination of these species occurring at a high percentage at alternating
390 (20/30°C) and constant (25°C) temperatures (I.S.T.A, 1983). All these forage crops are of
391 meaningful importance for biomass production in temperate grassland of Argentina and some other
392 countries. Likewise, a similar thermal behaviour was described by Basnizki and Mayer (1985) for
393 *Cynara syriaca* achenes; Thanos et al (1989) for *Glaucium flavum* seeds and Doussi and Thanos
394 (2002) for four species of the Muscari genus. All these species originated in the Mediterranean
395 basin have low germination at temperatures close to 20 °C. These authors stated that this is a
396 characteristic of Mediterranean species to fit germination to the humid condition prevailing in
397 autumn and winter.

398 Final germination was reduced at osmotic potentials from -0.6 MPa onwards (Fig. 6B). This
399 reduction was stronger when achenes were incubated at -0.8MPa and -1MPa; in these treatments
400 germination decreased to 30% and 10%, respectively. Our results agree with those shown by
401 Raccuia et al. (2004). These authors reported germination values ranging from 32 to 46% at -
402 0.6MPa, and values close to 20% at -0.9MPa, for eight *C cardunculus* achenes populations
403 collected at Catania (Italy). Bewley (1997) proposed that inhibition of germination under low soil
404 water availability constitutes an important survival mechanism until sufficient water is available for
405 successful seedling emergence.

406 Deep burial through soil tillage and the presence of cover on the soil surface are agronomic
407 practices that can be applied to reduce seedling emergence of many weeds (e.g. Amini et al., 2017).
408 Emergence of *C cardunculus* was limited to 36% of their maximum percentage by a burial depth of
409 6cm (Fig. 7A). However, the full prevention of seedling emergence was reached when achenes were
410 placed at 8cm deep. Since buried achenes may maintain the capacity to germinate for 5-7 years

411 (Fernandez & Curt, 2005), burial may be useful to reduce emergence before seting long term
412 pastures such as the mentioned above. The similar values of seedling emergence observed from the
413 soil surface up to 4cm of burial is consistent with the large size of Cynara achenes (1000 achenes
414 weight close to 37g). The possibility of large seeds germinating deep in the soil was described for
415 seeds of weed and cultivated species. This trait is based on the extent of energy reserves able to
416 support seedling growth (e.g. Benvevuti et al., 2001). Even though the current study does not
417 evaluate the reason/s that reduce/s *C. cardunculus* seedlings emergence, the main factors acting in
418 the reduction of weed seedling emergence, with an increasing depth, have been already summarized
419 by Benvenuti et al. (2001). These authors proposed factors such as, the lack of enough seed reserves
420 to reach the soil surface, hypoxia, presence of CO_2 and low rates of gaseous diffusion at increasing
421 depths that may induce secondary dormancy. The increment in the amount of stubble on soil surface
422 did not significantly reduce *C. cardunculus* seedling emergence (Fig. 7B). Some of the reasons
423 proposed by the reduction of seedling emergence by this practice are a prevention of light reaching
424 the seeds, physical obstruction provided by crop residue and allelopathy (e.g. Liebman & Davis,
425 2000). The ability of *C. cardunculus* achenes to germinate in the dark can explain the lack of
426 response to the presence of stubble on the soil surface. Anyway, this would be impractical in forage
427 crops production systems, in which crop cover is used to feed livestock. These results suggest that
428 the presence of crop residues on the soil surface or shallow burial depths are not effective to reduce
429 *C. cardunculus* seedling emergence. So, the main factors affecting the number of seedlings emerged
430 from natural soil seed bank would be those controlling germination, such as soil temperature and
431 soil water availability.

432 Flooding for 21d drastically reduced the emergence of *C. cardunculus* (Fig. 7C). Thus, this species
433 is sensitive to prolonged flooding and may not be able to persist in areas that remain waterlogged
434 for long periods. This may be the main reason for explaining why *C. cardunculus* is most frequent
435 in drained than flooded soils (CABI, 2017). The capacity to tolerate flooding is a highly species-
436 specific trait and *C. cardunculus* shows less tolerance than some species such as *Ipomea purpu*rea

437 (Singh *et al*., 2012) and *Agrostis stolonifera* (Zapiola & Mallory-Smith, 2010). For instance, the
438 latter keeps its capacity to germinate during a seventeen-weeks period long.

439 In conclusion, *C. cardunculus* achenes are dormant at dispersal and this state is effectively broken
440 by exposure to alternating temperatures. This dormant state (evaluated in relation to the requirement
441 of alternating temperature to germinate) can be broken by exposing the achenes to compounds able
442 to increase oxygen availability. *C. cardunculus* behave as many other winter annual species, in
443 which dormancy is alleviated through dry afterripening (the higher the temperature the higher the
444 dormancy release rates), while cold temperatures may provoke entrance into secondary dormancy:
445 although this response differ among *C. cardunculus* achenes populations. In addition, germination
446 of after-ripened achenes is inhibited at high temperatures (> 20°C), which may be instrumental for
447 avoiding germination of non-dormant achenes during the summer, delaying germination to the
448 autumn months. In parallel, germination is also affected by the reduction of water potential to -0.6
449 MPa. onwards. This may be another adaptation able to delay germination till autumn, when
450 temperatures together with the soil water availability are compatible with *Cynara* requirements for
451 germination. As part of an integrated control method, before the sowing of long term pastures, *C.*
452 *cardunculus* seedling emergence can be reduced by burying achenes deeper than 6cm. In contrast,
453 the presence of cover has not reduced the emergence of *C. cardunculus* seedling emergence along
454 of pasture production cycle. *C. cardunculus* did not show a great tolerance to flooding.

455

456

457

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461

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573

574 **Figure legends**

575 **Fig. 1** (A) Imbibition of *Cynara cardunculus* achenes incubated at fluctuating temperatures
576 (20/10°C, 12 h thermoperiod) (solid symbols) and constant temperatures (15°C) (empty symbols) in
577 relation to incubation time (d). (B) Cumulative germination time courses of *Cynara cardunculus*
578 achenes incubated at fluctuating (20/10°C, 12 h thermoperiod) (closed symbols) and constant
579 temperatures (15C) (open symbols). Data are means of triplicates± SE. When observed, vertical
580 bars are ± SE.

581

582 **Fig. 2** (A) Final germination of *Cynara cardunculus* achenes incubated in water at constant 15°C
583 after different H_2SO_4 soaking times and incubated in water at fluctuating temperatures (20/10ºC)
584 (black column). Data obtained at 15°C were fitted using an exponential plus linear regression model
585 (R^2=1), (B) Final germination of *Cynara cardunculus* scarified achenes incubated in water at
586 constant 15ºC (white column) and non-scarified achenes incubated in water at constant 15º C (white
587 column) and at fluctuating temperatures (20/10 ºC) (black column). T bars indicate SE. Different
588 letters at the top of each bar indicate significant differences according Tukey´s Test (α= 0.05).

589

590 **Fig. 3** (A) Germination of *Cynara cardunculus* achenes incubated at constant 15 °C in different
591 H_2O_2 concentrations and in water at fluctuating temperatures (20/10 °C) (black column). (B)
592 Germination of *Cynara cardunculus* achenes incubated in water at constant 15 C after different
593 NaClO soaking times and in water at fluctuating temperatures (20/10 ºC) (black column). Data
594 obtained at 15°C were fitted by an exponential plus lineal model (a + b*r^ x +c*x) with the
595 following parameters: a= 6.32, b= -3.34, c= -1.02 and r= 1.87 with a R^2= 0.99; SE=19. (C)
596 Germination of *Cynara cardunculus* achenes incubated at constant 15 ºC in a KNO_3 solution (white
597 column) and in water, at constant 15 C (white column) and at fluctuating temperatures (20/10 ºC)
598 (black column). T bars indicate the SE. Different letters at the top of each bar indicate significant

differences according multiple comparisons Krustal-Wallis's Test (A) and Tukey´s test (C) ⟨α= 0.05).

Fig. 4 Germination of *Cynara cardunculus* achenes collected at Olavarría in 2013 incubated in water at fluctuating temperatures (20/10°C) (black column) and at constant 15°C (white column). Figure in the first column indicate germination of fresh achenes. Second and subsequent columns represent germination throughout different durations of achenes storage (15, 30, 45, 60 and 75 days) whilst rows represent different storage conditions (6°C humid and dry, 15, 25°C). Bars indicate the SE.

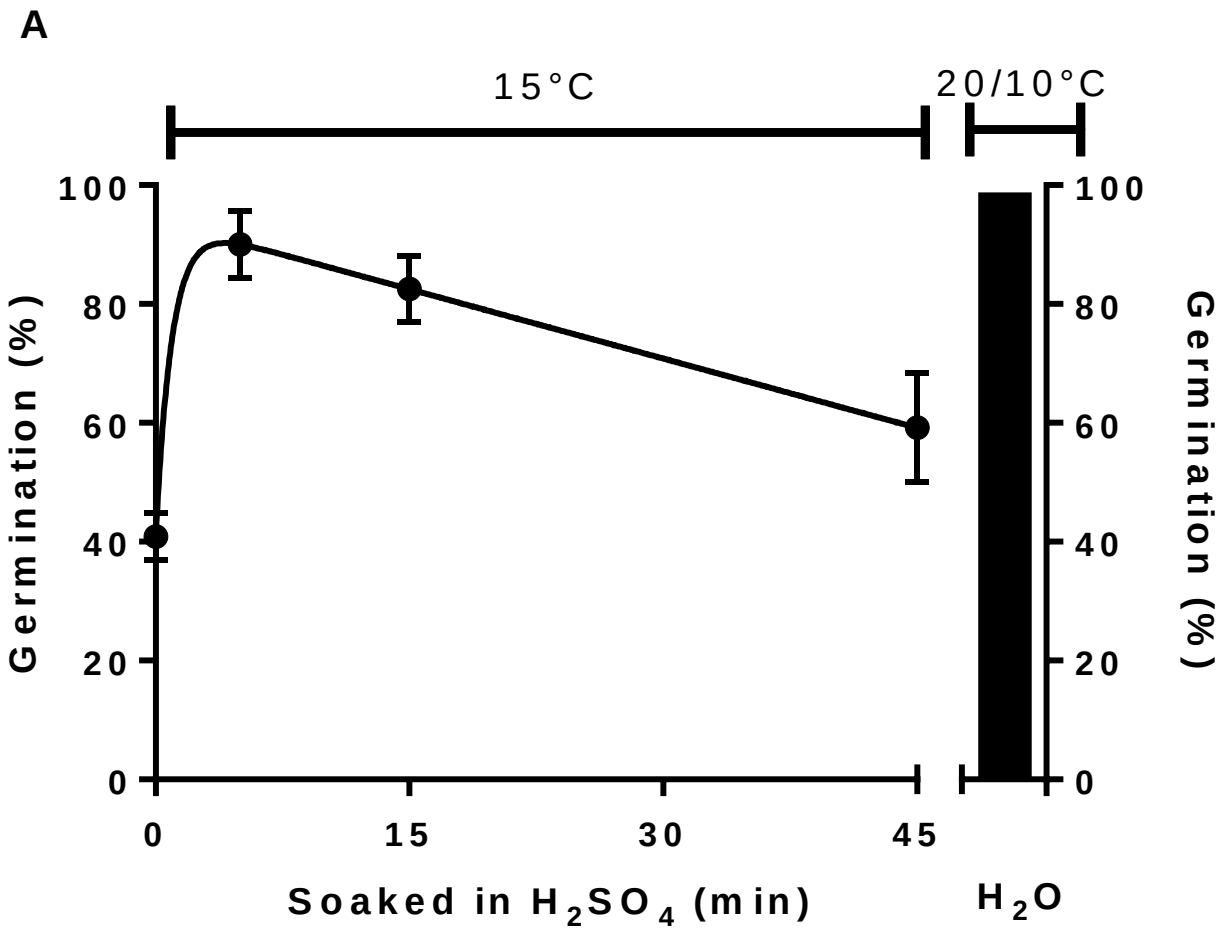
Fig. 5 Germination of *Cynara cardunculus* achenes collected at Abasto in 2013 incubated in water at fluctuating temperatures (20/10°C) (black column) and at constant 15°C (white column). Figure in the first column indicate germination of fresh achenes. Second and subsequent columns represent germination throughout different durations of achenes storage (10, 20, 30 and 40 days) whilst rows represent different storage conditions (6°C humid and dry, 15, 25°C) tested during storage. Bars indicate the SE.

Fig. 6 (A) Final germination of achenes incubated in water at a range of constant and fluctuating temperatures. T bars indicate the SE. Different letters at the top of each bar indicate significant differences according Tukey's Test ⟨α= 0.05). (B) Germination dynamics at a range of water potential (-MPa.). T bars indicate the SE. Data were fitted using a sigmoidal dose-response (variable slope) model ($Y= Bottom + (Top-Bottom)/1 + 10^{LogEC50-X}$) with the following parameters: Bottom = -68.23, Top= 118.8, LogEC50= 0.91, EC50= 8.29 with a R^2= 0.95.

623 **Fig. 7** (A) Effect of depth of burial on *Cynara cardunculus* seedlings emergence. T bars indicate the
624 SE. Data were fitted using an exponential (plateau followed one phase decay) model (Y= IF (X<X0,
625 Y0, Plateau+(Y0-Plateau) *exp (-K*(X-X0))) with the following parameters: X0=1, Y= 59.37,
626 Plateau= -179.2, K= 0.037 with a R^2= 0.58 and a SE 7.71. (B) Effect of different cover amounts on
627 *Cynara cardunculus* seedling emergence. T bars indicate the SE. Data were fitted using a lineal
628 model (slope = 1.44, Y-intercept = 57.7 and X intercept= -39.86. (C) Effect of different flooding
629 durations on *Cynara cardunculus* seedling emergence. T bars indicate the SE. Data were fitted
630 using a lineal model with a R^2= 0.97 (slope = -3.03, Y-intercept = 63.64 and X-intercept = 20.98).

631

Figure 2


A
15°C
20/10°C
100
80
60
40
20
0
Germination (%)
0
15
30
45
Soaked in H_2SO_4 (min)
H_2O

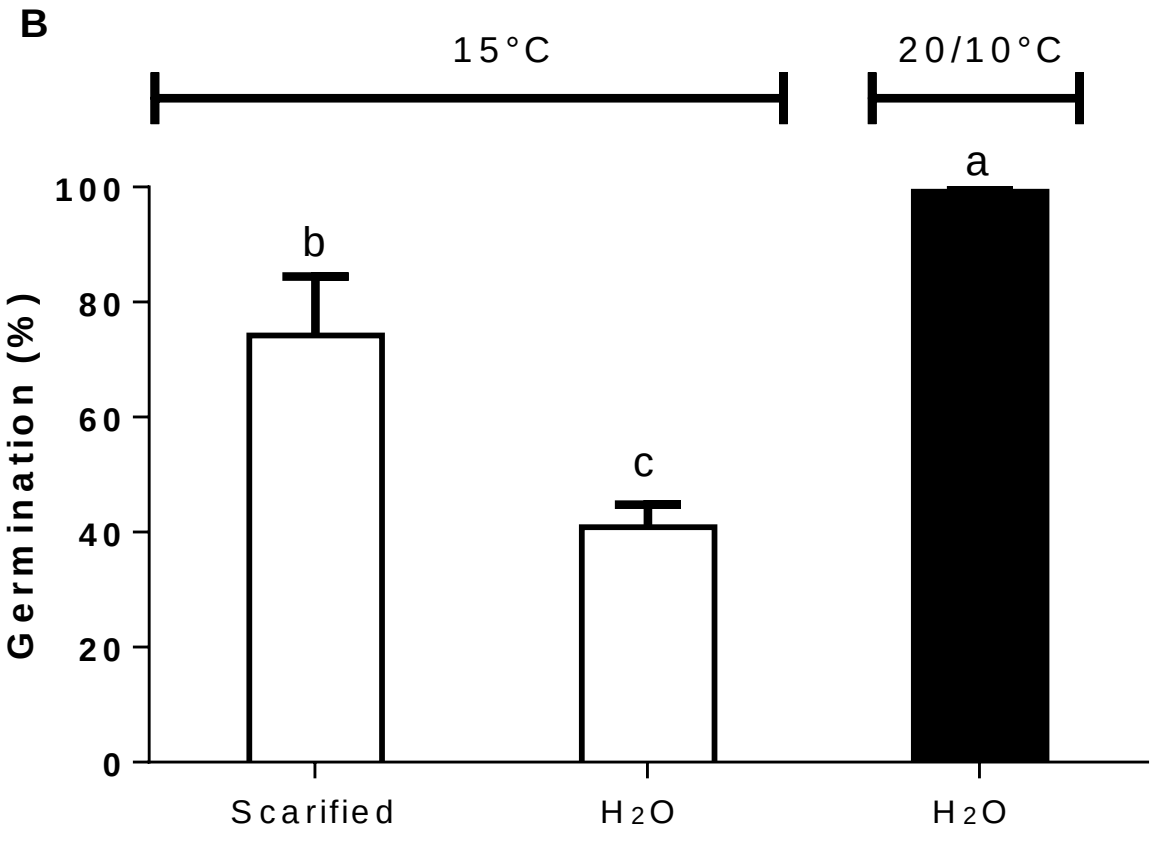

B
15°C
20/10°C
100
80
60
40
20
0
Germination (%)
b
c
a
Scarified
H_2O
H_2O

Figure 3

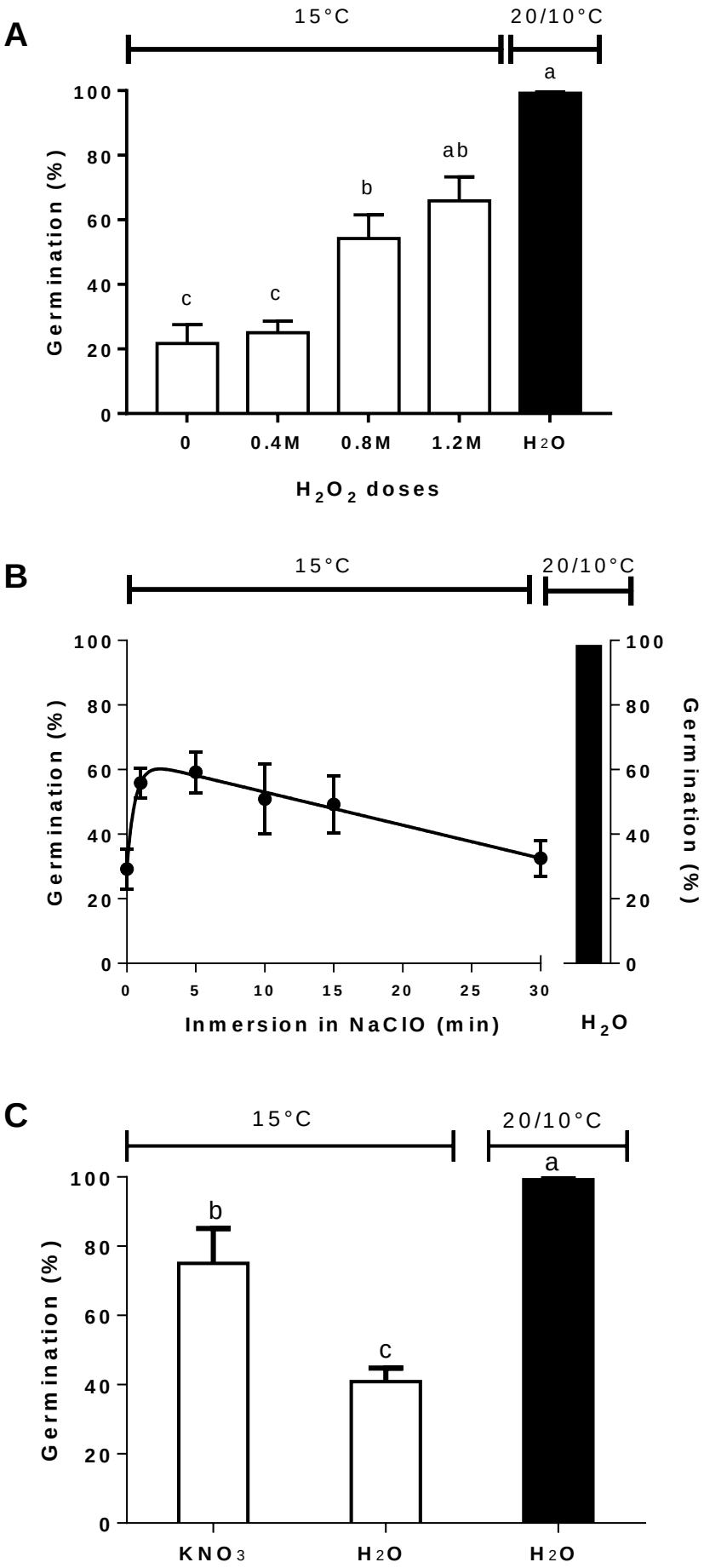

A
15°C
20/10°C
Germination (%)
100
80
60
40
20
0
a
ab
b
c
c
0
0.4M
0.8M
1.2M
H2O
H2O2 doses
B
15°C
20/10°C
Germination (%)
Inmersion in NaClO (min)
H2O
C
15°C
20/10°C
Germination (%)
b
c
a
KNO3
H2O
H2O

Figure 4

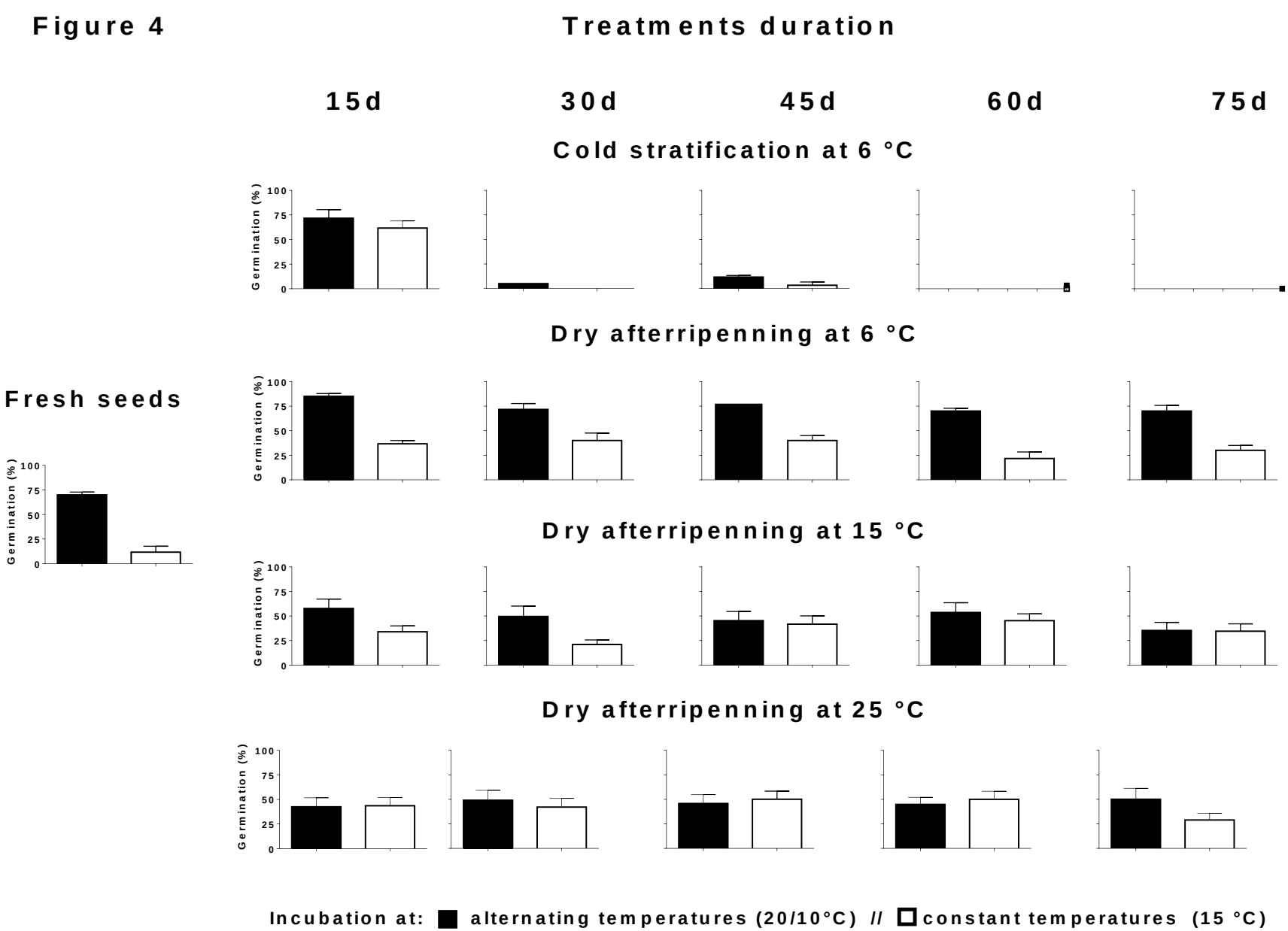

Treatments duration
15d
30d
45d
60d
75d
Cold stratification at 6 °C
Dry afterripenning at 6 °C
Dry afterripenning at 15 °C
Dry afterripenning at 25 °C
Fresh seeds
Germination (%)
100
75
50
25
0
Incubation at: alternating temperatures (20/10°C) // constant temperatures (15 °C)

Figure 5

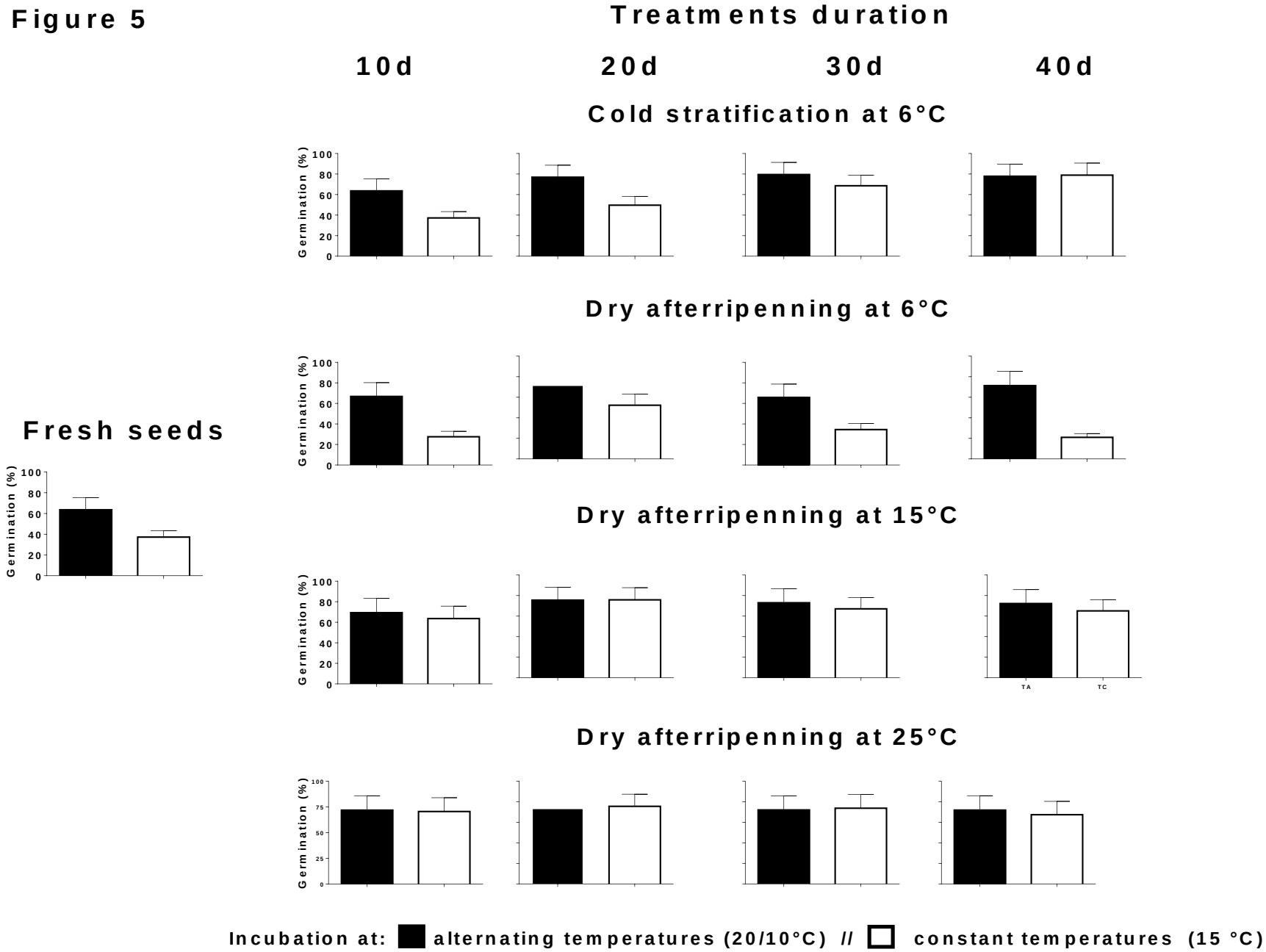

Treatments duration
10d
20d
30d
40d
Cold stratification at 6°C
Dry afterripenning at 6°C
Dry afterripenning at 15°C
Dry afterripenning at 25°C
Fresh seeds
Germination (%)
TA
TC
Incubation at: alternating temperatures (20/10°C) // constant temperatures (15 °C)

Figure 6

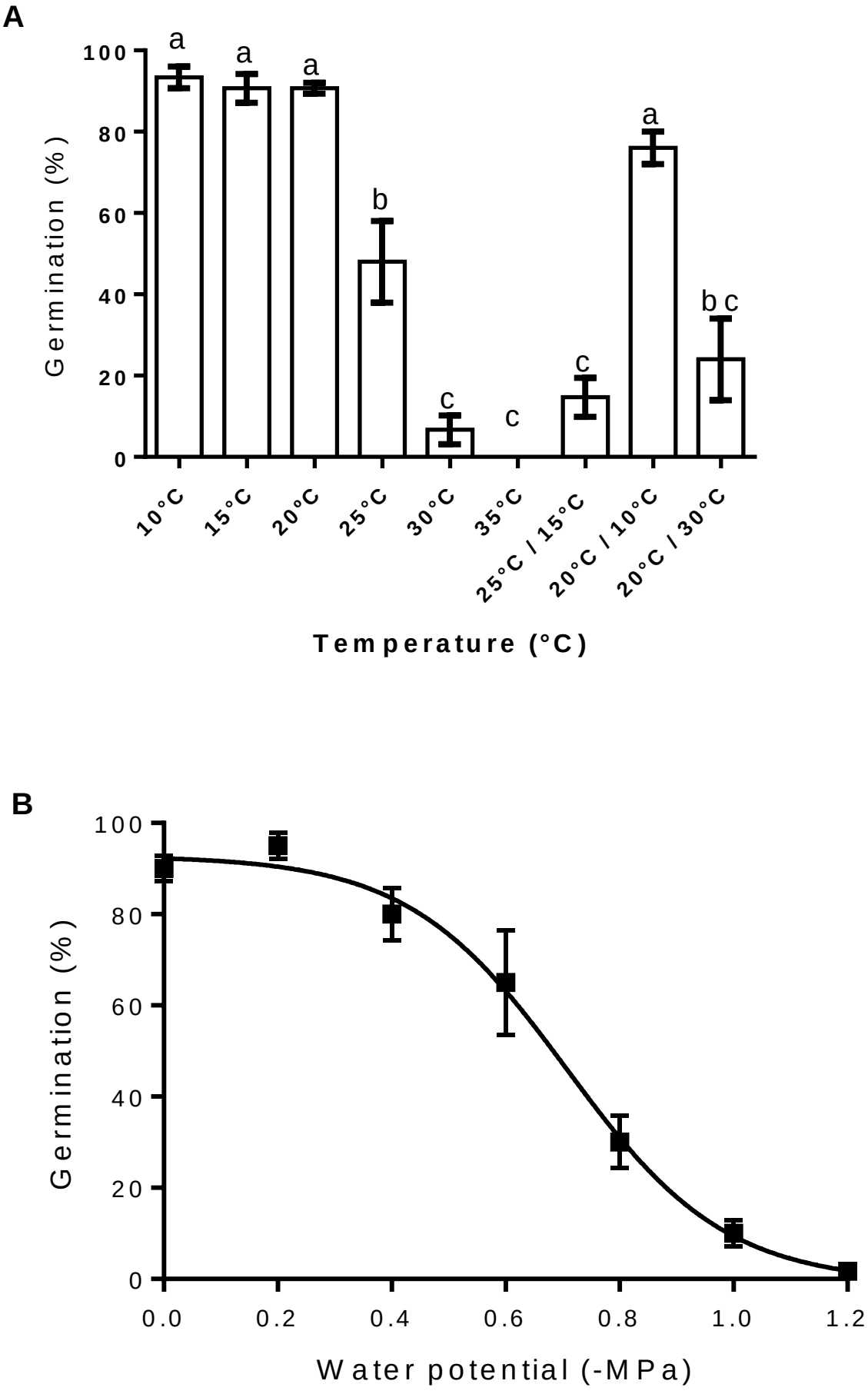

A
Germination (%)
100
80
60
40
20
0
a
a
a
b
c
c
c
a
b c
10°C
15°C
20°C
25°C
30°C
35°C
25°C / 15°C
20°C / 10°C
20°C / 30°C
Temperature (°C)
B
Germination (%)
100
80
60
40
20
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Water potential (-MPa)

Figure 1

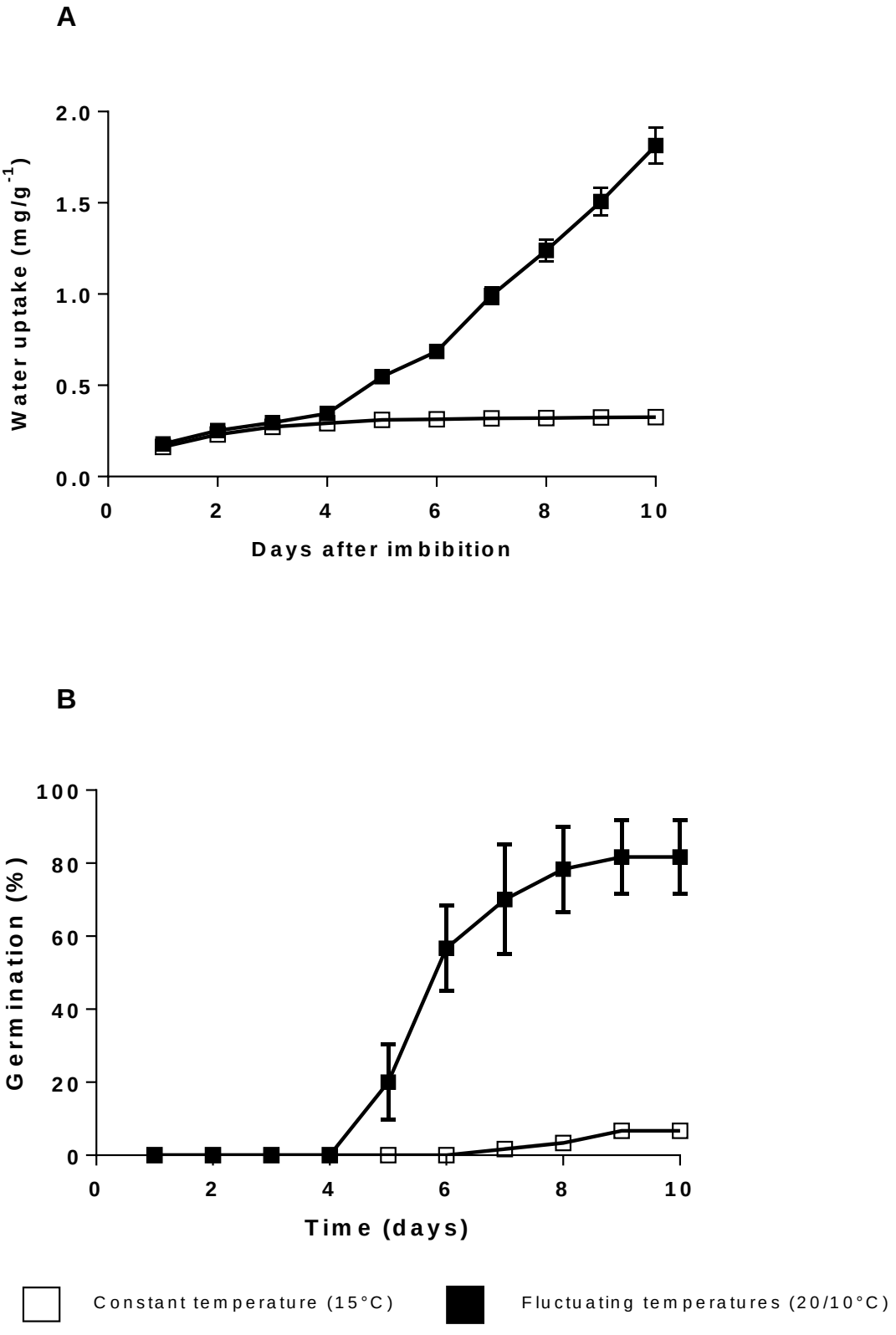
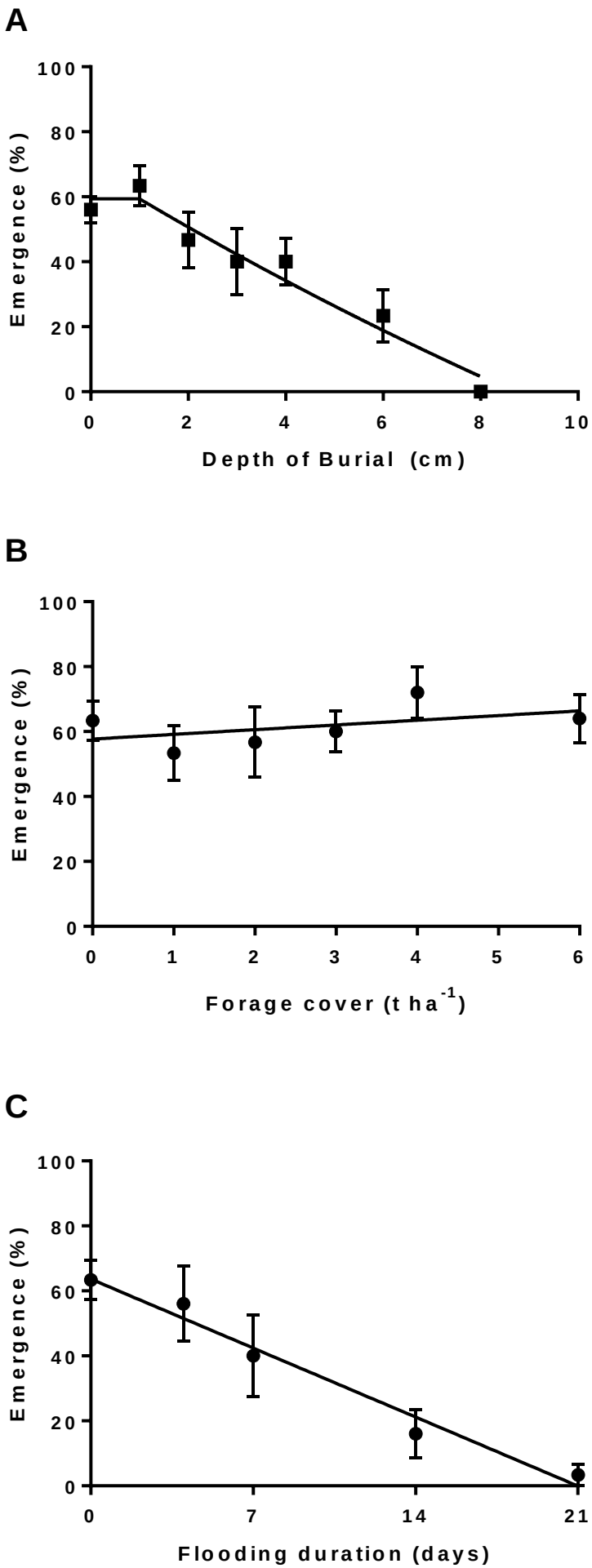

A
Water uptake (mg/g-1)
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
Days after imbibition
B
Germination (%)
100
80
60
40
20
0
0
2
4
6
8
10
Time (days)
Constant temperature (15°C)
Fluctuating temperatures (20/10°C)

Figure 7


A
100
80
60
40
20
0
Emergence (%)
0
2
4
6
8
10
Depth of Burial (cm)
B
100
80
60
40
20
0
Emergence (%)
0
1
2
3
4
5
6
Forage cover (t ha-1)
C
100
80
60
40
20
0
Emergence (%)
0
7
14
21
Flooding duration (days)