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Short Communication

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

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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:

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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.