Effects of nitrogen, light and wet and dry heat treatments, on the germination of six perennial plant species from western Spain

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Summary 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 30°C, 50°C and 100°C 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. The effect of nitrogen on germination was also investigated by applying $NaNO_2$, NH_4NO_3, KNO_3 and NH_4Cl at five increasing concentrations (1, 10, 50 and 100mM). Temperatures of 30°C were the most successful for germination of pre-imbibed seeds. *Dittrichia viscosa*, *Cistus albidus* and *C. ladanifer* increased germination under the wet treatment. Germination was higher after treatment at 30°C and 50°C that at 100°C for non-imbibed 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 100mM increased both level and rate of germination in most species. Continuous darkness inhibited germination in all the species. The increase in 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.
Keywords Germination, imbibition, desiccation, temperature, nitrogen, light and dark.

INTRODUCTION

One of many factors determining the colonising and competitive capacity of a given plant species is the ability to germinate when the environmental conditions are suitable and guarantee the development of the young plant, or of delaying germination when the environmental conditions are adverse (Angevine and Chavot 1979). Probably 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 (Derkx and Karssen 1993), and the presence in the soil of chemical compounds which act as limiting factors (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, suitable temperatures and hours of daylight all coincide (El-Sharkawi *et al*. 1989). In these enclaves there are recurrent fires of variable intensity, producing ash which temporarily raises the nutrient content of the surface soil. Fire also creates gaps in the vegetation which can be settled by new vegetation. However the relationship between fire intensity and the patterns of seedling establishment is not clear.

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 (Thompson *et al*. 1997), since an increase in the latter contributes to a decline in the soil's water potential and reduces the possibility of water uptake and seed imbibition.

In the present work, we describe the results of two experiments on the effects on germination of applying heat treatments to dry and imbibed seeds, and of applying nitrogenous compounds to intact seeds of five representative species of a woodland of Central-Western Spain. All of them are considered weeds in most fields in the Iberian Peninsula.

MATERIALS AND METHODS

Plant material Selected species were among the most representative and abundant in a typical Iberian 'dehesa' woodland: *Verbascum sinuatum* L. (Scrophulariaceae), *Hypericum perforatum* L. (Hypericaceae), *Dittrichia viscosa* (L.) Greuter (Asteraceae), *Cynara humilis* L. (Asteraceae), *Cistus ladanifer* and *C. albidus* (Cistaceae). Mature seeds were gathered at the end of the summer in 2001 and stored in opaque envelopes for two months at 12±1°C to provide a post-harvest ripening thus eliminating innate dormancy.

Wet heat treatment Seeds of each species were mixed and covered with 50 g of soil in 10 cm Petri dishes. The soil was then watered with 20 mL water and the seeds were left to imbibe for 2 hours at room temperature, when, the water was drained off. The

dishes were at then placed in forced air ovens at 30, 50, 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 soil's field capacity and transferred to a greenhouse with mean maximum and minimum temperatures of 32.6°C and 11.3°C respectively, and lighting periods of 14 h light and 10 h dark.

Dry heat treatment Unimbibed seeds were treated at the same temperatures and times, with the same replication, as described above. The seeds were placed on sterile cotton in Petri dishes and kept for 35 days in an incubator at 22/18°C during light and dark periods respectively. As controls, 80 untreated seeds of each species were sown in four replicates of 20 seeds per dish and incubated also for 35 days under the same conditions.

Application of nitrogenous compounds The effect of nitrogen on germination was investigated by applying $NaNO_2$, NH_4NO_3, KNO_3, and NH_4Cl at a series of five concentrations viz 1, 10, 25, 50 and 100mM. For each combination of concentration and nitrogenous solution, 80 seeds per species in replicates of 20 were sown in 10 cm Petri dishes on sterile cotton as inert substrate and incubated under the same conditions as above.

The combined effect of light and darkness and fertiliser on germination was tested by incubating the seeds in continuous darkness after having watered them with the nitrogenous compounds. Germination counts were made weekly and under green light (Hou and Simpson 1993). Due to seed shortages, *Cynara humilis* was not tested in these two experiments.

Data on percentage germination were analysed by one or two-way analysis of variance using StatView® (1992–98 SAS Institute Inc.). When needed, arcsine transformations were performed to improve normality.

RESULTS

Germination after imbibition in soil 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 (Figure 1). In general, heating at 50°C and 100°C reduced seed germination relative to the unheated controls. *Cistus albidus*, *C. 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 nil, with seed germination only in the 30°C for one day and 100°C for four days treatments. Germination of treated *Cynara humilis* and *Verbascum sinuatum* seed was always lower than the controls. The combinations of time and temperature, which led to the greatest germination percentages in *D. viscosa*, *C. albidus* and *C. ladanifer*, were 30°C for practically any period of time. Optimal germination occurred at 50 and 100°C for four days in both *Cistus* species. Statistical differences in final percentages of germination were detected between the control (non-treated seeds) and dry and wet heated seeds.

Dry heat treatment 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. In *Dittrichia viscosa*, *Hypericum perforatum*, and *Verbascum sinuatum* the greatest germination percentages were attained in seeds treated at 30 and 50°C independent of time (Figure 3). Cistaceae seeds reached their optimal germination, which was far greater than for the controls, at 100°C applied for four days.

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

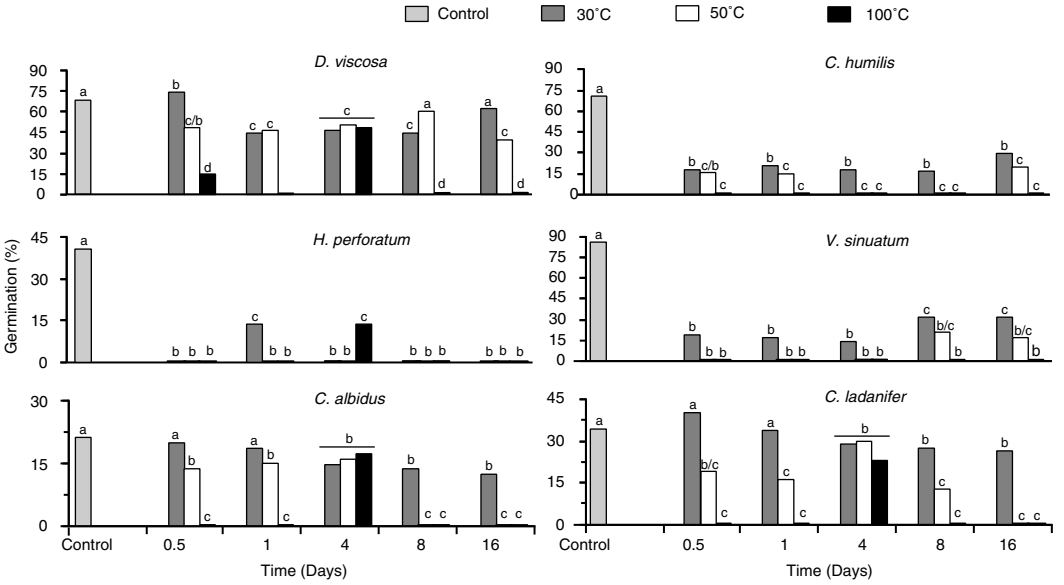

Figure 1. Germination percentages of six species subjected to imbibition in soil and dried at 30, 50 and 100°C for 0.5, 1, 4, 8 and 16 days. Control = not dried. Missing values not shown are 0% germination. Letters on top of the columns indicate significant differences after a multiple range test, after ANOVA.

Table 1. P values after ANOVA comparing the final percentages of germination of seeds germinated with the control and with four nitrogenous compounds.

Species	$NaNO_2$	KNO_3	NH_4Cl	NH_4NO_3
V. sinuatum	0.0346	0.0498	0.0498	0.0173
H. perforatum	0.0306	0.0089	0.0105	0.7035
D. viscosa	0.0001	0.3663	0.0001	0.0001
C. albidus	0.0001	0.0058	0.0493	0.5995
C. ladanifer	0.0001	0.0082	0.0004	0.0358

Combined effects of nitrogenous compounds and darkness The seeds of only three species germinated in darkness: *D. viscosa*, *C. albidus*, and *C. ladanifer*. In none of the cases, however, did the final germination percentages surpass the percentages attained in the trial under alternating light and dark. The statistical comparison between the final percentages in light and dark 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 germination of the seeds that were pre-imbibed and then dried was very low. The decline in water potential is detrimental in itself, but it could also cause rapid loss of the water accumulated in the seed. This loss of water in the seed then acts to inhibit germination since it is accompanied by deterioration of the cell structures (Hegarty and Ross 1980, Thompson *et al*. 1997).

Germination percentages of the controls of *Cistus albidus* were very low. This could be because the seeds had not managed to attain their physiological (Vleeshouwers *et al*. 1995) and/or environmental requirements for germination. Obviously, 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 in germination of the seeds thermally treated without imbibition indicate that this treatment acts inducing the germination of the Cistaceae seeds, especially *Cistus ladanifer*, as has been reported for other species of this genus (Pugnaire and Lozano 1997). Likewise, the 50°C dry heat treatment applied over any of the time periods activated *Dittrichia viscosa*, *Hypericum perforatum*, and *Verbascum sinuatum* germination. There was some germination shown by *V. sinuatum* under the seed imbibition treatment, although the values were much lower than the controls.

Heat treatments activate germination in this species, so that germination after imbibition in the soil is the result of the loss of water and heat shock.

The application of nitrogenous compounds led to high germination percentages in all the species. The differences in germination of *Hypericum perforatum* watered at high concentrations of NH_4NO_3 with respect to the controls, indicates that this salt contributed to the breaking of dormancy. Breaking of dormancy by ammonium nitrate can operate as a gap detection mechanism (Bewley and Black 1982).

In the two Cistaceae, we also observed nitrogen-regulated germination (very evident in *Cistus albidus*), although in most cases the effect of increasing fertiliser concentrations was to reduce and delay germination. 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 in these two species. This adds nitrogen to the environmental factors that seeds may use in detecting potential competition from previously established plants (Tilman 1980). For the specific case of these five species, there is the need for light, since its elimination led to an inhibition of germination which was total for *Verbascum sinuatum* and *Hypericum perforatum*.

In view of these results, one may picture a possible field situation (that coincides with direct observations). 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. When this occurs, and the temperatures at the level of the soil are not very high, there is a very rapid germination and growth of herb species.

As temperatures rise during the summer, there is a considerable decline in available water, and it will be the species which, by reason of their anatomical characteristics, are capable of retaining water that will be able to germinate.

When the vegetation is abundant, the amount of nitrogen in the soil is low, since it is being utilised by the adult individuals making up the community. Also the presence of vegetation reduces the amount of light reaching the substrate. 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.

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