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Simulation of germination of pioneer species along an experimental drought gradient

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Abstract: *The germination of ten plant species from the Iberian Peninsula was assessed along a water deficit gradient between -0.1652 (moist) and -0.4988 MPa (dry) of osmotic potential, created by addition of increasing concentrations of polyethylene glycol (PEG 6000) to distilled water in which plants were grown hydroponically. The level and rate of germination of Daucus carota and Thapsia villosa significantly decreased with decreasing Ψ. Seeds of Dactylis glomerata and Dittrichia viscosa had positive germination responses to low osmotic potentials; germination of Epilobium hirsutum was not affected by osmotic potential. Germination of Medicago arabica, Cynosurus cristatus, Cistus ladanifer and Cistus albidus, was no favored by the addition of polyethylene glycol (PEG). Germination of Foeniculum vulgare and Thapsia villosa was inhibited by PEG.*

Key words: *Drought tolerance, Generalized linear models, Germination, Osmotic potential, PEG.*

Introduction

Seed production has to guarantee recombination of genetic material, maintenance of plant populations and communities and enable species dispersion (Bradbeer, 1988; Leck *et al.*, 1989). In harsh and unpredictable habitats soil seeds banks facilitate the survival of plant species by providing opportunities for germination when environmental conditions are suitable for seedling survival (Ungar, 1987; Auld, 1995; Baskin and Baskin, 2001). However, lack of correlation between potential plant communities represented in the soil seed bank and the plant community observed above-ground has been reported (Smith and Kadlec, 1983; García Fayos *et al.*, 2001; Pugnaire and Lazaro, 2000; Pérez-Fernández *et al.*, 2001). Drought is a common stress in the semi-arid part of the Iberian Peninsula (Ferrer-Castán and Vetaas, 2003). Water shortage is commonly observed in two seasons: summer, when rains are scarce and soil water retention is minimal, and winter, when the water is frozen and remains inaccessible to the plants. Under conditions of extreme water deficit, only those species that are able to make an optimal use of water in the stages of germination and early growth can succeed (Redente, 1982; Pérez-Fernández *et al.*, 2001). We hypothesize that (i) the capacity to withstand alternating periods of water availability and drought during germination and seedling establishment could be the main factor accounting for plant species' colonizing capability, and (ii) that species with anatomical structures (i.e. hairs, appendages or membranes) on the seeds that can capture water, germinate more under water stress conditions than species without those seed structures.

The objectives of this study are (i) to analyze the level (total number and/or final percentage of seed germination) and rate (speed) of germination of ten species grown on a water gradient between -0.1652 (moist) and -0.4988 MPa (dry) of osmotic potential, generated by the addition of increasing concentrations of polyethylene glycol (PEG), and (ii) to compare the germinative responses of ten species representative of the Iberian Peninsula, to determine which ones show a greater capacity of establishment under conditions of extreme water stress.

Specifically, we present evidence for (i) species colonizing ability and their water requirements, being those species less tolerant to periods of drought at germination, species with a low colonizing capacity and/or high water requirements in an adult state; and (ii) the positive relationship between anatomical structures in seeds (i.e. hairs, appendices or membranes), which are able to retain water, and level and rate of germination.

Materials and Methods

Plant materials: The following species were used: *Daucus carota* L., *Foeniculum vulgare* Miller, *Thapsia villosa* L., *Dittrichia viscosa* (L.) Greuter, *Epilobium hirsutum* L., *Cistus albidus* L., *Cistus ladanifer* L., *Medicago arabica* (L.) Hudson, *Dactylis glomerta* L. and *Cynosurus cristatus* L. These species were selected because they are very well represented in a wide range of environments of the Iberian Peninsula and in other parts of the world (Devesa, 1995; Thanos, 1999). The selected species were comprised of annual, biennial and perennial herbs, and shrubs (Table1). *Epilobium hirsutum*, *Medicago arabica*, *Dactylis glomerata* and *Cynosurus cristatus* are more often distributed in environments with a high humidity unlike the remaining species that can be found in both humid and dry habitats (Devesa, 1995). Mature fruits of each species were

Table – 1: Anatomical and ecological characteristics of the ten studied species. Nomenclature follows Devesa (1995).

Species	Family	Life form	Habitat	Diaspore mass (mg)	Studied diaspore	Appendages
Daucus carota L.	Apiaceae	Annual	Ruderal	0.76	Fruit	Short hairs
Foeniculum vulgare Miller	Apiaceae	Perennial	Uncultivated land	3.05	Fruit	No
Thapsia villosa L.	Apiaceae	Perennial	Pathways	8.29	Fruit	Wings
Dittrichia viscosa (L.) Greuter	Asteraceae	Perennial	Ruderal	0.32	Fruit	Hard hairs
Epilobium hirsutum L.	Onagraceae	Perennial	Irrigation canals	0.14	Seed	Hairs
Cistus albidus L.	Cistaceae	Perennial	Calcareous	1.18	Seed	No
Cistus ladanifer L.	Cistaceae	Perennial	Shrubs	0.22	Seed	No
Medicago arabica (L.) Hudson	Fabaceae	Annual	Wet meadows	3.29	Seed	No
Dactylis glomerata L.	Poaceae	Annual	Fertile meadows	0.39	Fruit	No
Cynosurus cristatus L.	Poaceae	Annual	Wet meadows	0.03	Fruit	No

Table – 2: Amounts of PEG added per litter of distilled water to create water potential gradients.

PEG g/l	g PEG/ g H20	Ψ
212	0.212	- 0.4988
182	0.182	- 0.3676
152	0.152	- 0.2564
122	0.122	- 0.1652

collected between May and October 2001. In some species (*M. arabica*, *E. hirsutum*, *C. albidus* and *C. ladanifer*) seeds were studied, whereas fruits were studied in others. By agreement, in order to simplify the notation, we will refer to them as seeds. These seeds were extracted and stored for nine months in paper envelopes at 18±1ºC and 20% RH. Batches of 100 seeds were weighted (Table 1).

Effect of water potential in seed germination: Polyethylene glycol (PEG) is an inert, water binding polymer with a non ionic and virtually impermeable long chain (Couper and Eley, 1984) that accurately mimics drought stress under dry-soil conditions. The higher the concentration of PEG, the lower the water potential achieved, thus inducing higher water stress in a watery medium. The effect of osmotic potential on germination was tested at four levels of water potential, (-0.4988, -0.3676, -0.2564 and -0.1652 MPa). These values are within the wide range observed in soils of the Iberian Peninsula (García-Fayos *et al.*, 2001). Water potential was simulated using polyethylene glycol (PEG 6000) (García-Fayos *et al.*, 2001). The appropriate concentrations of polyethylene glycol to achieve each water potential were determined following Michel and Kaufmann (1973) and further modifications by Hardegree and Emmerich (1990). Final equation used to determine amounts of PEG was:

$$\Psi = 0.130\,[PEG]^2\,T - 13.7\,[PEG]^2$$

where the osmotic potential (Ψ) is a function of temperature (T). Given the characteristics of the species selected, the optimal average temperature for the germination of its seeds is 20± 1ºC (ISTA, 1993), which is why the estimation of the final PEG concentrations was carried out for this temperature. The amounts of PEG 6000 added per liter of distilled water to create the corresponding water potential are indicated in Table 2. Control treatment consisted of distilled water.

Germination experiments were conducted in 90 mm diameter petri dishes. Each water potential treatment was replicated four times for each species. Replicates consisted of a Petri dish, watered with 10 ml of the appropriate solution and 20 seeds of each selected species floating in the solution. To prevent from variations in Ψ, dishes were sealed with parafilm and the PEG solutions were renewed weekly by pouring out the existing PEG in the petri dish and adding the same amount of fresh solution (Pujol *et al.*, 2000; García-Fayos *et al.*, 2001). The experiment was repeated in the same way with the seeds of *D. viscosa, S. officinalis, T. villosa* after removing their appendices manually, aided by a pair of pliers. Previous to the germination experiment, seeds with impermeable coats (i.e. *M. arabica, C. albidus* and *C. ladanifer*) were treated to make them permeable to water by soaking them in boiling water. Petri dishes were kept in an incubation chamber (SELECTA Hotcold-ggl- 4000702, Germany) at 20 ± 1ºC in light (12 hr) and at 18±1ºC in darkness (12 hr) (Rodríguez-Echeverría and Pérez-Fernández, 2001; Pérez-Fernández and Rodríguez-Echeverría, 2003). Germination was assessed daily. Germination was determined by radicle emergence (Thanos and Rundel, 1995; Letnic *et al.*, 2000). After 32 days, non germinated seeds were washed with distilled water and then returned to germinate under the same experimental conditions, being watered with distilled water. At the end of this second germination test, non germinated seeds were individually dissected to determine their viability. Viable seeds were those with the embryo and endosperm intact and without discoloration (Cochrane *et al.*, 1999).

Data analyses: The rate or speed of germination was calculated using a modification of Timson's index (IT) (Timson, 1965; Pujol *et al.*, 2000):

$$IT = \Sigma(G/t)$$

where G is the percentage of germination of seeds every two days, and t indicates the total time during which seeds were germinating (32 days). The percentage of recovery of the germination (PRG) and Timson's index for the recovery of the germination (ITRG) were determined by the following expressions (Pujol *et al.*, 2000):

$$PRG = [(a - b) / (c - b)]100$$

$$ITRG = [d / (a - b)]\,100$$

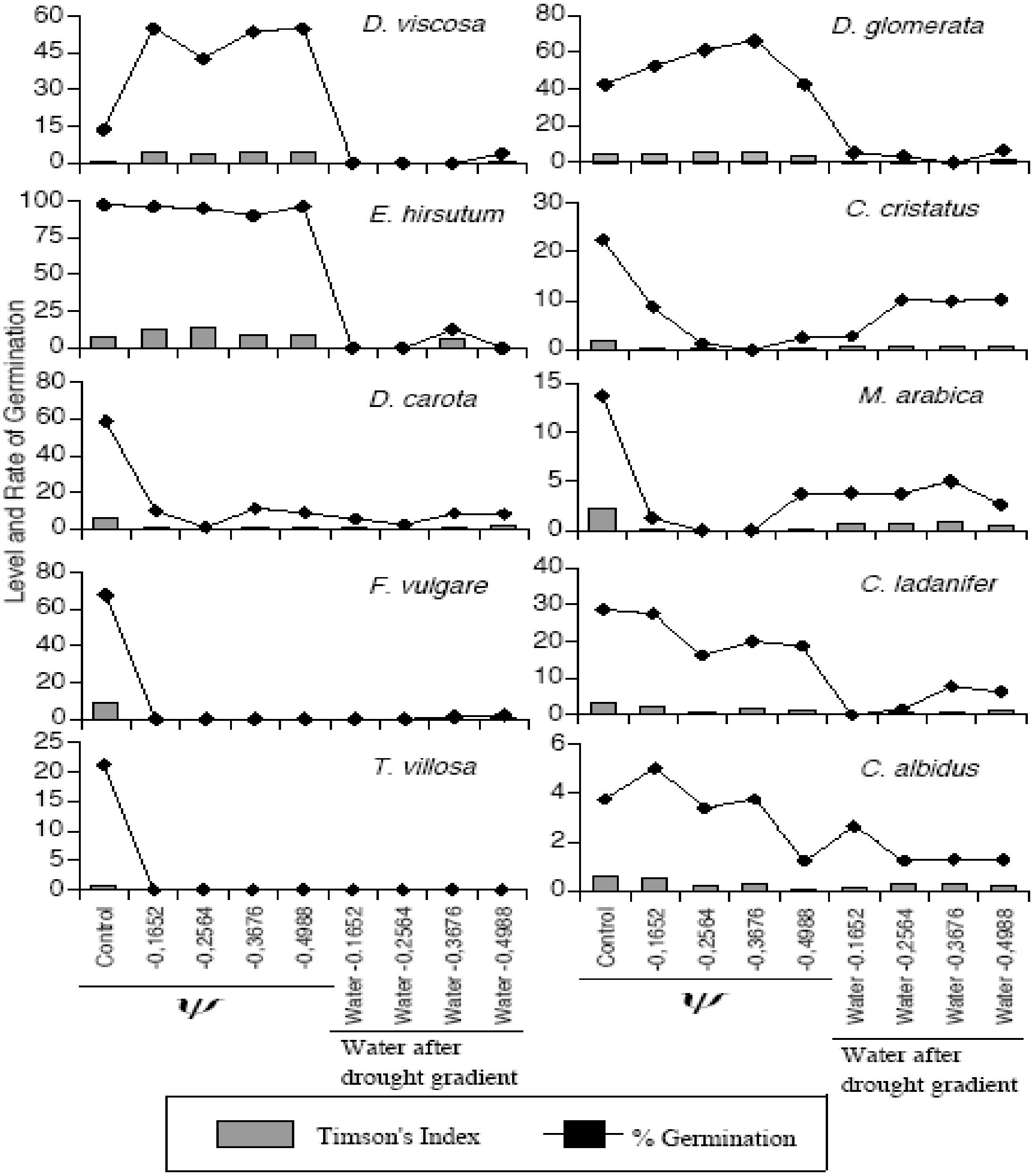

Fig. 1: Level and rate of germination of seeds of ten plant species under water stress created by increasing concentrations of PEG. Seeds sown with structures of water retention. PRG, calculated as above, is presented as % of final germination and the Index of Timson for recovery of germination (ITRG) is given as Timson's Index.

where *a* indicates the total number of seeds germinated after the transference to distilled water, *b* represents the total number of germinants at different concentrations of PEG, *c* is the total number of seeds and *d* is the value of Timson's index for the seeds germinated in distilled water (control). The effects of water potential on the germination level and rate for each species were determined using analysis of variance (ANOVA) (Pérez-Fernández and Rodríguez-Echeverría, 2003). Tukey tests were applied to compare germination levels and rates among treatment Ø. Similar analyses were performed to compare the germination of seeds incubated in water after the PEG had been washed out. Percentages, adjusted to number of viable seeds, were arcsine-square root transformed prior to analyses. Normality and homoscedasticity of data were tested by means of David's and Cochran's tests, respectively.

Generalized linear models (GLM; McCullagh and Nelder, 1989; Dobson, 1990) were used to describe the drought response gradient. Because the data obtained were discrete

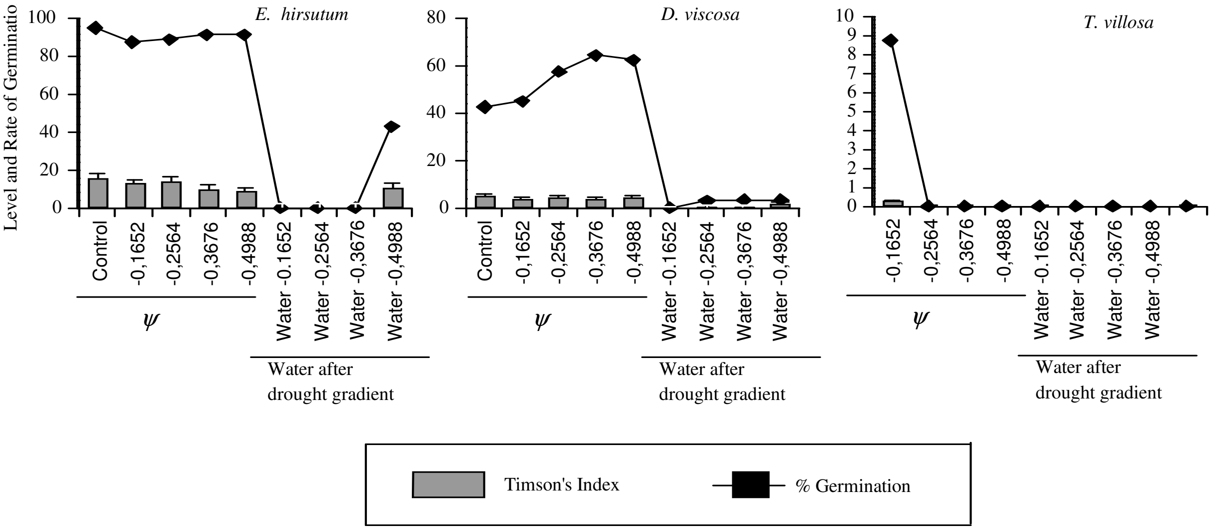

Fig. 2: Rate of germination of seeds of 11 plant species under water stress created by increasing concentrations of PEG. Seeds sown with structures of water retention. PRG, calculated as above, is presented as % of final germination and the index of timson for recovery of germination (ITRG) is given as Timson's index.

discrete (counts), a Poisson distribution of errors was assumed, which requires a logarithmic link (McCullagh and Nelder, 1989; Ferrer-Castán *et al.*, 1995). Thus it was also ensured that the fitted values were positive (Hastie and Pregibon, 1993). The first and second order polynomials of the explanatory variable (PEG concentration) were tested against a null model and against each other. The change in deviance between nested models was tested by the F-test to control for over dispersion in the data. All models were obtained using S-Plus 6.1 for Windows (Anonymous, 2002).

Results and Discussion

The level (final percentage) and rate (speed given by the index of Timson) of germination for the 10 species germinated at four concentrations of PEG are presented in Fig. 1 Ø (intact seeds) and 2 (seeds without appendages). PRG, calculated as above, and presented as % of final germination and ITRG is given in Fig. 1 and 2 as Timson's Index. Germination varied between species, though similar trends were found within Apiaceae and within Cistaceae. *Daucus carota* reached the maximum level and rate of germination in the control, and control was the only treatment under which *F. vulgare* and *T. villosa* germinated. The recovery of germination is also very low (next or equal to zero) for these three species. When the germination of *T. villosa* without seed appendages was recorded (Fig. 2), the results were similar, but with a decrease in the level and rate of germination in the control compared with the same treatment on intact seeds. In these three species, significant differences in the rate of germination between the control and all the treatments with PEG were detected (Table 3). *Epilobium hirsutum*, with or without seed appendages, was the best germinator (ca. 100%) under any concentration of PEG, with no significant differences between the control and the treatments with PEG (Table 3). *Dittrichia viscosa*, also reached high levels of germination under any PEG concentration, being the highest values of germination (64.5%) for seeds without appendages (Fig. 1 and 2). Significant differences in the rate of germination were also found (Table 3). The two *Cistus* species followed the same germination response, with a significant reduction of germination at increasing values of water potential. Maximum germination of *C. ladanifer* (29.8%) was reached in the control treatment as well as at water potential of -0.1652. In both species, the rate of germination under these two treatments was significantly higher than that under any other treatment (Table 3).

Cynosurus cristatus and *Medicago arabica* had poor level and rate of germination in both the control and PEG treatments (22 and 14 %, respectively). These two species were able to slightly recover their germination when washed and transferred to distilled water (Fig. 1 and 2). Even though the final percentages of germination of washed seeds were low, the rate of germination was significantly higher than for those under the PEG treatments and significantly lower than for those in the control (Table 3).

Germination of *D. glomerata* increased with increased PEG concentrations. The rate of germination at the two intermediate concentrations of PEG (which determines osmotic potentials of -0.2564 and -0,3676 MPa) was higher than and significantly different from those at the other concentrations (Table 3). Recovery of germination after transfer to distilled water was almost zero.

The effect of decreasing water potential on seed germination differed among species (Table 4). Significant relationships between germination of intact seeds of *D. carota*, *C. cristatus* and those of *D. viscosa* without structures of water retention, clearly depend on the water potential of the medium surrounding the seeds. Fig. 3 shows the trends in germination

Table – 3: Values of Timson's indices for seeds of 10 species germinated in a gradient of osmotic potential created by increasing concentrations of PEG. Same letters next to each value indicate absence of statistical differences; different letters indicate significant differences after a test-T (* seeds without structures).

	Water Potential (MPa)				
Species	**0**	**-0.1652**	**-0.2564**	**-0.3676**	**-0.4988**
D. carota	5.59^{a}	0.53^{b}	0.32^{b}	0.59^{b}	0.43^{b}
F. vulgare	9.37^{a}	0^{b}	0^{b}	0^{b}	0^{b}
T. villosa	0.71^{a}	0^{b}	0^{b}	0^{b}	0^{b}
*T. villosa**	0.27^{a}	0^{b}	0^{b}	0^{b}	0^{b}
D. viscosa	0.89^{a}	4.56^{b}	3.91^{b}	4.27^{b}	4.57^{b}
*D. viscosa**	4.98^{a}	3.59^{a}	4.71^{a}	3.86^{b}	4.14^{b}
E. hirsutum	7.84^{a}	12.72^{a}	14.30^{a}	8.83^{a}	8.80^{a}
*E.hirsutum **	15.68^{a}	12.77^{a}	14.32^{a}	9.86^{a}	8.82^{a}
C. albidus	0.62^{a}	0.49^{a}	0.23^{b}	0.28^{a}	0.04^{a}
C. ladanifer	3.34^{a}	2.12^{a}	0.91^{b}	1.56^{a}	1.25^{a}
M. arabica	2.17^{a}	0.08^{b}	0^{b}	0^{b}	0.18^{a}
D. glomerata	4.40^{a}	4.63^{a}	5.34^{b}	5.48^{c}	3.72^{a}
C. cristatus	1.86^{a}	0.47^{a}	0.08^{b}	0^{b}	0.11^{b}

responses of three species. The rates and levels of germination of *D. carota* and *C. cristatus* diminish significantly as the concentration of PEG increased (i.e. as the water potential diminishes), whereas *D. viscosa* without anatomical structures of water retention shows a positive significant response to the decrease in water potential. The remaining species (*M. arabica*, *E. hirsutum*, *D. glomerata*, *C. ladanifer* and *C. albidus*) with or without structures, as well as *D. viscosa* with structures, did not show significant differences between level and rate of germination and water potential. The germination of *F. vulgare* and *T. villosa* was completely inhibited by all the treatments with PEG.

Water availability is without no doubt one of the most important factors in determining germination and seedling establishment under stressful conditions, and the results presented in this study corroborate it. Nine of the ten species did not germinate when water potential was at the wilting point (-0.4988 MPa) and they germinated at higher values, with

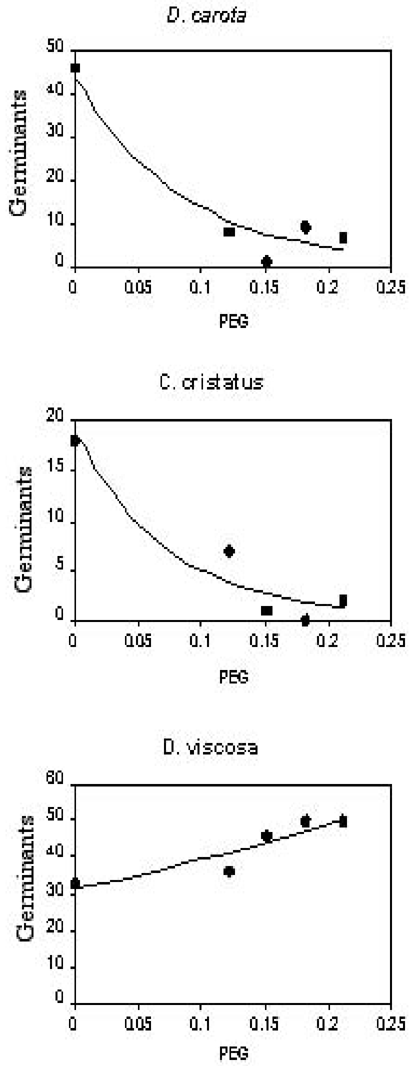

Fig. 3: Level of germination of seeds of four plant species under water stress created by increasing concentrations of PEG. Seeds sown without structures of water retention. PRG, calculated as above, is presented as % of final germination and the Index of Timson for Recovery of Germination (ITRG) is given as Timson's Index.

Table – 4: Summary of the results after the general linear models on the germination of three species treated with four water potential levels (values are means). Only significant results are included.

Species	**Treatment**	**Residual deviance**	**df**	**F**	***p***
D. carota	with structures				
Null Model (total *deviance*)		75.54	4		
PEG		14.18	3	15.8	0.02
C. cristatus	with structures				
Null Model (total *deviance*)		37.59	4		
PEG		7.38	3	15.3	0.03
D. viscosa	without structures				
Null Model (total *deviance*)		6.11	4		
PEG		1.03	3	15.27	0.03

maximum germination at a water potential of -0.1652. Therefore, these species are expected to germinate when the water potential of the ground is above the wilting point, as has been shown previously for some of these species (Pérez-Fernández *et al.*, 2003). The water potential of the soil in the surroundings where these plants grow and from where seeds were collected is close to the indicated value (-0.1652 MPa). As the water potential increases, the probability of germination, emergence and later establishment of seedlings also decreases. In ecosystems of Mediterranean type, rain events are frequently followed by high temperatures which determine a fast reduction of the water potential in the soil. This situation induces a reduction in the rate and level of germination and increases the number of days needed for the onset of germination. In fact, in our experiment, both the rate and the level of germination were diminished in all the species at water potential values above the wilting point. These results agree with those from García-Fayos *et al.* (2001), for other representative species of the Mediterranean environments, where the reduction in the water potential induces a reduction in the level of germination and increases the number of days taken for the germination to start.

It is possible then to ask why some species are better colonizers than others. Species with a better colonizing ability have among their attributes a high efficiency in the use of water and nutrients for germination (Newsome and Noble, 1986; Witkowsky, 1991). Except *D. glomerata*, species lacking anatomical structures showed poor drought tolerance, being unable to germinate, or reaching low percentage of final germination at a water potential of -0.2564 MPa. On the other hand, *D. viscosa* and *D. hirsutum*, species with seminal anatomical structures, showed a greater tolerance to drought, germinating even at water potential of -0.4988 MPa. The presence of anatomical structures surrounding the seed seems to be the most plausible explanation for the increased germination in these two species. This fact has been verified for other species; in particular of Asteraceae (McIvor and Smith, 1973), where the presence of sticky and mucilaginous villis helps to reduce the superficial tension between the ground and the seed, and therefore they are able to retain a greater amount of water in a smaller period of time. This way, soil water becomes almost immediately available for seed, thus enhancing germination. This fact could explain the greater germination of *D. viscosa* and *E. hirsutum* bearing water-holding structures. Therefore, the structures described in these species seem to have a double function, the traditionally accepted one of seminal dispersion and also the water retention to favor germination and early establishment. Against all predictions, *D. viscosa* showed a positive relation between the level of germination and the decrease in water potential when hairs were removed (Fig. 2 and 3). Also, and contrary to the behavior of the other species, the villi as well as the seed themselves are impregnated with a sticky and hydrophobic substance. The hydrophobic nature of this structure is easily observable since when introduced in water the seeds are not imbibed even after seven days in direct bonding with water (personal observation). Therefore, when eliminating the villi, any amount of water can reach the seed, activating its germination even at extremely low values of water potential.

Although water availability acts like a trigger in the germination of these species, we cannot forget other environmental factors which directly influence seeds and act as germination regulators too. Nutrient concentration and availability in the soil, especially nitrogen, is crucial in the regulation of the germination of numerous species including some of those studied in this work (Pons, 1989; Witkowsky, 1991; Pérez-Fernández *et al.*, 2002). Also, changes in the soil salinity, the erosion processes and the occurrence of other disturbances have to be observed at the same time under field conditions in order to predict the establishment of these species. As no clear pattern of germination was observed according to life forms or to environments, reasons for the behavior observed have to be found in other environmental cues or in the physiological characteristics of the species.

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