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Germinations of selected perennial plant species from western Spain under nitrogen, light and wet and dry heat treatments

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Key words: germination, imbibition, desiccation, temperature, nitrogen, light and dark.

Abstract

Imbibed seed of six common species from the Central West of Spain were placed in Petri dishes in moist soils and heated in ovens set to 30ºC, 50 ºC and 100 ºC for 0.5, 1, 4, 8 and 16 days. After heating, seeds were incubated for 35 days on a 10/14 dark/light regime and germination was recorded. The same duration of heating treatment was applied to non-imbibed seeds. It was also investigated the effect of nitrogen on germination, by applying $NaNO_2$, NH_4NO_3, KNO_3 and NH_4Cl at five increasing concentrations (1mM, 10mM, 25 mM, 50mM and 100mM). Temperature of 30ºC was the most successful for germination of pre-imbibed seeds. *Dittrichia viscosa, Cistus albidus* and *Cistus ladanifer* increased germination under the wet treatment. Germination is positively correlated to soil water content for the aforementioned species and negatively correlated in *Verbascum sinuatum*. Germination was higher after treatment at 30ºC and 50ºC that at 100ºC for non-imbibed seed except for *Cynara humilis* and *Cistus albidus.* The maximum temperature required to prevent germination varied among species and was variable with duration of heating. Fertilisation with KNO_3 and NH_4Cl at 25mM and NH_4NO_3 at 10mM and 100 mM increased both level and rate of germination in most species. Continuous darkness inhibited germination in all the species. The increase of temperature activates the germination of non-imbibed seeds and also the increase of nitrogenous compounds in the field trigger the germination of nutrient-requiring species.

Introduction

One of the variety of factors determining the colonising and competitive capacity of a given plant species is its possibility of germinating when the environmental conditions are suitable to guaranteeing the development of the young plant, or of delaying germination when the environmental conditions are adverse (Angevine and Chavot, 1979). One of the most important mechanism by which seeds may "detect" gaps in the vegetation is regulation of the processes of dormancy and germination by temperature, which may act independently of light (Thompson and Grime, 1983; Derkx and Karssen, 1993), and the presence in the soil of chemical compounds which act as limiting factors (Tilman, 1980; Jornsgard et al., 1996). Nitrogen may break dormancy in many species, both alone and in combination with alternating temperatures and/or light-dark cycles (Bewley and Black, 1982).

In arid climates, especially those of the Mediterranean type, seed germination and plant growth are limited to the favourable times of year when availability of space for the establishment of new plants, sufficient supply of water and mineral nutrients, and suitable temperatures and hours of daylight, all coincide (Koller, 1969; El-Sharkawi et al., 1989).

Many studies have reported that in arid enclaves fire is an important factor creating gaps suitable for plant species colonisation. In eliminating a great part of the vegetation, fire leaves ash, which temporarily raises the nutrient content of the surface soil creating the favourable environment to be occupied by new species (Keeley and Keeley, 1988; Thanos et al., 1989; Trabaud and Oustric, 1989; Whelan, 1988; Pugnaire and Lozano, 1997). The heat of the fire stimulates the germination of certain species which require this thermal treatment to eliminate seed dormancy (Evans and Etherington, 1990; Buckland

et al., 1997; Martínez-Chersa et al., 1997). It is thus natural to expect that such species will show tolerance to thermal shock, an activation of their germination under the effect of high temperatures, a capacity to recover after imbibition and desiccation (Buckland et al., 1997), and the possibility of detecting gaps in the vegetation in which to establish themselves.

Given the intimate relationship between the dispersed seeds and the substrate lodging them, there could exist a regulation of germination as a consequence of water availability and soil temperature (Hegarty and Ross, 1980; Evans and Etherington, 1990; Thompson et al., 1997). In Southwestern Spain, most rain falls in sporadic rainfall events during autumn and spring. In these environments, most seeds remain at the soil surface or are shallowly buried, so that seeds are likely to become imbibed with water after every rain event but could dry out before the next rains, affecting both the rate and the level of final germination.

High soil temperatures can activate or inhibit germination according to the intensity and duration of the heat treatment that might range from low temperatures to over 100ºC (Zammit and Zedler, 1988; Keeley and Keeley, 1987; Pugnaire and Lozano, 1997). Likewise, the post-fire addition of ash to the soil leads to a rise in soil N content, in response to which some species may increase their germination level (Grove et al., 1986; Johnson and Elliott 1997).

In the present work, we tested the hypotheses that (i) the application of high temperature dry heat to seeds of six perennial species would promote their germination; (ii) imbibition treatment of seeds and further desiccation in soil at different temperatures would lead to a decline in germination, and (iii) breaking the seed dormancy of six perennial species is activated by nitrogen, so that this element may act as a detection mechanism for gaps in the vegetation, with the consequence that species capable of responding to increased nitrogen levels will germinate rapidly and increase in numbers in a given community.

Material and methods

a) Study area and plant material

The study zone was an open woodland formation of *Quercus ilex* subsp *ballota* (Desf.) Samp. Located in the vicinity of Badajoz, central-west Spain. The intermediate stratum consists of multispecies Cistaceae scrub on a very acidic substrate, with *Cistus ladanifer* L. and *Cistus albidus* L. as the dominant species. These plants are present in varied ages and sizes with a roughly random distribution giving coverages of between 15% and 40%. The lower stratum consists of numerous species representing numerous botanical families, giving a coverage of up to 85%. The species selected for the present study were among the most representative and abundant in both, the field sampled in particular and the area in general, for which there is a lack of information regarding the particular mechanisms involved in their ability to colonise new environments: *Verbascum sinuatum* L. (Scrophulariaceae), *Hypericum perforatum* L. (Hypericaceae), *Dittrichia viscosa* (L.)Greuter (Asteraceae), *Cynara humilis* L. (Asteraceae), *Cistus ladanifer* (Cistaceae) and *Cistus albidus* (Cistaceae). Mature seeds were gathered at the end of the Summer in 1998 and stored in opaque envelopes at room temperature (12 ± 1 °C) for two months so as to provide a post-harvest ripening that eliminates the innate dormancy. The seed characteristics of each species are listed in Table 1.

b) Wet heat treatment

Seeds of each species were mixed and covered with 50g of soil in 10 cm Petri dishes. The soil was then watered with 20 ml distilled water and the seeds were left to imbibe for 2 hours at room temperature. After this imbibition period, the water was drained from the dishes which were then placed in forced air ovens at 30°C, 50°C, and 100°C for 0.5, 1, 4, 8, and 16 days. Eighty seeds of each species were used for each combination of time and temperature in four replicates of 20 seeds. After the heat treatment, the Petri dishes were watered with distilled water to the field capacity of the soil and transferred to a greenhouse with mean maximum and minimum temperatures of 32.6°C and 11.3°C, respectively, and a photoperiod regime of 14 h daylight and 10 h darkness. These conditions coincide with the natural day-length and temperatures in the field over the time when seeds are more likely to germinate (Cabezas-Fernández, 1991). We also estimated the soil moisture content for each time and temperature combination of drying. For this purpose, 20 Petri dishes containing soil to which the same quantity of water had been added as in the imbibition treatments, were placed in the oven at each of the temperatures used for the imbibed seeds. The wet weight of the soil was recorded, as well as the weight after each of the drying periods (0.5, 1, 4, 8, and 16 days). The moisture content was calculated as [(moist wt - dry wt)/(dry wt)] x 100 (i.e. expressed on a dry weight basis rather than a total weight basis).

c) Dry heat treatment

Non-imbibed seeds of the six species were treated at the same temperatures and for the same time periods as those described in section (b), and the same number of replicates were prepared per species and per treatment as in the previous trial. The seeds were placed on sterile cotton in Petri dishes rather than in soil and kept for 35 days in an incubator at 22°C during the period of light and 18°C during the period of darkness. As controls, 80 untreated seeds of each species were sown in 4 replicates of 20 seeds per dish and incubated for 35 days under the same conditions as described above.

Table 1. Lengths, diameters, and mean weights of the seeds of the six species.

	SEED ATTRIBUTES		
SPECIES	**LENGTH (mm)**	**WIDTH(mm)**	**MASS (mg)**
Cynara humilis	6.95	4.11	6.08
Verbascum sinuatum	0.70	0.44	0.104
Hypericum perforatum	0.82	0.35	0.062
Dittrichia viscosa	1.93	0.49	0.326
Cistus albidus	1.53	1.04	1.18
Cistus ladanifer	0.97	0.55	0.22

d) Application of nitrogenous compounds.

We did not use C*inara humilis* in this and the following experiment due to a lack of seeds for a sufficient number of replicates.

The effect of nitrogen on germination was investigated by applying nitrogenous solutions of $NaNO_2$, NH_4NO_3, KNO_3, and NH_4Cl at a series of five concentrations; 1 mM, 10 mM, 25 mM, 50 mM and 100 mM. These nitrogenous compounds have previously been demonstrated to have an effect on the germination of species from the Iberian Peninsula (Pérez-Fernández et al., 1994). Table 2 lists the values of the pH of the solutions used. For each of the solutions, 80 seeds per species in replicates of 20 were sown in 10 cm Petri dishes on sterile cotton as inert substrate. The dishes were kept in an incubator at 24°C and 18°C under a photoperiod of 14 h light / 10 h dark for 35 days.

e) Combined effect of darkness and fertilizer.

An additional experiment was performed to investigate the combined effect of light and darkness and fertilizer on germination. To this end, we followed the same procedure as in the study of the effect of the nitrogenous compounds on germination, but incubating the seeds in darkness under identical temperature regimes. The seeds used in this experiment were only exposed to direct white light at the time of sowing. The weekly germination counts were made under green light to avoid the possibility of phytochrome activation and subsequent germination (Corbineau et al., 1992; Hou and Simpson, 1993).

Daily counts were made of germination, taking the protrusion of the radicle as the criterion for germination (Baskin et al., 1993; Derkx and Karssen, 1993).

f) Data analysis

Data on percentages of germination were analysed by one or two-way analysis of variance using StatView® (1992-98 SAS Institute Inc) to determine the treatment effects of temperature, length of temperature treatment and nitrogenous compounds. Arcsine transformations were performed on the percentages of germination data prior to analyses. Where one-way ANOVA showed significant effects at 5%, Fisher's least significant differences were determined for all replicates showing some germination. A correlation analysis was performed to assess the degree of association between soil water content and seed germination.

g) Nomenclature

Nomenclature of the scientific names follows Tutin et al.,1964-80.

Table 2. Values of the pH of the nitrogenous solutions of $NaNO_2$, NH_4NO_3,KNO_3, and NH_4Cl at five concentrations.

	1mM	10mM	25mM	50mM	100mM
$NaNO_2$	6.25	6.55	6.57	6.55	6.60
NH_4NO_3	6.15	5.80	5.53	5.36	5.30
KNO_3	6.13	5.76	5.83	5.86	6.01
NH_4Cl	6.10	5.65	5.76	5.30	5.83

Results

a) Germination after wet heat treatment

For most of the species, we observed a twofold influence on the final germination percentages of the combined action of temperature and duration of heating of seeds imbibed directly in the soil. In general, heating at 50ºC and 100 ºC reduced seed germination relative to the unheated controls. Figure 1 presents the final germination percentages of the six species. The response to temperature and duration of heating was variable at the lowest temperature (30°C). The two *Cistus* species, *Cistus albidus* and *Cistus ladanifer*, and *Dittrichia viscosa* were the species that attained the greatest germination percentages under the different combinations of temperature and duration of heating. Germination of *Hypericum perforatum* was practically null, with seed germination only at 30°C for 1 day and 100°C for four days. Germination of *Cynara humilis* and *Verbascum sinuatum* was always lesser in the seed treated at any of the temperatures than that in the controls. The combination of temperature and duration of its application which led to the greatest germination percentages in *D. viscosa*, *C. albidus*, and *C. ladanifer* were 30°C for practically any period of time, and 50° and 100°C for four days in both *Cistus* species (Figure 1). Statistical differences in final percentages of germination were detected between the control (non-treated seeds) and dry and wet heated seeds (Figure 1).

The Asteraceae and Cistaceae treated at 30°C needed the shortest time to initiate germination (Table 3); the other two species required very prolonged times before showing any germination. It was difficult to compare the time lag required for germination between species and length of treatment of the 50°C and 100°C temperatures because of the low percentages observed. The mean number of days required for the onset of germination rose significantly ($p < 0.005$) in all species with the treatment of imbibition in comparison with the controls (Table 3). The percentage of water in soil decreased with increasing temperatures. At 30ºC the soil lost all moisture within 4 days. Therefore at temperatures of 50ºC and 100ºC the soil dried out much quicker, needing only two days at 50 ºC to dry out and presumably less than that at 100ºC.

Figure 2 shows the relationship between the soil moisture content in the soil treated at 30°C for 0.5, 1, 4, 8 and 16 days and the germination of *Dittrichia viscosa, Cistus ladanifer, Cistus albidus, Verbascum sinuatum* and *Cinara humilis.* In the first three species, there was a clear relationship between soil moisture content and seed germination, with a greater germination percentage for greater moisture content. On the contrary, *V. sinuatum* had the opposite response, with germination decreasing as the moisture content rose. In all five cases, the indices of correlation were close to unity, suggestive of the high degree of association between these two variables. There was significant no relationship of either type for the germination of *Cynara humilis* or *Hypericum perforatum*. Neither did we detect any significant relationship between the germination of the six species and the loss of moisture from the soil at 50°C and 100°C.

b) Germination after dry heat treatment

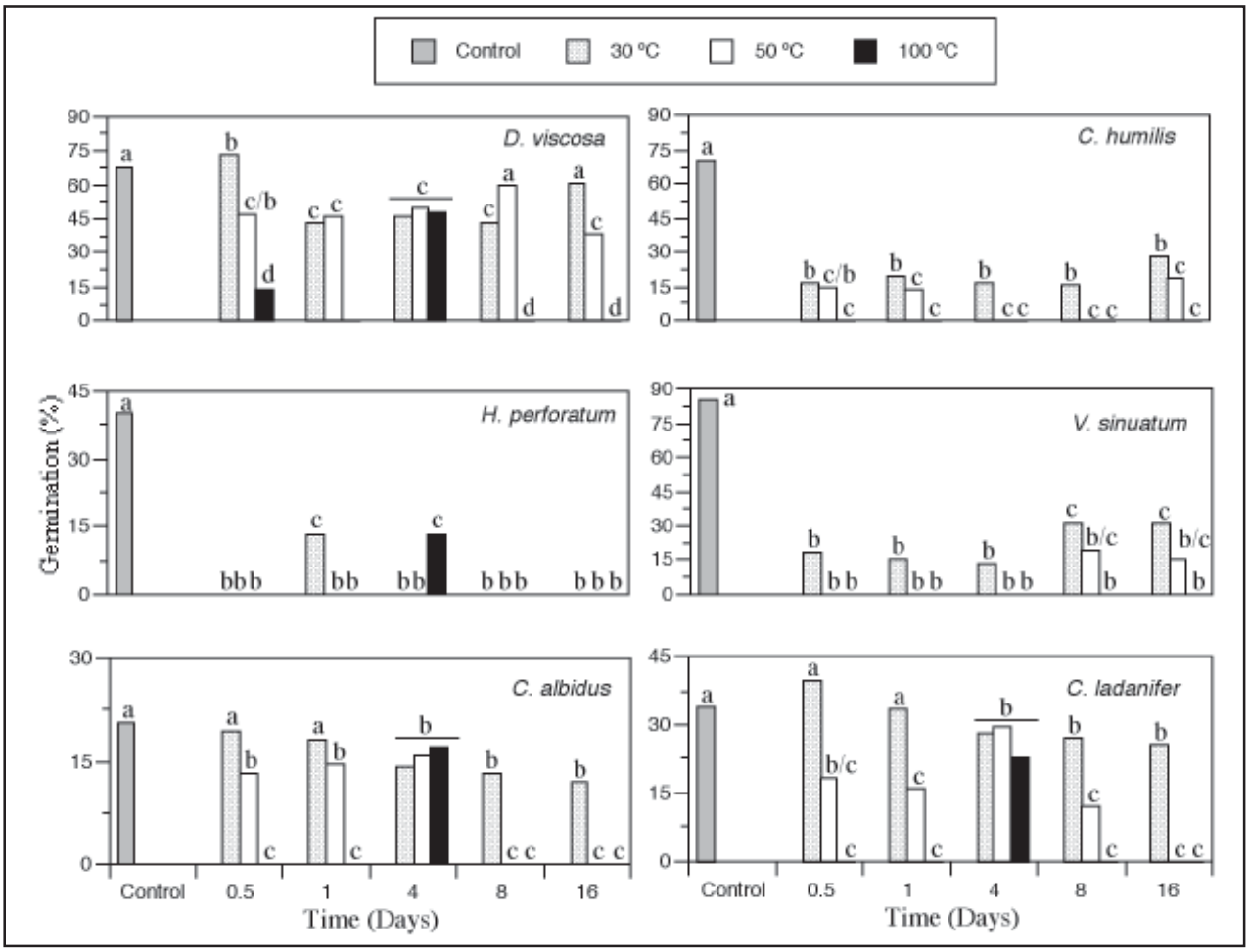

Figure 1. Germination percentages of various species subjected to imbibition in soil and dried at 30°C, 50°C, and 100°C for 0.5, 1, 4, 8, and 16 days. Control = non-dried seeds. Missing values not shown are 0% germination. Letters on top of the columns represent significant differences between and within treatments, including the control, after a multiple range test performed after the ANOVA.

The final germination percentages of the seeds that were heat treated without any prior imbibition were far greater than those of seeds which were imbibed before heat treatment (Figures 1 & 3). In *Dittrichia viscosa, Hypericum perforatum*, and *Verbascum sinuatum*, the greatest germination percentages were attained in seed heated at 30° C and 50 ° C independently of the length of time the treatment was applied (Figure 3). Cistaceae seeds reached their optimal germination, which was far greater than for the controls, at the temperature of 100°C applied for 4 days.

The species which initiated their non-soaked germination in the shortest space of time were *Dittrichia viscosa* and *Cistus albidus.* The remaining species required a longer period of time for the onset of germination. *Hypericum perforatum* took the longest for the onset of germination (Table 3).

c) Germination after application of nitrogenous compounds

Final germination percentages

Significant differences were found in the final germination percentages between species, treatments, and concentrations of nitrogenous compounds (Figure 4). The germination of *Dittrichia viscosa* was slightly activated (no significant differences) by KNO_3 and NH_4Cl at 10, 25 and 50 mM and inhibited by the highest concentrations of $NaNO_2$ NH_4NO_3. The germination response of *Verbascum sinuatum* was similar in all the treatments except for $NaNO_2$ at 25, 50 and 100 mM, KNO_3 at 50 mM and NH_4Cl at 10 and 50 mM. The most heterogeneous response was found in *Hypericum perforatum*. Watering with distilled water applied to the controls leaded to a 40% of germination, which was similar to that of watering with $NaNO_2$ at 1 mM and KNO_3 at 10 mM. The NH_4NO_3 solution applied at increasing concentrations significantly augmented germination, with the greatest response at 100 mM. Germination of this species treated with the remaining salts at any of the concentrations reduced final percentage of germination. The germination of *Cistus albidus* was only significantly increased with the concentration 25 mM KNO_3 and NH_4Cl. Concentrations of 25, 50 and 100 mM of $NaNO_2$, as well as for 1 mM of KNO_3 significantly inhibited germination. No statistically significant differences were detected in *Cistus ladanifer* germination, although there was a clear inhibition of germination when seed were treated with the concentrations 50 and 100 mM of $NaNO_2$ and KNO_3, and 25, 50 and 100 mM of NH_4NO_3.

Number of days for the onset of germination

There was a similar behaviour in the five species regarding the number of days to start germination. The greater the activation of germination by a particular combination of salt and its concentration, the fewer days

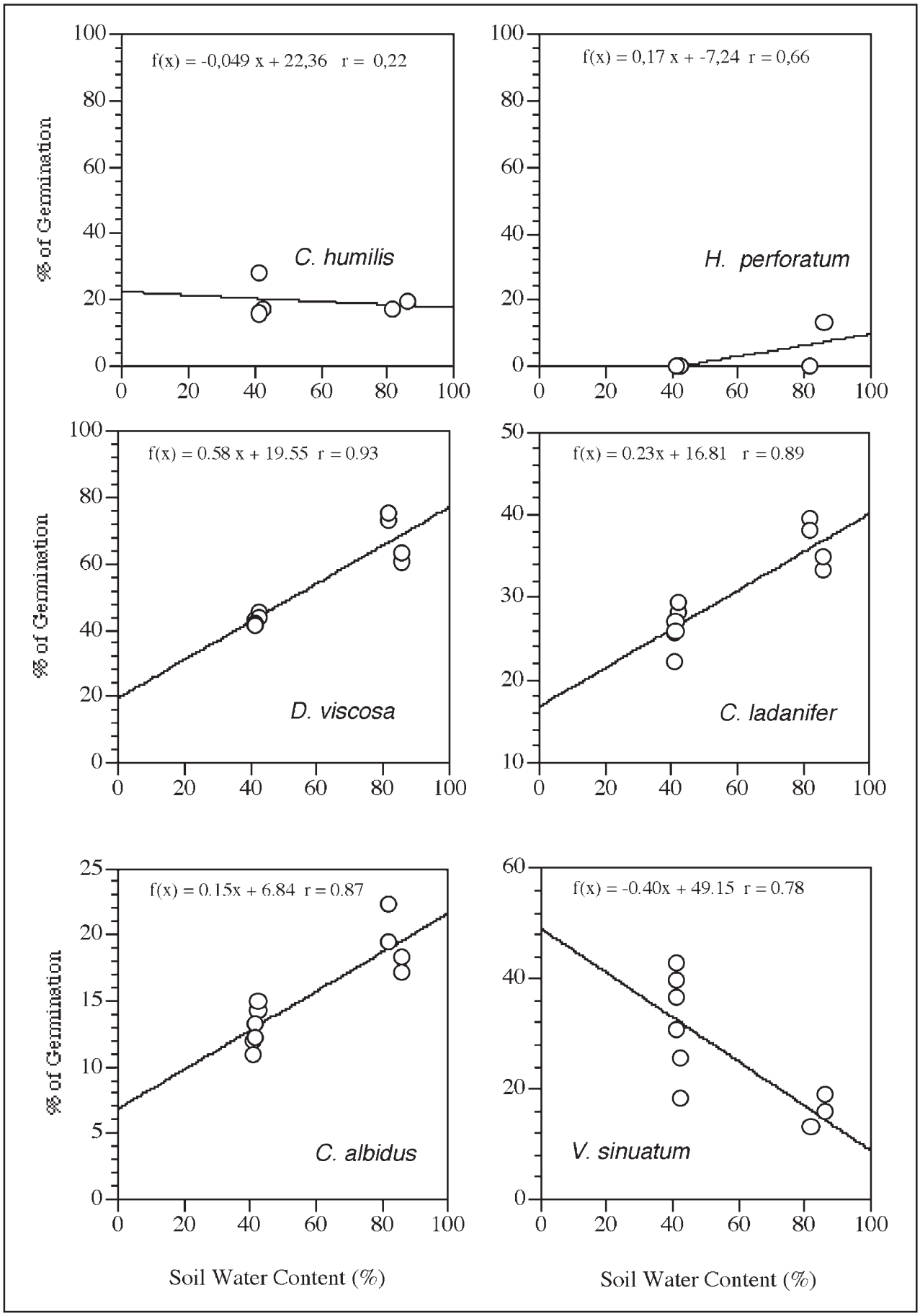

Figure 2. Relationship between seed germination and soil moisture content after treatment at 30°C for 0.5, 1, 4, 8, and 16 days of drying.

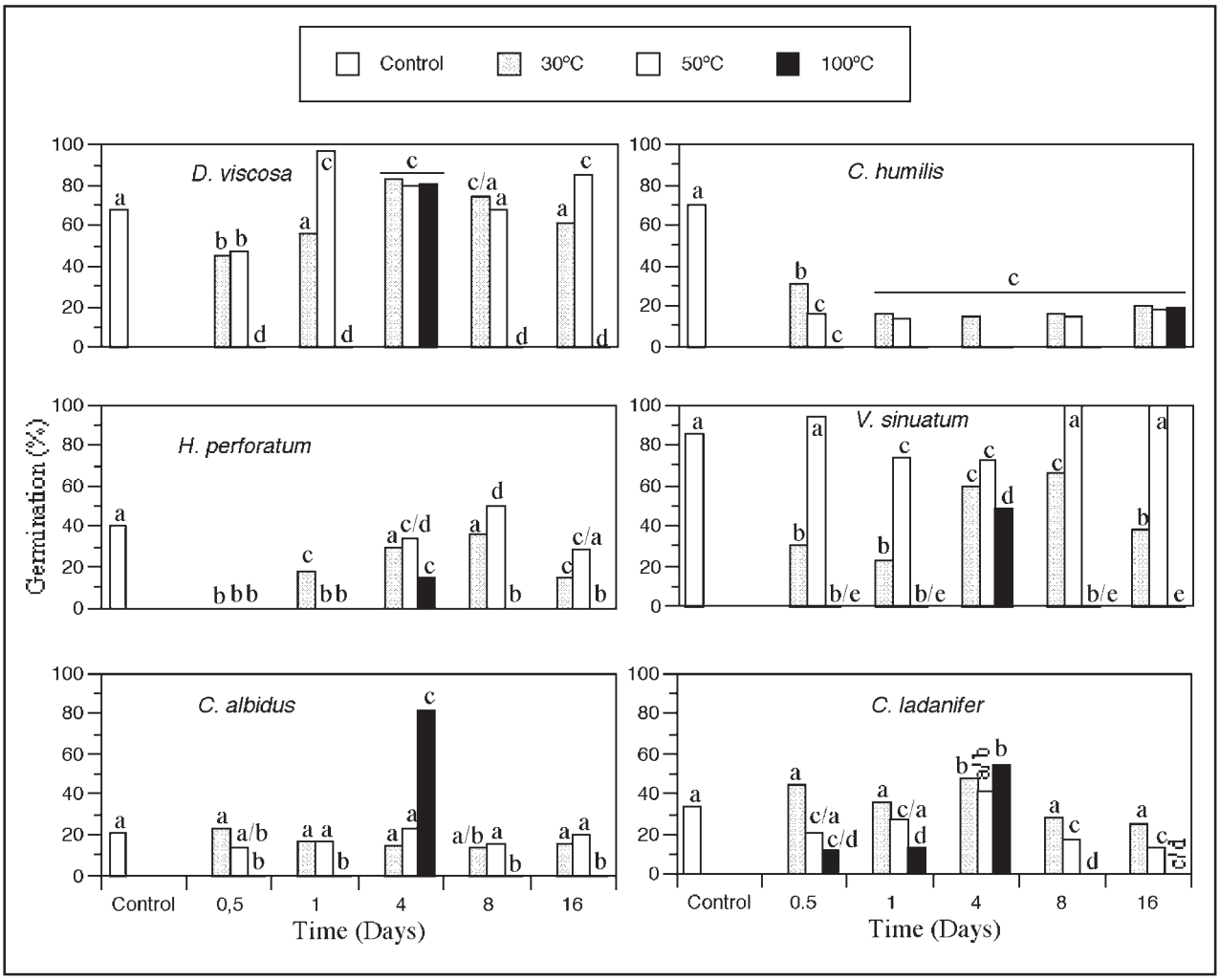

Figure3. Germination percentage of various species subjected to dry heat treatment (30^{0}C, 50^{0}C and 100 ^{0}C) for 0.5, 1, 4, 8, and 16 days. Missing values correspond to 0% germination. Letters on top of the columns represent significant differences between and within treatments, including the control, after a multiple range test performed after the ANOVA.

Table 3. Mean number of days required for the onset of germination of six species subjected to two treatments, imbibition in soil and dried at 30°C, 50°C, and 100°C for 0.5, 1, 4, 8 and 16 days, and dry heat treatment at the same temperatures and for similar periods of time. (C = control).

				30º					50º					100º		
SOAKED	**C**	**0.5**	**1**	**4**	**8**	**16**	**0.5**	**1**	**4**	**8**	**16**	**0.5**	**1**	**4**	**8**	**16**
Verbascum sinuatum	3^a	16^b	21.5^b	12^b	18^b	19^b	—^{b}	—^{b}	—^{b}	12^c	12.3^c	—^{b}	—^{b}	—^{b}	—^{b}	—^{b}
Hypericum perforatum	7.5^a	—^{b}	—^{b}	—^{b}	—^{b}	—^{b}	—^{b}	—^{b}	—^{b}	—^{b}	—^{b}	—^{b}	—^{b}	25.5^c	—^{b}	—^{b}
Dittrichia viscosa	2.5^a	3.2^a	3.5^a	5.7^b	6^b	6.5^b	$11.2^{a/b}$	$8.2^{a/b}$	6^b	11.7^b	16^b	25.5^c	—^{b}	7^b	—^{b}	—^{b}
Cinara humilis	5^a	$6.6^{b/c}$	7.3^c	4^a	9.3^d	$6^{b/a}$	—^{b}	—^{b}	—^{b}	—^{b}	—^{b}	Ø	Ø	Ø	Ø	Ø
Cistus albidus	3.5^a	4^a	6.2^a	—^{b}	6.3^a	9^a	14^b	25^c	—^{d}	—^{d}	—^{d}	—^{b}	—^{b}	13^a	—^{b}	—^{b}
Cistus ladanifer	2.7^a	$5.5^{a/b}$	7^a	$4^{a/b}$	2.2^a	13.7^b	14.7^b	2.5^a	—^{c}	—^{c}	23^d	—^{b}	—^{b}	12.7^c	—^{b}	—^{b}
NON-SOAKED	**C**	**0.5**	**1**	**4**	**8**	**16**	**0.5**	**1**	**4**	**8**	**16**	**0.5**	**1**	**4**	**8**	**16**
Verbascum sinuatum	3^a	10^b	7.7^a	8.2^a	7.7^a	5.7^a	7^a	7.5^a	8.5^a	7.5^a	11^b	—^{b}	—^{b}	12.7^c	—^{b}	—^{b}
Hypericum perforatum	7.5^a	—^{b}	14.2^c	$10.3^{a/c}$	13.5^c	22^c	—^{b}	—^{b}	23^c	17.2^c	15.7^c	—^{b}	—^{b}	19.6^c	—^{b}	—^{b}
Dittrichia viscosa	2.5^a	4.5^b	5^b	5^b	4.5^b	4^b	$6.7^{b/c}$	10^c	4^b	7.5^c	7^b	—^{b}	—^{b}	16.5^c	—^{b}	—^{b}
Cinara humilis	5^a	4^a	6^a	19^b	8^a	8^a	—^{b}	—^{b}	—^{b}	—^{b}	11^b	Ø	Ø	Ø	Ø	Ø
Cistus albidus	3.5^a	5^a	2^a	6^a	3^a	12^b	6^a	18.5^b	4^a	6^a	16^b	—^{b}	—^{b}	9.5^c	—^{b}	—^{b}
Cistus ladanifer	2.7^a	6^b	$4.5^{a/b}$	6.7^b	6^b	6.5^b	16.5^b	22^b	24.3^b	5.6^a	10^b	19^b	21^b	6.5^a	—^{c}	—^{c}

Table 4. Days for the onset of germination under control conditions and under four different nutrient solutions at five concentrations (mM). Different letters in the same row indicate significant differences between treatments. (C = control).

		$NaNO_2$					KNO_3					NH_4Cl					NH_4NO_3				
	C	1	10	25	50	100	1	10	25	50	100	1	10	25	50	100	1	10	25	50	100
Verbascum sinuatum	3^a	3^a	3.5^a	5.5^a	18^b	30^b	4^b	3^a	3.5^a	3^a	3.5^a	3.5^a	3.5^a	5^b	5.5^b	4^a	3.5^a	3.5^a	3.5^a	18^b	30^b
Hypericum perforatum	7.5^a	16^b	7.5^a	17^b	30^c	30^c	7^a	15^b	10^b	15^b	27^c	12.5^a	15^b	11.5^a	16.5^b	30^c	11.5^a	12^a	7.5^a	12^a	13^a
Dittrichia viscosa	2.5^a	3^a	3^a	4.75^a	7.2^b	25.75^c	3.25^a	3^a	3^a	3^a	3^a	2.7^a	3^a	2.7^a	3.75^b	6.5^c	3^a	3^a	4.5^a	7.5^b	8.5^b
Cistus albidus	4^a	5^a	5^a	30^b	30^b	30^b	30^b	7^a	4^a	11^a	6^a	3^a	6^a	5^a	4^a	17.5^b	3^a	6^a	5^a	3^a	8^a
Cistus ladanifer	3.75^a	2^a	3.5^a	4.25^a	6.25^b	30^b	2^a	4.75^a	3.25^a	6.25^a	18.25^a	3.25^a	2^a	5^b	5^b	4^a	2.5^a	3^a	4.75^b	4.5^a	4.5^a

required for its onset (Table 4). Table 5 includes *p* vales after significant ANOVA. The fastest germination was for *D. viscosa* which needed only an average overall number of 5.2 days to initiate the germination under the nitrogenous treatments. This species was followed by *C. ladanifer* and *V. sinuatum,* species that needed an average of 6 and 7.5 days, respectively to start germination respectively. There was a lag in the onset of germination in *C. albidus* and *H. perforatum,* with values that were always greater than those achieved with the control treatment (11 and 15.8 days respectively).

Table 5. p values after ANOVA comparing the number of days needed to start germination by seeds in the control and seeds germinated in four different solutions.

	$NaNO_2$	KNO_3	NH_4Cl	NH_4NO_3
Verbascum sinuatum	0.0346	0.0498	0.0498	0.0173
Hypericum perforatum	0.0306	0.0089	0.0105	0.7035
Dittrichia viscosa	0.0001	0.3663	0.0001	0.0001
Cistus albidus	0.0001	0.0058	0.0493	0.5995
Cistus ladanifer	0.0001	0.0082	0.0004	0.0358

d) Combined effects of nitrogenous compounds and darkness

The seeds of only three species germinated in darkness: *D. viscosa, C. albidus*, and *C. ladanifer* (Figure 5). In none of the cases, however, did the final germination percentages surpass the percentages attained in the trial under alternating light and darkness. The statistical comparison between the final percentages in light and darkness showed highly significant differences ($p< 0.00001$; $F = 1.6332$). The most noticeable feature of these results is that in none of the three species did the control seeds germinate. The application of nitrogenous compounds in darkness did activate germination, while darkness alone contributed to seed dormancy.

Discussion

The effect of temperature treatments on the pre-imbibed seed germination was not linear. The combined effect of heat and length of its application may have several explanations. Rapid decline of the water potential in the soil during the drying treatments would explain the fall in the final germination percentages of three species, *Dittrichia viscosa, Cistus albidus*, and *Cistus ladanifer*. The decline in water potential is detrimental in itself, and it also increases the rate of water loss from seeds. This loss of water in the seed then acts inhibiting germination since it is accompanied by deterioration of the cell structures (Hegarty and Ross, 1980; Thompson et al. 1997). It has occasionally been observed that, even in wet media, the conditions at the surface are more severe for germination than at a few centimetres depth, due to the moisture fluctuations at the soil/air interface which result in unfavourable conditions for germination (Evans et al., 1967; Mott, 1974). In the case of the species which germinate in the controls but not in soil, the present case is similar to that described by Harper and Benton (1966) for *Helichrysum cassinianum*. The germination of this species is favoured over other species since it can modify the tension at the seed/soil interface thanks to the presence of hairs (Mott, 1974). This is also the case for the seeds of *D. viscosa*. Such pattern of behaviour, however, was not followed by *C. humilis*, an Asteraceae with villi. This is probably because the villous coat of the seeds had been removed, so that it was not available to act as a water-retaining agent.

Germination percentages of the controls of *Cistus albidus* were very low, most likely because the seeds had not attained their physiological (Vleeshouwers et al., 1995) and/or environmental requirements for germination. These requirements are different from the combinations of light and temperature in the incubator in which the seeds were kept for germination after the different treatments.

The differences observed in the germination of the seeds treated thermally without prior imbibition indicate that this treatment induces the germination of the Cistaceae seeds, especially *Cistus ladanifer*, as has been reported for other species of this genus (González-Rabanal and Casal, 1995; Pugnaire and Lozano, 1997). Likewise, the 50°C dry heat treatment applied for any of the lengths of time activated *Dittrichia viscosa, Hypericum perforatum,* and *Verbascum sinuatum* germination. Only a low percentage of germination was achieved by pre-imbibed seeds of *V. sinuatum*. Nevertheless, that some germination was achieved and that this increased with decreasing soil moisture content, together with the final germination percentages reached in the dry heat treatments without imbibition, are indications of coherence in the present results. Heat treatments activate germination in this species, proving that germination after imbibition in the soil is the result of the loss of water and subsequent baking of the soil, which is what induces germination rather than any relationship with the moisture content of the soil.

The application of nitrogenous compounds led to high germination percentages in the six species. The response

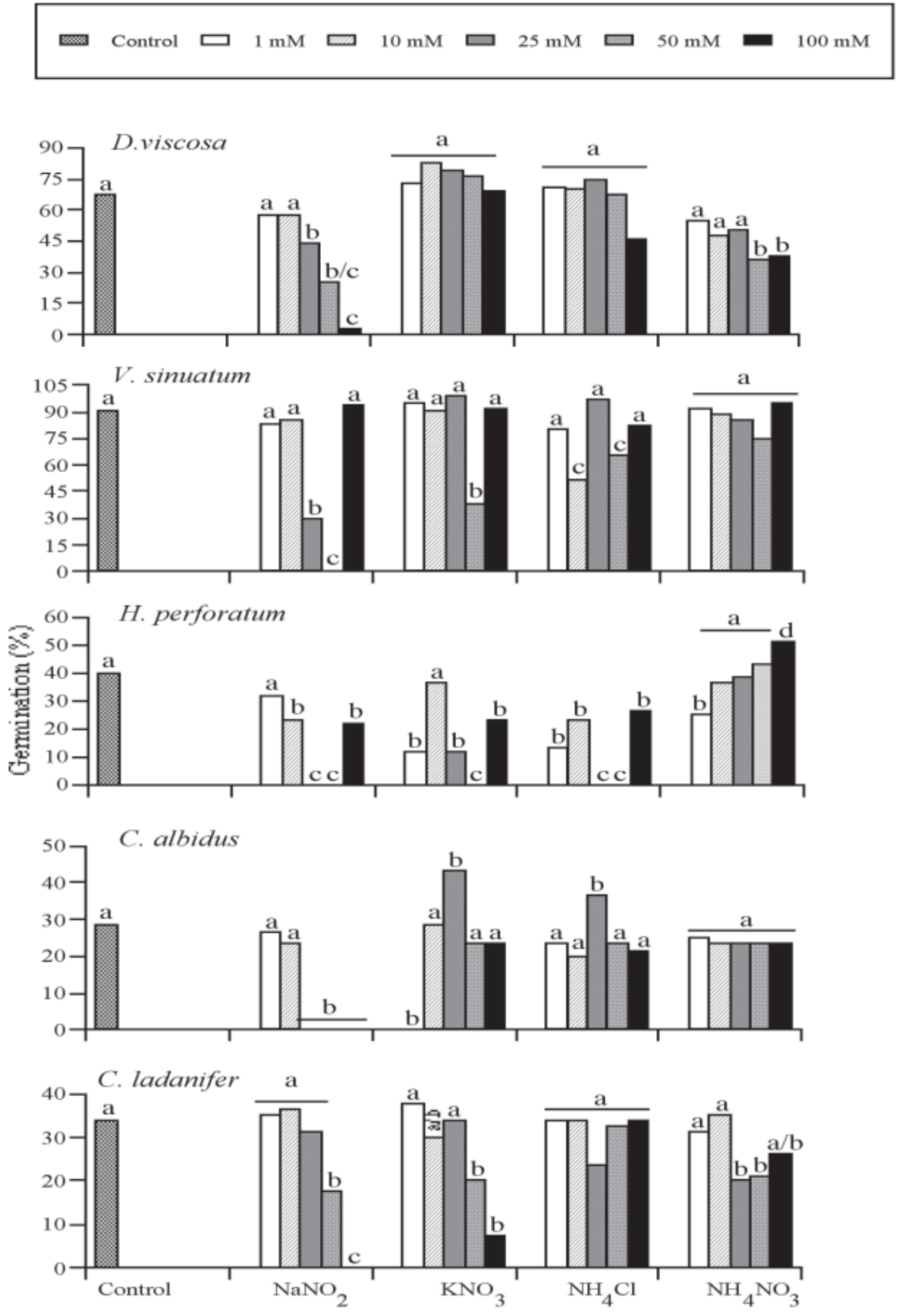

Figure 4. Final germination percentages of the five species after application of $NaNO_2$, KNO_3, NH_4Cl, and NH_4NO_3 at concentrations of 1 mM, 10 mM, 25 mM, 50 mM, and 100 mM. Missing values not shown are 0% germination. Letters on top of the columns represent significant differences between the control and the five concentrations of each treatments after a multiple range test performed after the ANOVA.

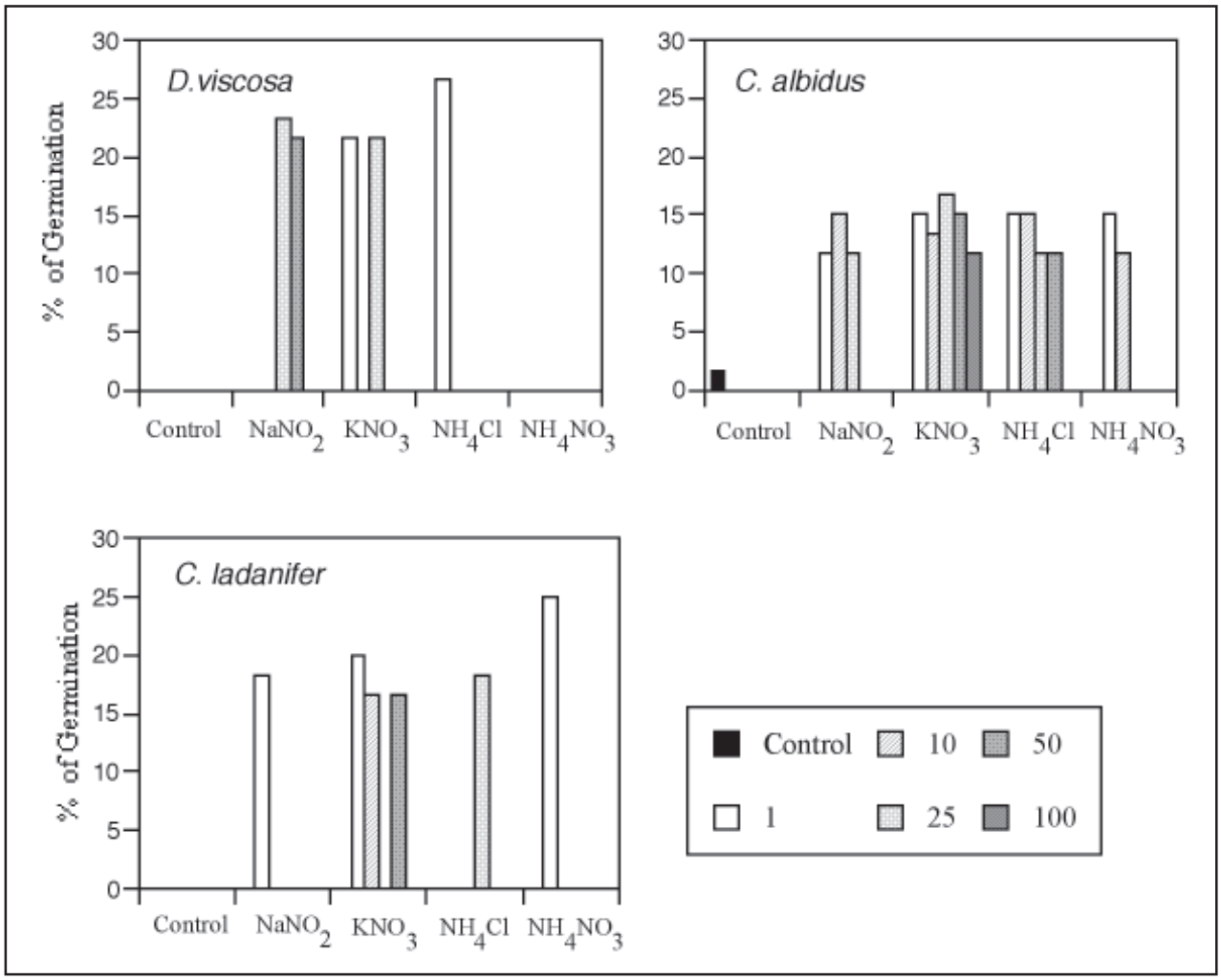

Figure 5. Germination percentages of the species germinated in darkness as influenced by 1, 10, 25, 50 and 100 mM nitrogen solutions.

of *Dittrichia viscosa* and *Verbascum sinuatum* was to considerably reduce the number of days required to initiate germination. Fast germination confers to these species an advantage in colonising gaps in the vegetation as it has been seen in the field (personal observation). High concentrations of NH_4NO_3 clearly breaks dormancy of *Hypericum perforatum*. Breaking of dormancy by ammonium nitrate can operate as a gap detection mechanism for this species. This mechanism has been observed in annual species that can detect potentially competing plants (low levels of nitrogen in the soil) and inhibit germination until these plants disappear (increase in the levels of nitrogen in the soil) (Bewley and Black, 1982; Pons, 1989).

The two Cistaceae, showed nitrogen-regulated germination (very evident in *Cistus albidus*), with a reduced and delayed germination at increasing fertiliser concentrations. The mechanism by which the seed detects variable concentrations of nutrients in the soil might be related to the pH of the medium. In the field, *Cistus ladanifer* and *C. albidus* grow on highly acidic soils. The addition of the solutions employed here, although they were slightly acidic, tended to raise the pH. Therefore, as postulated by Mustar and Cowling (1993), the actual distribution of plant species may have as one of its basic explanations the relationship with the characteristics of the substrate housing the seeds at the moment they begin to germinate. Thus an excessive rise in pH would induce a decrease in germination. On the other hand, as suggested by Pons (1989), the nutrient balance provides the ideal conditions that the seeds must identify as being suitable or not to ensure the establishment of plants, so that the availability of nutrients in the medium results in an increased germination. With this in mind, together with the present results, one can state that in *C. albidus* and *C. ladanifer* there is regulation of germination by the presence of nitrogen, whether in the form of nitrate or ammonium ion. This regulation may act as a mechanism for the detection of inhospitable conditions. This adds nitrogen to the environmental factors that seeds use in detecting potential competition from previously established plants (Tilman, 1980; Thompson and Grime, 1983). Also, for the specific case of these five species, as is common in other herbs of Mediterranean-type environments (Boojh and Ramakrishnan, 1982; Juan et al., 1995), there is also the necessity for light, since its elimination led to an inhibition of germination which was total in the particular cases of *Verbascum sinuatum* and *Hypericum perforatum*.

In view of these results, one may picture a possible field situation, which indeed coincides with direct observations in the ecosystems in which the selected

species are found. In zones unaffected by any kind of perturbation, there is a homogeneous spatial distribution of water, which provides the conditions needed for the germination and establishment of species. Thus, when the temperatures at the level of the soil are not very high, there occurs a very rapid germination and growth of species that have few pre-requisites for their germination. That is the case observed in the controls and the heat-treated seeds where they had sufficient moisture supply. *D. viscosa, C. humilis* and *V. sinuatum* act as first colonisers, creating softer environments and safe sites for the germination and development of herbaceous species. Annuals find under the canopy of these three perennials shelter against excessive light.

As temperatures rise during the summer season, available water in soil declines; thus only the species with particular anatomical characteristics suited for retaining water will be able to germinate. In the likely event of a fire (or there being a prolonged and high degree of baking of the soil), species in the Cistaceae will be the first to germinate and will do so in greater proportions, since they best resist both drought and heat stress. The Cistaceae Family is one of the best-represented botanical families in the ecosystems of the central-west of the Iberian Peninsula. The capability of their seeds to germinate after a heat shock ensures the presence of vegetation when no other species can colonise perturbed soils or soils with very little humidity. One of the roles of the *Cistus* species in the studied ecosystems is to guarantee the permanence of plant communities under stressful conditions of high temperatures and extreme drought.

When the vegetation is abundant, there is little nitrogen available in the soil. Adult plants in community use the nitrogen, depleting it. The dense vegetation also reduces the amount of light reaching the substrate. Thus, reduced nitrogen and diminished light, impede the germination of the species studied. When the above-ground biomass is removed, the available nitrogen in the soil rises together with the amount of light reaching the soil. Both of these are factors required for germination. In the event of a fire, it eliminates of the above-ground biomass, and simultaneously adds ashes to the soil, contributing to increasing the nitrogen content and soil pH. Under these circumstances, practically all of the species we studied will germinate. The isolated or combined effects of a fire, such as the increase of soil temperature, the acceleration of the loss of soil moisture, and the rise in nitrogen concentration in the soil, would provoke a rapid germination of all of the species. Presumably, however, such effects would be more promotive in *Cistus albidus, Hypericum perforatum*, and *Verbascum sinuatum*, since these are the species that have most constraints on the germination of their seeds.

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