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Short Communication

Effects of salinity and ingestion by ducks on germination patterns of *Juncus subulatus* seeds

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Abstract

Changes in the nature of the seed coat caused by gut passage are considered to be the proximate causes of changes in germination patterns. Modifications of seed coat permeability may be decisive in saline environments (e.g. Mediterranean wetlands), in which the response of the seed to osmotic stress, or its resistance to toxicity caused by some ions may be affected. We analysed how passage through a duck gut can change the response pattern of seeds of a Mediterranean helophyte *Juncus subulatus* to an experimental salinity gradient. Gut passage produced (1) a significant loss of seed viability (about 30%) across the whole salinity gradient and (2) no change in the intrinsic variability of the dormancy period or germination rate in *J. subulatus* seeds. However, *J. subulatus* seeds clearly have the potential to disperse via birds. We compare our results with those obtained previously for sympatric species of saline environments.
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1. Introduction

Wetlands in semiarid environments are frequently temporary, saline and isolated water bodies. Bird-mediated dispersal between wetlands plays an important role in wetland ecology and in the maintenance of genotypic diversity (see Figuerola and Green, 2002; Green et al., 2002; Amezaga et al., 2002).

The passage of seeds through the digestive tract of birds has a wide variety of effects on germination patterns (percentage of germination and germination rate) amongst the range of plant species studied (Traveset, 1998; see review in Traveset and Verdú, 2002).

Changes in the nature of coat caused by gut passage have been considered by some authors as the proximate causes of changes in germination patterns (see Traveset, 1998; Traveset et al., 2001). Modifications of seed coat permeability may be decisive in saline environments (e.g. Mediterranean wetlands), in which the response of the seed to osmotic stress, or its resistance to toxicity caused by some ions may be affected (Ungar, 1978; Baskin and Baskin, 1998). Recent work indicates the need to study the germination process in the field or at least while simulating different environmental conditions which can influence germination pattern, seedling survival and plant performance (Traveset et al., 2001; Santamaría et al., 2002; Espinar et al., 2004; Rodriguez-Girones et al., 2005; Figuerola et al., 2005). The microhabitat where the seeds are deposited may play an important role in the final outcome of the dispersal process. Espinar et al. (2004) established that gut passage changed the response pattern to salinity during germination in seeds of helophyte species living in saline environments (*Scirpus litoralis* and *S. maritimus*).

In this paper we analysed the effects of salinity and ingestion on the germination patterns of seeds of *Juncus subulatus*, a perennial emergent plant very common in saline and seasonal wetlands of Mediterranean areas.

2. The procedure

J. subulatus Forsskal is one of the major components of the perennial vegetation in saline areas of the temporary marshes within Doñana National Park, south-west Spain (37°N, 6°W). On August 2001, seeds of *J. subulatus* were collected in the study area. The seeds were separated from the spikes and were stored in darkness at 4 °C in order to stimulate germination (Clevering, 1995).

Twenty semicaptive mallards *Anas platyrhynchos* were distributed in individual cages with a mesh floor and given unlimited access to food (broken rice) and drinking water. Removable metal trays were placed under the cages to recover any droppings. The next morning, approximately 500 seeds of *J. subulatus* were force fed to the birds. The day after, faeces were collected from the trays and transported in plastic bags to the laboratory. The faeces collected were washed in a sieve then dried at room temperature. Intact seeds were separated and stored in darkness at 4 °C.

Saline solutions were prepared by adding different quantities of sea salt to demineralized water to produce a range of salinities (0, 1, 2, 4, 8, 16 and 32 dS/m). Both the ingested seeds (passage) and noningested seeds (controls) were randomly distributed into groups of 25 seeds, using four groups for each of the seven salinity treatments employed (four replicates with a total of 100 seeds for each combination of ingestion treatment and salinity, i.e. 700 ingested seeds and another 700 controls in total. Ingested seeds were subsampled from those ingested by different ducks so that each of the 20 individuals contributed 35 ingested seeds per plant species to the germination trials.

Each group of seeds was placed in a Petri dish containing a Watman #1 filter paper and saline solution. The dishes were placed in a germination chamber, with a 12 h/12 h light–darkness photoperiod and a light intensity of 200 μmol photons m^{-2} and temperature cycles of 25 °C/10 °C. Every 2 days, the number of seeds germinated on each dish/flask was counted so as to obtain, after 30 days, the total percentage germination (G_T). For each treatment, we calculated the number of days taken for the first seed to germinate (T_0) as an estimate of dormancy, and the time (in days) taken for half of those seeds that germinated by the end of the experiment to germinate (T_{50}) as an estimate of the speed of germination (Barnea et al., 1991; Traveset et al., 2001).

The great number of seeds employed in the experiment (1700) and the size of seeds (0.3 ± 0.005 mm, $N = 100$; J.L. Espinar, unpublished data) prevent us from applying a tetrazolium test to determine seed viability. To detect a possible loss of viability of ingested seeds that did not germinate in the various treatments, they were placed in a freshwater medium for a month at 4 °C and in darkness. We then placed the seeds in a nonsaline medium for germination using the above protocols (recovery test), calculating the total percentage germination after 30 days (G_R).

The response of the different germination parameters to passage and salinity was modelled by using generalized lineal models (GLMs). A Poisson error distribution with log-link was applied (Dobson, 2002). For each response variable, the model included two fixed independent variables and the interaction between them. The bird treatment was modelled as a categorical variable with two categories (ingested and control seeds), and salinity as a continuous variable. The Akaike Information Criterion (AIC) was used to assist in model selection (i.e. those being more parsimonious and with a better fit, minimizing the AIC values). The significance of salinity (lineal, quadratic and cubic), passage, and first-order interaction (salinity × passage) effects were tested by using GLM (Wald tests). The sign of parameters having significant effects was used to interpret the results.

The effect of passage on the intrinsic variability in the response of seeds of each species to the experimental salinity gradient was tested by using a nonparametric approximation (bootstrap, Mooney and Duval, 1993) to Lewontin's (1966) test. The effect of the recovery period on the ingested seeds of each species was analysed with a Wilcoxon test. We controlled the 'familywise'error rate (FWER) at the 5% level when repeated tests were carried out (García, 2004).

Overall, salinity and gut passage explained a highly significant fraction of the observed response in the germination parameters studied ($p < 0.0005$ in all cases). Germinability was best explained by treatment levels (90% of variance), whereas germination rate was least explained (almost 50% of variance)(Table 1).

Seed germinability (G_T) was not affected by salinity treatment, except for that of 32 dS/m, which significantly reduced the percentage of germination (Fig. 1, Table 1). Ingestion enhanced the deleterious effect of high salinity on seed germination (i.e. the salinity × passage interaction was significant-Fig. 1, Table 1). We did not find significant differences in the total number of seeds that germinated before and after the recovery period ($p < 0.11$, Table 2). This lack of differences suggests that almost 30% of the seeds that were defecated apparently intact and were used in the experiments were in fact irreversibly affected by gut passage.

Table 1
Log-linear regression models of the values of germination parameters G_T (total percentage germination), T_0 (number of days taken for the first seed to germinate) and T_{50} (the time taken for half of those seeds that germinated by the end of the experiment to germinate) in *Juncus subulatus*, as a function of the salinity in the medium (S), passage (P), and the interaction between both factors (SP)

	Whole model		Factors				
	AIC	p	Effect	Coefficient	S.E.	Wald's χ^2	p
G_T	433.2	**0.000**	S^3	−3E-05	0.020	185.3	**0.000**
			P	−0.136	2E-06	47.7	**0.000**
			SP	−0.007	0.002	15.6	**0.000**
T_0	218.9	**0.000**	S	0.030	0.005	37.6	**0.000**
			SP	0.009	0.003	6.6	0.010
T_{50}	260.5	**0.000**	SP	0.020	0.004	31.4	**0.000**
			P	0.294	0.053	29.9	**0.000**

Akaike's Information Criterion (AIC) values and significance for the overall models; value, S.E. and significance (Wald test) for effect coefficients are shown. p values which remained significant after controlling the familywise error rate (FWER) at the 0.05 level are shown in bold. The SP term of the T_0 parameter has been preserved because its p value is very close to the corrected significance threshold (see Fig. 1).

The delay in germination caused by salinity (T_0) was the same in control seeds and those remaining viable after gut passage, while the germination rate (T_{50}) was significantly reduced both by an increase in salinity and by the effect of gut passage, in a more or less additive way i.e. without a significant interaction between these effects (Fig. 1, Table 1).

Table 3 shows the overall effect of gut passage on the average value and relative variability of the response of *J. subulatus* seeds across the whole salinity gradient. The effect of passage on relative variability was not significant for any of the studied germination parameters, but the average values of both G_T and T_{50} were significantly reduced by gut passage.

3. Germination, salinity and gut passage

In *J. subulatus*, an increase in water salinity produces a decrease in the percentage of seeds that germinate and an increase in the duration of seed dormancy, as previously observed in studies of other species from saline habitats (Ungar, 1978; Marañón et al., 1989; Espinar et al., 2004) and in the same species (Espinar et al., 2005). Nevertheless, the effects of salinity on germinability were only significant at the highest salt concentration, which indicates that *J. subulatus* is a high salinity resistant species at the germination stage. The values of salinity tolerance are similar to *Juncus gerardii* Lois (0–26.4 dS m^{-1}, see Amiaud et al., 2000).

J. subulatus gut passage significantly reduced the average germination rate of seeds across the whole salinity gradient, without producing any significant change in the duration of dormancy, the response of seeds to the salinity gradient, or the relative variability of germination parameters. Furthermore, the irreversible loss of viability in about 30% of the apparently whole seeds after digestion suggests that gut passage produces deleterious effects in this species. In contrast, for *Juncus gerardii* (a similar species

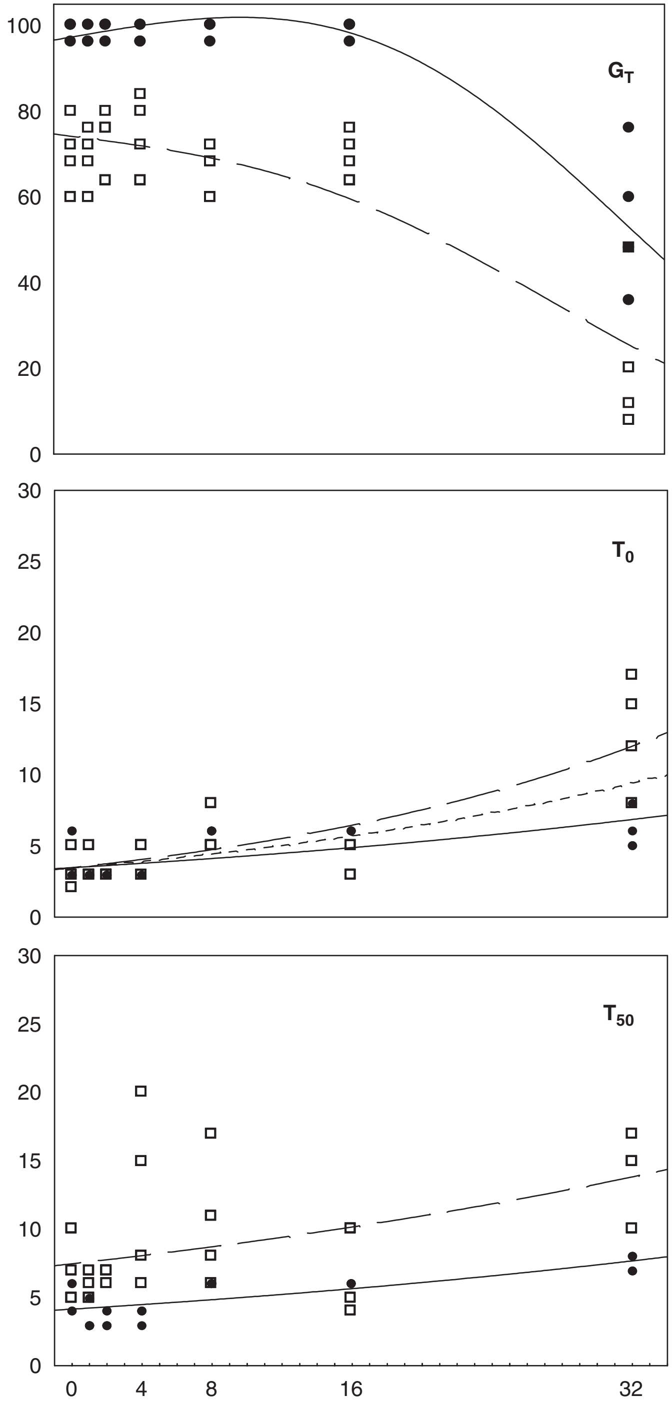

Fig. 1. Values of germinability (G_T, %) and number of days until the 1st seed germinated (T_0) and 50% of total germination (T_{50}) was reached, for ingested (empty squares) and control (solid circles) seeds of *Juncus subulatus*. Distance weighted least squares regression lines (McLain, 1974) are shown.

Table 2
Results of the recovery test for ingested *Juncus subulatus* seeds, showing the salinity used for the initial treatment (salinity), percentage of seeds that recovered after transfer to nonsaline conditions (N_R), and cumulative percentage of seeds that germinated after both periods (G_{FINAL})

Salinity	N_R	G_{FINAL}
0	0	70
1	3	70
2	0	74
4	0	75
8	12	71
16	0	70
32	44	56
Average	8	69

Table 3
Overall response (mean and coefficient of variation, CV, across the whole studied salinity gradient) of *J. subulatus* seeds to gut-passage treatment

	G_T	T_0	T_{50}
Mean			
Control	92	5	5
Passage	64	5	9
p	0.000	0.740	0.000
CV			
Control	0.18	0.36	0.31
Passage	0.30	0.7	0.49
p	0.065	0.048	0.052

to *J. subulatus*) the seed found in bovine faeces doubled their germination rate after digestive transit (Amiaud et al., 2000). In our study area, some terrestrial ungulates (R. Soriger, personal observation; J.L. Espinar, personal observation) and rabbits (Rogers et al., 1994) frequently eat spikes of *J. subulatus*. The role of herbivores should be considered in future research about the dispersion pattern of this important species in Mediterranean wetlands.

Previously we described how the passage of seeds through the digestive tract of *A. platyrhynchos* directly affected the duration of the dormancy in two *Scirpus* species (*S. maritimus and S. litoralis*), causing a reduction in the reaction time of seeds under conditions favourable for germination without affecting the total proportion of seeds that germinate (Espinar et al., 2004). In ecosystems in which the major environmental variables change in an unpredictable way, the increase in variability of the response of seeds itself could favour the survival of plants (Izhaki and Safriel, 1990; Traveset et al., 2001; Santamaría et al., 2002).

Scirpus spp. seeds are, amongst those of helophytes, the most frequently and systematically consumed by waterfowl in marshes of the Mediterranean region (Suarez and Urios, 1999; Green et al., 2002; Fuentes et al., 2004) and intact *Scirpus* seeds are often found in waterfowl faeces in Doñana (Figuerola et al., 2003; J. Figuerola, A.J. Green and M.I. Sánchez unpublished data). The vegetative parts of *J. subulatus* are consumed by greylag geese *Anser anser* in Doñana (Espinar, personal observation) and *Juncus inflexus* and *J. compressus* seeds are known to be consumed by ducks elsewhere (Pirot, 1981; Thomas, 1982). We therefore expect that *J. subulatus* seeds are consumed and dispersed by waterbirds, although not as often as *Scirpus* seeds.

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