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Short Communication

Factors influencing seed germination of *Cyperus capitatus*, inhabiting the moving sand dunes in southern Europe

S. Redondo-Gómez*, L. Andrades-Moreno, R. Parra, E. Mateos-Naranjo, A.M. Sánchez-Lafuente

Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Apartado 1095, 41080 Sevilla, Spain

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ABSTRACT

Cyperus capitatus Vandelli (Cyperaceae) is distributed in coastal sandy habitats and mobile dunes of south Europe. Its seed germination ecology is not known, despite its potential to be used in re-vegetation projects. Laboratory experiments were conduced to assess the effects of salinity, light regime, cold stratification and burial on seed germination of this species. Overall, increasing salinity delayed germination, increased seed dormancy and mean time to germination (MTG), and reduced final germination percentage, inhibiting it completely above 1% of salinity; although it did not affect seed viability. *C. capitatus* seeds exhibited their greatest germination at levels between 0 and 1% in non-stratified seeds, and between 0 and 0.5% for stratified seeds. Thus, the effect of salt was greater for stratified seeds at 5 °C. Germination in light/darkness conditions was similar to that in full darkness. Finally, burial in sand of *C. capitatus* seeds appeared to have a significant effect on cumulative percentage of germination. Seeds buried at depths greater than 2 and 3 cm showed a lower germination success than those on sand surface or buried at shallower depths. Burial also affected the beginning and speed of seed germination.

1. Introduction

Cyperus capitatus Vandelli (Cyperaceae) is a perennial grass that occurs in coastal sandy habitats and mobile dunes of southern Europe (Castroviejo, 1990). This species produces extensive rhizomes, and it is one of the earliest species to colonize newly deposited dunes contributing to the initial stabilization of sand; it has been also described growing on dune slopes (Galal and Fawzy, 2007). Therefore, in arid and semiarid coastal areas, where desertification is becoming a serious problem, *C. capitatus* might be useful in re-vegetation projects.

Establishment from seeds is an especially critical phase in the life cycle of plants inhabiting dry environments (Huang et al., 2004; Yang et al., 2010). Habitats like sand dunes in arid and semiarid regions are characterized by spatio-temporal variation in soil salinity and superficial fresh water availability (Balestri and Cinelli, 2004; Jefferies et al., 1979). In these environments, increase in soil salinity may occur by the incorporation of salt from tidal flooding and aerosol spray, while changes in fresh water availability are primarily determined by seasonal rainfall. Thus, survival of new plants in these areas is related mainly to mechanisms that ensure germination and seedling development at the right time and in a suitable place (Huang and Gutterman, 1998). To date the seed germination ecology of *C. capitatus* is not known. Hence, this study was carried out (1) to asses the interactive effects of salinity (NaCl), light and cold on seed germination of *C. capitatus*, and (2) to investigate the effect of burial at different sand depths on germination, which is one of the most important abiotic factors that may lead to a decline in establishment rates in many dune plants (Maun, 1998).

Dry inflorescences of *C. capitatus* were collected in May 2010 from a population of several hundred individuals growing on a moving sand dune at Odiel Marshes (37°15′N, 6°58′W; SW Spain). This area is subject to a Mediterranean climate, with oceanic influences, mild winters (mean temperature ca. 11 °C in January) when most rainfall occurs (mean 510 mm year^{-1}) is mainly in winter, which has mild temperatures (mean temperature ca. 11 °C in January), and long and dry summers (ca. 25 °C; Redondo et al., 2004).

In the laboratory the inflorescences were manually shaken and the naked seeds fell out and were collected. All seeds were surface-sterilized by vigorous shaking in sodium hypochlorite solution (5% w/v) for 2 min, then washed with sterilized water. With these seeds we proceeded as follows:

* Corresponding author. Tel.: +34 95 4557165; fax: +34 95 4615780.
E-mail address: susana@us.es (S. Redondo-Gómez).

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2. Materials and methods

2.1. Effects of salinity on germination under a variable light regime

We distributed 1000 seeds in 40 Petri dishes with filter paper, in groups of 25. Each 8 dishes were randomly assigned one of 5 salinity levels (i.e. 8 replicates per treatment level), and the seeds contained submerged in a 3 ml solution of 0 (i.e. distilled water), 0.5, 1, 2 or 3% (w/v) NaCl, respectively. Salinity concentrations were chosen to mimic the natural variation recorded during summer and early-autumn on sand surface in the Mediterranean region (Balestri and Cinelli, 2004). Four replicates of each salinity level were wrapped with parafilm, placed in a germinator (ASL Aparatos Científicos M-92004, Madrid, Spain), and exposed to a regime of 16 h of light (25 °C, 400–700 nm, 35 μmol photon m^{-2} s^{-1}) and 8 h of darkness (15 °C). The remaining four replicates of each salinity level were exposed to 24 h darkness (dishes were covered with several sheets of foil). Both light regimes were held for 30 d. The temperature chosen for each regime again mimicked the autumn and spring conditions in which seeds germinate in the Odiel Marshes. Seeds incubated in the light/darkness regime were inspected daily and germinated seeds counted and removed. Seeds incubated in full darkness were only checked once at the end of the experiement. Seed germination was accepted when the radicle was visible (Redondo et al., 2004).

2.2. Effects of cold stratification and salinity on germination

Four 25-seeds replicates per treatment were placed on a filter paper in 9 cm Petri dishes and submerged in 3 ml solutions of 0, 0.5, 1, 2 and 3% (w/v) NaCl. Dishes were wrapped with parafilm and placed in a chamber in darkness for 30 d at 5 °C. Following the stratification period, dishes were placed in the germinator under the same light/darkness conditions as the previous experiment for a further 30 d. Germinated seeds were counted and removed daily during this period.

2.3. Recovery experiment

In July 2010 a recovery experiment was carried out to determine whether salinity inhibits germination permanently. All seeds not germinated in the previous experiments were immersed in 3 ml of distilled water in new dishes, and then maintained for another 30 d in distilled water in the germinator, under the same light/darkness regime described before.

2.4. Effects of burial on germination

We tested the effect of seed burial on germination using five shallow trays (30 × 20 × 6 cm deep), filled with aquarium sand to a depth of 4 cm, and wetted until moisture was ca. 15–20%. Each tray was randomly assigned to a different burial level: 0 (i.e. surface), 0.5, 1, 2 and 3 cm depth, and divided into four compartments. In each compartment 25 seeds were placed (i.e. four 25-seeds replicates per tray) at the corresponding depth. Trays were covered with a thin transparent plastic to prevent water evaporation. Trays were then placed in the germinator for 60 d, under the same light darkness regime described for previous experiments. Germinated seeds were counted daily.

2.5. Statistical analyses

Germination likelihood of individual seeds was modelled as a binomial dependent variable (germinated vs. not-germinated), and analysed using generalised linear models with a logit link function. Salinity and light regime, and salinity and cold stratification were used as fixed predictors in their respective analyses. Similar analyses were carried out on the not-germinated seeds remaining from this experiment following the recovery treatment. We calculated germination curves for the different levels of each treatment, using the Kaplan–Meier estimator (Kaplan and Meier, 1958). Germination curves were subsequently compared using longrank tests, in order to evaluate if the distribution of germination times differed among levels within treatments.

Two more variables related to germination success were also analysed. These were the time to first germination and the mean time to germination (MTG), calculated as:

$$\mathrm{MTG} = \sum_i (n_i \times d)/N$$

where n is the number of seeds germinated at day i, d is the incubation period in days and N is the total number of seeds germinating in the treatment (Brenchley and Probert, 1998; Redondo-Gómez et al., 2007). The lower the value, the more rapid the germination. These 3 dependent variables followed a normal distribution, and thus were analysed using general linear models. As the 2 analyses were carried out using the same seed pool, to minimize the probability of 'false positives' a sequential Bonferroni procedure was used to correct probabilities (Redondo-Gómez et al., 2008).

All statistical analyses have been carried out using the R environment for statistical computing (version 2.11.1; R Development Core Team, 2010), with the "survival" package (version 2.35–8).

3. Results and discussion

Salinity was the main factor responsible for germination success in our experimental procedures. Thus, saline concentration significantly explained differences in germination likelihood (Deviance = 896.42, df = 4995, $p < 0.001$) in conditions of both light/darkness and full darkness. However, the light regime was not significant, either alone (Deviance = 464.21, df = 1994, $p = 0.52$) or in interaction with salinity (Deviance = 464.56, df = 4990, $p = 0.94$). Significant differences in the effect of salinity on germination likelihood were observed between concentrations of 0–1% NaCl and higher concentrations (i.e. 2% and 3% NaCl), for which seed germination was completely inhibited (Table 1, Fig. 1).

The effect of salinity on germination differed, however, in relation to the cooling treatment. Thus, although saline concentration had a significant effect on germination in the cooling experiment (Deviance = 1018.05, df = 4995, $p < 0.001$), and the cooling treatment alone was not significant (Deviance = 586.70, df = 1994, $p = 0.26$) a significant interaction between both treatments (Deviance = 599.15, df = 4990, $p < 0.009$) suggests an influence of the latter on the former. Nevertheless, the effect of cooling on salinity seemed to be restricted to the intermediate saline concentration, as the germination likelihood of seeds in 1% NaCl in ambient temperature was significantly higher than that of seeds in the same concentration but previously cooled (Table 1). No significant effects of cooling were found for saline concentrations below 1% NaCl while, as found in the previous experiment, germination was completely inhibited for concentrations above 1% NaCl.

We found significant differences among all levels of the salinity treatment in median germination time for both seeds germinated with and without a previous cooling treatment ($Chi^2 = 196$, df = 5, $p < 0.001$). But interestingly enough, when comparing germination curves between seeds subjected, and not subjected, to the previous cooling treatment, we found that the effect of cooling in germination timing could be comparable to that of an increased salinity.

Table 1
Final germination (%), days to first germination and mean time to germination (MTG) of *Cyperus capitatus* in response to treatment with a range of NaCl and dark/light and darkness conditions, and after stratification at 5 °C for 30 d. Values are means ± S.E ($n = 4$).

	Salinity treatment (%)	Final percentage in dark/light	First germination (days)	MTG	Final percentage in darkness
Effects of salinity	0	86 ± 4.2^{a}	7 ± 0.3^{a}	12 ± 0.2^{a}	89 ± 1.9^{ab}
	0.5	89 ± 3.0^{a}	9 ± 0.0^{b}	14 ± 0.2^{b}	95 ± 2.5^{a}
	1	76 ± 7.8^{a}	13 ± 0.3^{c}	20 ± 0.8^{c}	82 ± 3.8^{b}
	2	0 ± 0.0^{b}	–	–	0 ± 0.0^{c}
	3	0 ± 0.0^{b}	–	–	0 ± 0.0^{c}
Effects of cold stratification in darkness and salinity	0	80 ± 2.8^{a}	7 ± 0.3^{a}	12 ± 0.4^{a}	
	0.5	76 ± 2.3^{a}	11 ± 0.4^{b}	18 ± 0.4^{b}	
	1	26 ± 6.6^{b}	22 ± 1.6^{c}	25 ± 0.7^{c}	
	2	0 ± 0.0^{c}	–	–	
	3	0 ± 0.0^{c}	–	–	

a, b and c indicate means within an analyzed variable that are significantly different from each other (Tukey test; $p < 0.05$).

Thus, median germination time (and 95% confidence interval) of seeds in distilled water and subjected to previous cooling (12, 11–13) did not differ from that of seed in 0.5% NaCl without previous cooling (12, 12–13). The same pattern was found in the case of seeds germinated in a solution of 0.5% NaCl with a previous cooling treatment (19, 17–20), when compared to those in1% NaCl and no previous cooling (20, 19–22; Fig. 1A and B).

The complete inhibition of germination at salinities beyond the tolerance limits of the species (Ungar, 1991) may be attributed to decreasing osmotic potential of the solution by salinity that would avoid seed hydration (Ramoliya and Pandey, 2002). Additionally, an increase in seed dormancy induced by increasing salinity stress has been widely reported (Pujol et al., 2000; Redondo et al., 2004).

Salinity did not permanently inhibited seed germination, as found for not-germinated seeds recovered with distilled water. We found that, in both the cases of different light and cooling treatments, seeds whose germination was previously inhibited by high saline concentrations (i.e. 2% and 3% NaCl) germinated at a comparable rate than those with lower saline concentrations following the recovery process (77–92%; see Tables 1 and 2).

Therefore, the salts concentrations tested in our study did not affect seed viability during the study period. As for several halophytes and desert species, in *C. capitatus* inhibition by salinity (Balestri and Cinelli, 2004) would be of ecological value preventing seeds from germination after occasional rains, which occasionally occurs in late summer. In a Mediterranean climate, seedling emergence appeared associated with periods of greater precipitation (i.e. autumn and spring). As reported for other Mediterranean dune plants (Maun, 1994) this emergence pattern may be of ecological value, distributing germination at a time when

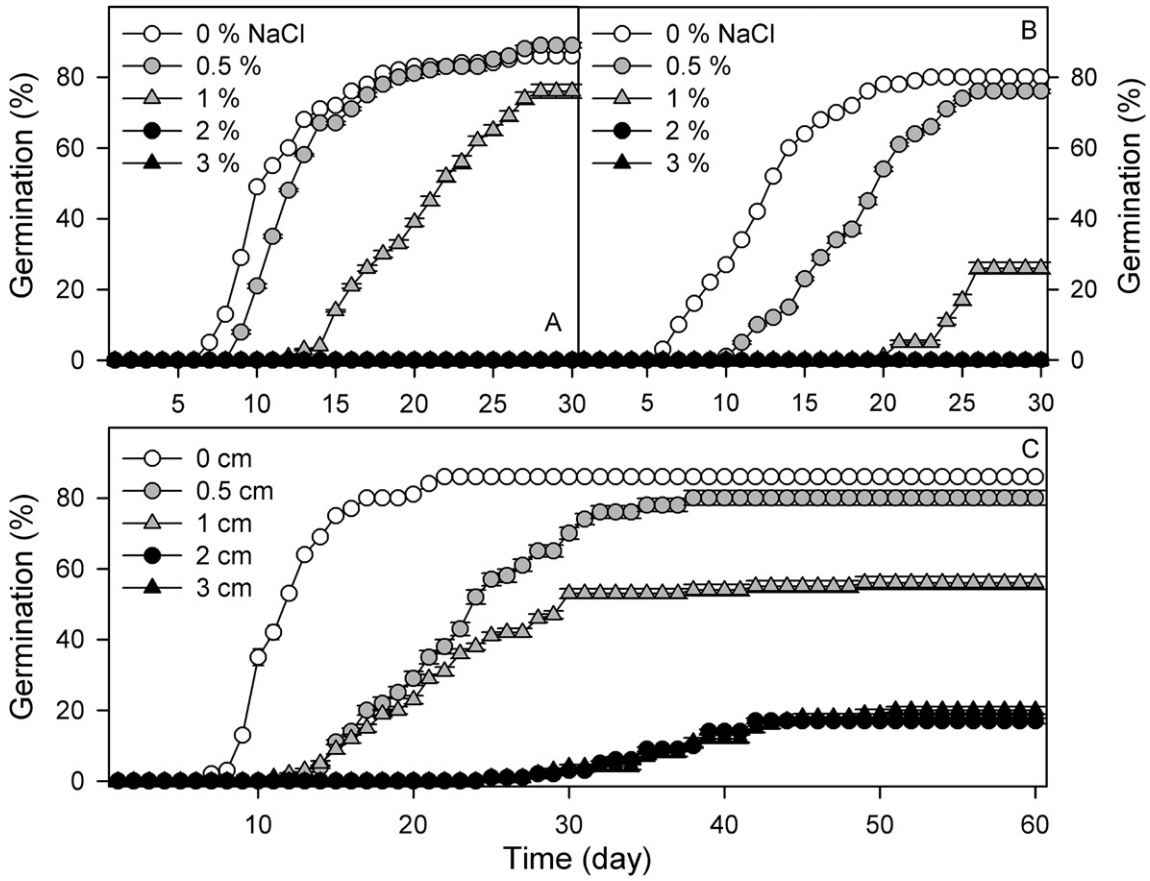

Fig. 1. Cumulative germination of *Cyperus capitatus* in response to: treatment with a range of NaCl in dark/light conditions (A) and after cold stratification in darkness (B) for 30 d, and burial at different depths (C) for 60 d. Values are means ± S.E ($n = 4$).

Table 2
Final germination (%) of *Cyperus capitatus* for seeds in distilled water after four salinity pre-treatments and dark/light and darkness conditions, and after stratification at 5 °C (recovery of germination) for 30 d. Values are means ± S.E. ($n = 4$).

Salinity treatment (%)	Final percentage after the following pre-treatments:		
	Dark/light	Darkness	Cold stratification in darkness
0.5	2 ± 1.2^{a}	1 ± 1.0^{a}	7 ± 3.4^{a}
1	10 ± 2.0^{b}	6 ± 2.6^{a}	58 ± 10.0^{b}
2	86 ± 5.3^{c}	91 ± 3.4^{b}	88 ± 3.7^{b}
3	92 ± 1.6^{c}	85 ± 5.7^{b}	77 ± 3.0^{b}

a, b and c indicate means within an analyzed variable that are significantly different from each other (Tukey test; $p < 0.05$).

conditions are more favourable for seedling establishment and subsequent plant growth (Balestri and Cinelli, 2004; Redondo-Gómez et al., 2008; Yang et al., 2010).

On the other hand, Ungar (1991) noted that most seeds of salt-tolerant species exhibited their greatest germination in fresh water. However, Balestri and Cinelli (2004) found that seeds of *Pancratium maritimum* exhibited similar final germination at 0 and 0.4% salinity. Interestingly, there were no significant effects of salinities from 0 to 1% NaCl on final germination of *C. capitatus* seeds under light/darkness conditions (ca. 84%) and full darkness (ca. 89%; Table 1). Nonetheless, seed dormancy and the number of days to first germination increased for this NaCl range, and germination speed decelerated (i.e. higher MTG; Table 1). Furthermore, germination in darkness was similar to that recorded under alternate light/darkness conditions for all salinity treatments (two-way ANOVA, incubation conditions: $p > 0.05$). Therefore, *C. capitatus* did not require light for germination, in contrast to other *Cyperus* species, including *Cyperus erythrorhizos*, *Cyperus flavicomus* and *Cyperus squarrosus* (Baskin et al., 1993, 2004).

Seed burial in sand appeared to have an important effect on seed germination (Deviance = 182.98 df = 4495, $p < 0.001$; Fig. 1C). In particular, we found significant differences in germination likelihood between seeds located on, or near, the sand surface (mean ± SE at 0 and 0.5 cm depth; 0.86 ± 0.03 and 0.80 ± 0.04), compared to those located 1 cm (0.56 ± 0.05), while those buried at a higher depth had a significantly lower germination success probability (2 and 3 cm: 0.17 ± 0.04 and 0.20 ± 0.04). We also found significant differences among burial levels in time and duration of germination (logrank test: $Chi^2 = 290$, df = 4, $p < 0.001$). Thus, although final germination likelihood did not show differences between 0 and 0.5 cm treatments, not-buried seeds germinated significantly earlier [median and confidence interval in days: 12 (10, 13)] than those buried at 0.5 [23 (21, 24)] and 1 cm depth [21 (20, 24)] and, in turn, these germinated significantly earlier than those buried at 2 [35 (33, 39)] and 3 cm depth [38, (35, 43)] (Fig. 1C). Similar results were found by Huang et al. (2004) for *Psammochloa villosa*. They explained that seeds may require shallow burial because it increases soil contact and relative humidity around the seeds, thus enhancing the probability of germination. Moreover, Pemadasa and Lovell (1975) explained that the percentage of seeds in enforced dormancy increased with depth. Many factors, such as oxygen content, CO_2 levels and aeration, and sand water content may cause enforced dormancy. This seed dormancy might have ecological advantages for species of sand dune by maintaining a long-term seed bank in the sandy soil, in that seedlings can be produced when erosion reduces the depth of sand (Huang et al., 2004).

C. capitatus demonstrated adaptation to salinities up to 1% NaCl during germination of non-stratified seeds under light/darkness conditions and full darkness; however, germination was inhibited at higher salinities. Otherwise, increasing salinity induced an increase of seed dormancy, although seed viability was not affected. Furthermore, the synergic effect of salinity and cold stratification enhanced this seed dormancy. This would be of ecological value associating seedling emergence with periods of greater precipitation (i.e. autumn and spring). Finally, *C. capitatus* did not require light for germination, but its final germination percentage was reduced under increasing sand thickness.

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