

Seed germination in response to chemicals: Effect of nitrogen and pH in the media

M. A. Pérez-Fernández¹, E. Calvo-Magro¹, J. Montanero-Fernández² and J.A. Oyola-Velasco²

¹Ecology Area, ²Mathematics Department, University of Extremadura, Avenida de Elvas s/n, 06071 Badajoz, Spain

(Received: 24 January, 2005 ; Accepted: 30 July, 2005)

Abstract: Seed germination generally presents a peak in the next growing season after a fire. Among other factors associated with fire are the increase of soil nitrogen and changes in the pH of the soil. In this study, we addressed the question, whether or not the germination response of eight species is linked with the increase in pH and nitrogenous compounds in the germination media? We assessed the separate and combined effects of nitrogenous compounds and pH on the percentage and rate of germination of seeds of *Medicago arabica* (L.) Hudson, *Epilobium hirsutum* L., *Foeniculum vulgare* Miller, *Daucus carota* L., *Thapsia villosa* L., *Cynosurus cristatus* L., *Dactylis glomerata* L. and *Rumex crispus* L.. All these species are well represented in the Mediterranean ecosystems of the central-west Spain. Water and CaCl₂ were used as controls. Nitrogenous compounds increased percent germination (level) and rate in three of the species studied. High pH negatively affected the germination rate of seeds from most species, but had no effect on the per cent germination of any of the species. The higher concentration of the nutritious solutions affected negatively the germination level and rate. The different germination responses of seeds of the studied species could not be exclusively attributed to pH values in the media, whereas the amount and form of Nitrogen in the media has a greater effect on it. These differences in germination are species dependent.

Key words: Nitrogen, Potassium, Calcium, pH, Germination, Herbaceous species

Introduction

It is well known that plants in the Mediterranean-type ecosystems are adapted to the effects of wildfires (Keeley, 1994; Whelan, 1995; Eshel *et al.*, 2000; Goubitz *et al.*, 2003). Survival of plant species after a fire is achieved by re-sprouting, establishing seedlings or both. Thus, for certain species the process of post-fire succession is based on germination during the first winter (Eshel *et al.*, 2000). Seed germination can be affected by several fire-related factors such as high temperature (González-Rabanal and Casal, 1995; Núñez and Calvo, 2000), ash and charred wood (Reyes and Casal, 1998; Pérez-Fernández and Rodríguez-Echeverría, 2003), litter cover (Lamont *et al.*, 1993) or soil water potential (Ne'eman *et al.*, 1999; Pérez-Fernández *et al.*, 2000). Seed dormancy can be broken by nitrate (Thanos and Rundel, 1995; Bungard *et al.*, 1997; Pérez-Fernández and Rodríguez-Echeverría, 2003). The positive effect of nitrogen in breaking seed dormancy has been observed in both soils (Roberts and Benjamin, 1979; Freijssen *et al.*, 1980; Franco-Viacaino and Sosa-Ramírez, 1997) and on filter paper wetted with water of nitrate solutions (Bell *et al.*, 1999; Pérez-Fernández and Rodríguez-Echeverría, 2003). Similarly, pH plays an important role in breaking dormancy and seed germination (Keeley and Fotheringham, 1998; Goubitz *et al.*, 2003).

Whether it is pH or nitrogen which triggers the germination of several species typical of the Mediterranean-type ecosystems has long been a point of debate. Thanos and Rundel (1995) reported that nitrogenous ions stimulated germination in *Emmenanthe penduliflora* and *Phacelia grandiflora* (two post-fire annuals). On the other hand, Keeley and Fotheringham (1998) demonstrated that KNO₃, NO₂⁻ and gibberellin solutions buffered at acidic pH induced germination

in the same species. Pérez-Fernández and Rodríguez-Echeverría (2003) observed enhanced germination of seven annuals and perennials after exogenous application of KNO₃ and NH₄Cl. Goubitz *et al.* (2003) concluded that high pH negatively affects seed germination in *Pinus halepensis*, whereas Killi (2004) proved germination enhancement of *Gossypium* spp. at increased pH.

The assumption that only one mechanism accounts for germination in all species leads to confusion. We consider that there is no general pattern in germination and that both, pH and nitrogen might enhance or inhibit germination singly or in conjunction and that the possible effects can be independent or combined. In this study we investigated the influence of pH and a range of nitrogen- and non-nitrogen containing compounds on the germination characteristics of eight annual species, in order to improve our understanding of how these two factors may act, and interact, to enhance seed germination. To this end, we conducted an experiment in which seeds were germinated in the presence of two forms of nitrogen (KNO₃ and NH₄NO₃) at two concentrations, all adjusted at four values of pH. As controls we used distilled water and a solution of CaCl₂ at the two concentrations, and distilled water adjusted at the same values of pH.

Materials and Methods

Species: Seeds of *Medicago arabica* (L.) Hudson, *Epilobium hirsutum* L., *Foeniculum vulgare* Miller, *Daucus carota* L., *Thapsia villosa* L., *Cynosurus cristatus* L., *Dactylis glomerata* L. and *Rumex crispus* L. were hand-harvested in natural populations of west Spain. All species but *T. villosa* and *E. hirsutum* grew in a *Quercus rotundifolia* Lam. woodland (39°10'N, 7°00'W, 240 meters above sea level). *T. villosa* was

Table – 1: Anatomic and ecological attributes of the studied species. Nomenclature follows Devesa (1995).

Species	Family	Habitat	Seed length (mm)	Seed width (mm)	Seed mass (mg)	Appendages
<i>Medicago arabica</i> (L.) Hudson	Fabaceae	Wet pasturelands	2.75	1.68	3.29	No
<i>Epilobium hirsutum</i> L.	Onagraceae	Hygro-nitrofilous	9.94	4.47	0.14	Hairs
<i>Foeniculum vulgare</i> Miller	Apiaceae	Uncultivated land	4.57	1.68	3.05	No
<i>Daucus carota</i> L.	Apiaceae	Ruderal	4.68	4.09	0.76	Short hairs
<i>Thapsia villosa</i> L.	Apiaceae	Country roads	10.31	7.62	8.29	No
<i>Cynosurus cristatus</i> L.	Poaceae	Wet pasturelands	4.24	0.69	0.03	Hairs
<i>Dactylis glomerata</i> L.	Poaceae	Pasturelands rich in N	3.75	0.61	0.39	No
<i>Rumex crispus</i> L.	Polygonaceae	Pasturelands rich in N	4.18	3.60	3.17	Membranes

collected from a degraded forest of *Quercus pyrenaica* Willd. (42°23'60"N, 5°37'60"W) whereas *E. hirsutum* was collected next to a creek (42°21'16"N, 2°3'40"W). Seeds were collected at the end of the summer of 2003, coinciding with the dispersal period of these species (Table 1) and bulk stored in paper bags at room temperature. Seeds of Asteraceae and Cistaceae were dry-stored under laboratory conditions at 18 ±1°C and 20% relative humidity to ensure the post-harvesting ripening needed prior to germination.

Nitrogen salts treatment: Solutions were made daily in freshly purified water. Nitrogenous compounds were applied as aqueous solutions of KNO₃ and NH₄NO₃, each at concentrations of 25 mM and 50 mM. Four replicates each with 20 seeds were prepared for each treatment. Solutions were adjusted to pH of 4.7, 5.7, 6.7 and 7.7 with HCl or NaOH. The control treatments were performed using untreated seeds of each species (four Petri dishes per species, with 20 seeds each) that were incubated on cotton disks watered only with distilled water and an aqueous solution of CaCl₂ adjusted to each of the selected values of pH. The aqueous solution of CaCl₂ was applied at the same concentrations as the nitrogenous compounds (25 and 50 mM).

Germination experiments: Germination experiments were conducted in plastic Petri dishes of 9 cm diameter, lined with sterile cotton disks (Pérez-Fernández and Rodríguez-Echeverría, 2003; Killi, 2004) and moistened with 10 mL of distilled water or the appropriate salt solution, according to the trial. All experiments were carried out in an incubator equipped with cool-white fluorescent tubes under a thermoperiod and photoperiod of 10 hrs at 18±1°C in darkness and 14 hrs at 24±1°C in light. These experimental conditions were chosen to emulate the environmental thermo- and photo-periods that seed would experience on the soil surface, in the germination season after forest fires are most likely to occur (García-Fayos et al., 2001).

Germination was expressed as a percentage from four replicates of 20 seeds each. Criterion of germination was the visible protrusion of the radicle (Thanos and Rundel, 1995; Cochran et al. 1999). Germination was recorded daily over a period of 32 days, and germinated seeds were removed

promptly. At the end of the period, non-germinated seeds were individually dissected to check for their viability (embryo and endosperm intact and not discoloured) (Cochrane et al., 1999; ISTA, 2003). The final results were adjusted to account for non-viable seeds. Any loss of moisture during the experiments was replaced with deionized water. The germination rate was determined using a modification of Timson's index (Khan and Rizvi, 1994) of germination velocity.

$$\text{Germination velocity} = \sum G/t$$

where G is the percentage of 2-day-interval germinated seeds and t is the total germination period. The greater the index value, the higher the germination rate.

Data analyses: Nested ANOVA was used to test for differences in level (total by 32 d) and rate (time to 50% of final) of germination between nutritious compounds, concentrations and pH values, for each individual species. Arcsine transformations were performed on the percentage germination data prior to analysis. Where ANOVA showed significant effects at 5% (p<0.05), Fisher's least significant differences (Lyman, 1998) were determined. Normality and homocedasticity of data were tested by means of tests given by David et al. (1954) and Cochran (1941), respectively. Overall comparative analyses on germination for all the species under different nutritious salts and pH values were performed by means of correspondence analyses.

Results

Results on the average germination of the eight species, considering independently the effects of nutritious salts, salts concentrations and pH are presented in Fig. 1. The overall effect of exogenous application of nitrogen and calcium was negative for germination (Fig. 1a). Similarly, pH had no effect in the final level of germination of the eight species (Fig 1c). However, intra and inter-specific differences in the level of germination were detected for both salt and concentration treatments and pH (Figs. 1b and 2). *C. cristatus*, *M. arabica*, *E. hirsutum* and *D. glomerata*, were the species that presented a higher inherent germination.

Nested ANOVA results indicate that the germination percentages of the eight species were significantly different depending on the nutritious solution applied (Table 2).

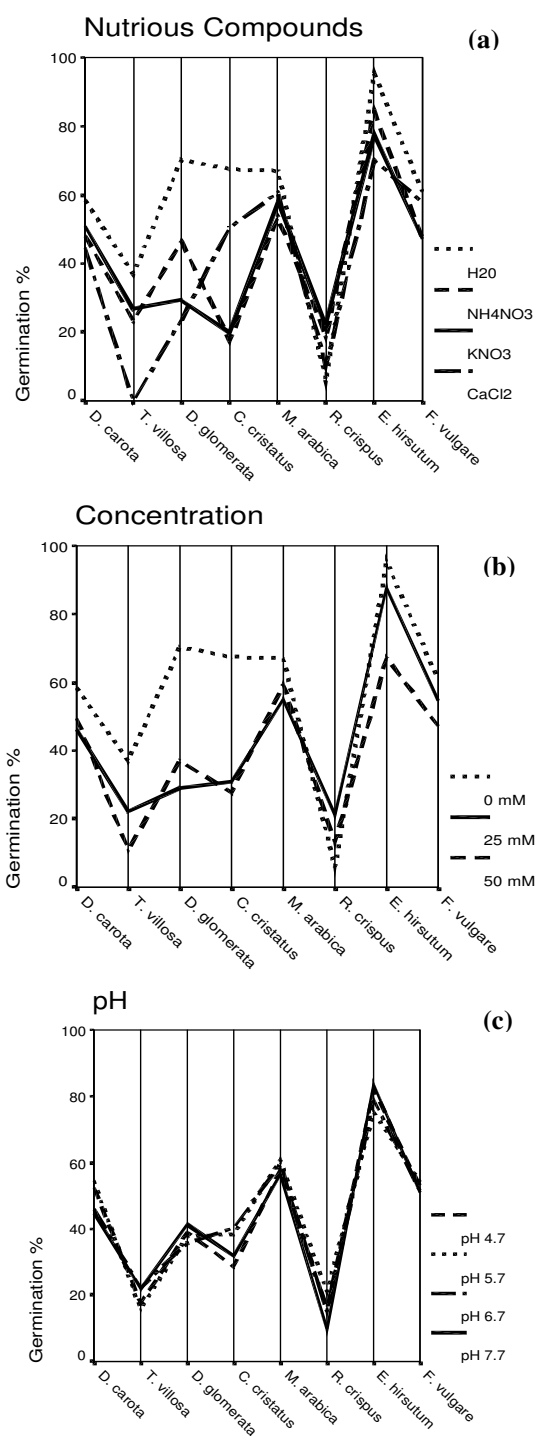


Fig 1: Overall proportions of germination of the eight studied species treated with three nutritious solutions (NH₄NO₃, KNO₃ and CaCl₂) at two concentrations (25 and 50 mM) and adjusted at four values of pH (4.7, 5.7, 6.7 and 7.7). (a) patterns of germination considering only the nutritious solutions; (b) patterns of germination considering only the concentrations of the nutritious solutions; and (c) patterns of germination considering only the pH of the nutritious solutions.

Exogenous application of NH₄NO₃ at concentration 25 mM significantly enhanced germination of *E. hirsutum* at pH of 4.7 and *R. crispus* at pH of 5.7 (Fig. 2). Similarly, this salt at 50 mM significantly increased germination of *D. carota* at pH of 5.7 and germination of *R. crispus* at pH of 6.7. Potassium nitrate (KNO₃) only at a concentration of 50 mM and adjusted at pH of 6.7 enhanced germination of *M. arabica*, *F. vulgare*, *T. villosa* and *E. hirsutum*. The same salt adjusted at pH of 5.7 enhanced germination of *D. carota*. Application of CaCl₂ had a clear inhibiting effect of germination in seven of the eight species, regardless the pH at which it was adjusted. The exception was *M. arabica*.

The correspondence analysis (Fig. 3) for the eight species treated with the three nutritious solutions at the two concentrations shows three different responses in germination. The control treatment differs from that of the nutritious salts, conforming the first group. The nitrogenous compounds present a similar germinative profile in the eight studied species thus conforming a second group. Finally, CaCl₂ at 25 and 50 mM, induces another germination profile, different of that induced by water and nitrogenous compounds. These patterns are validated by a significant Chi-squared and by the fact that the two principal axes of the analysis explain the 76.9% of total inertia (Table 3).

There were significant differences in the rate of germination for each species (Fig. 4). In most cases, these differences were due to a retarding effect in germination due to the exogenous application of nutritious salts. The exogenous application of NH₄NO₃ resulted in a significant negative effect in *M. arabica*, *E. hirsutum*, *C. cristatus* and *D. glomerata* at any combination of concentration and pH and had almost no effect in the remaining species (Fig. 4), where columns for the Timson's indexes are lower for this salt than for those in the control. Potassium nitrate significantly retarded germination of *E. hirsutum*, *D. glomerata* and *C. cristatus* at any combination of concentration and pH. The effect of this salt in the remaining species varied according to the combination of concentration and pH, the effect being negative or similar to that of the control treatments.

Only germination in *R. crispus* was accelerated due to the application of most combinations of salts, concentrations and pH. The exceptions were NH₄NO₃ and KNO₃ 50 mM at pH of 4.7 and 5.7 and any combination of pH and concentration of CaCl₂.

Discussion

Our results showed that pH does not have any effect on the germination level and rate of the eight species studied. Even though differences in level and rate of germination were not constant in all the species, germination responses were associated with combined nutritious compounds, their concentration and pH.

After a fire, the accumulation of charred wood and ash layer cause increase in pH of the topsoil (Goutbitz *et al.*, 2003). Low values of pH can positively affect germination of annuals

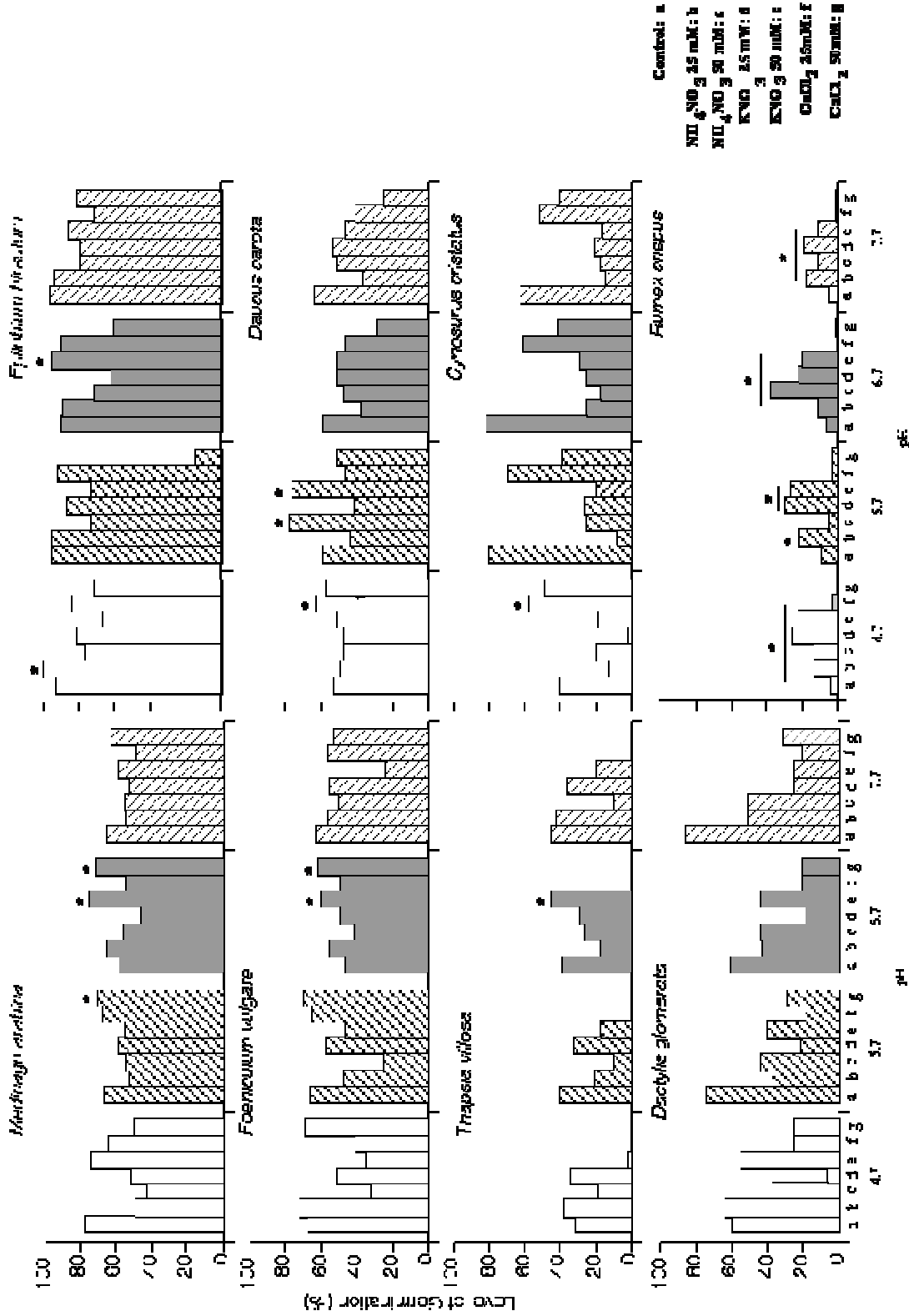


Fig. 2: Final percentages of germination of *M. arvensis*, *E. hirsutum*, *F. vulgare*, *D. carota*, *T. villosa*, *C. cristatus*, *D. glomerata* and *R. crispus* (after 32 days of incubation) treated with three nutritious solutions (NH₄NO₃, KNO₃ and CaCl₂) at two concentrations (25 and 50 mM) and adjusted at four values of pH (4.7, 5.7, 6.7 and 7.7). Asterisks on top of columns indicate significant positive differences (Fisher's P ≤ 0.05) between treatment and control at a similar value of pH of the nutritious solution. Control consisted in distilled water adjusted at the same pH values.

Table – 2: Results of the nested ANOVAs comparing level of germination of the eight species treated with three nutritious compounds (NH_4NO_3 , KNO_3 and CaCl_2) at two concentrations (25 and 50 mM) adjusted at four pH values. (4.7, 5.7, 6.7 and 7.7).

Species	Variable	g.l.	F	p
<i>Medicago arabica</i>	Salt	3	3.432	0.0200
	PH	3	0.468	0.7051
	Concentration (pH)	7	1.374	0.2250
	Residual	98		
<i>Epilobium hirsutum</i>	Salt	3	7.440	0.0002
	PH	3	0.663	0.5952
	Concentration (pH)	7	7.049	0.0001
	Residual	98		
<i>Foeniculum vulgare</i>	Salt	3	4.105	0.0086
	PH	3	0.161	0.9220
	Concentration (pH)	7	1.667	0.1260
	Residual	98		
<i>Daucus carota</i>	Salt	3	4.210	0.0076
	PH	3	4.026	0.0095
	Concentration (pH)	7	3.308	0.0033
	Residual	98		
<i>Thapsia villosa</i>	Salt	3	42.284	0.0001
	PH	3	1.175	0.3232
	Concentration (pH)	7	3.622	0.0016
	Residual	98		
<i>Cynosurus cristatus</i>	Salt	3	66.756	0.0001
	PH	3	3.954	0.0104
	Concentration (pH)	7	2.647	0.0150
	Residual	98		
<i>Dactylis glomerata</i>	Salt	3	31.265	0.0001
	PH	3	0.498	0.6846
	Concentration (pH)	7	1.542	0.1622
	Residual	98		
<i>Rumex crispus</i>	Salt	3	33.645	0.0001
	PH	3	1.541	0.2087
	Concentration (pH)	7	1.605	0.1430
	Residual	98		

Table – 3: Results of the analyses of correspondences for all the species considering treatments with nutritious compounds.

Dimension	Inertia	d.f.	χ^2	Proportion of Inertia	
				p	Explained Accumulated
1	0.048			0.542	0.542
2	0.020			0.227	0.769
3	0.010			0.115	0.884
4	0.007			0.084	0.968
5	0.002			0.028	0.996
6	0			0.004	1.000
Total	0.089	42	694.256	0.000	1.000

and perennials (Keeley and Fotheringham, 1998; Pérez-Fernández and Rodríguez-Echeverría, 2003), whereas high values of pH inhibit (Mayer and Poljakoff-Mayber, 1989) or enhance (Killi, 2004) germination by several mechanisms of action (Mayer and Poljakoff-Mayber, 1989). Our results are not conclusive in this respect. Germination of seeds moistened

exclusively with water (controls) was enhanced at low values of pH in *M. arabica* and *F. vulgare*; germination of *D. carota*, *T. villosa*, *C. cristatus* and *D. glomerata* was enhanced at high values of pH and the germination of *E. hirsutum* and *R. crispus* was similar at both low and high values of pH. The germination rates of seeds were negatively affected by high pH only in *E. hirsutum*.

Germination of seeds treated with the nutritious compounds was very low compared with the germination percentages of the controls. Both the level and rate of germination varied with species, indicating that each species attains their physiological or environmental requirements for germination at different times (Vleeshouwers *et al.*, 1995). The fact that some of the studied species showed positive response to the addition of nitrogenous salts indicates that these requirements also differ from the nutritious state of the seed and the surrounding environment (Bewley and Black, 1982; Pons, 1989).

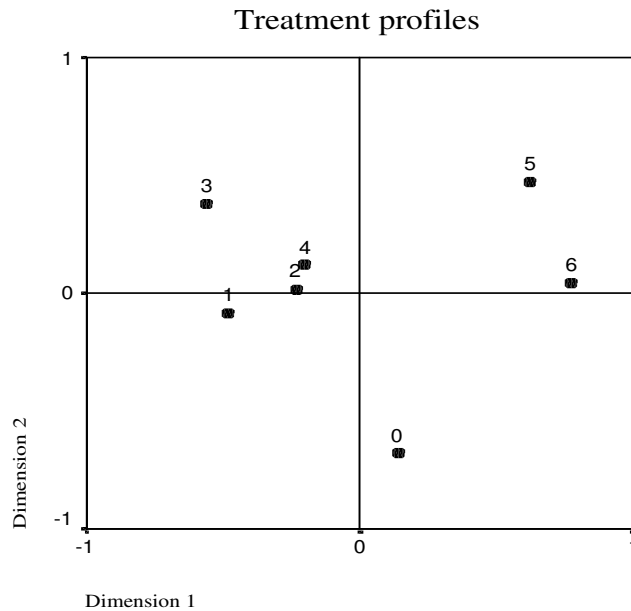


Fig. 3: Results of the correspondence analysis for the eight species treated with the three nutritious solutions at the two concentrations showing three different responses in germination. (0: Control; 1: NH₄NO₃ 25 mM; 2: NH₄NO₃ 50 mM; 3: KNO₃ 25 mM; 4: KNO₃ 50 mM; 5: CaCl₂ 25 mM; 6: CaCl₂ 50 mM).

Application of nitrogen, more specifically in its KNO₃ form and at the concentration of 50 mM, to some extent enhanced germination in *M. arabica*, *E. hirsutum*, *F. vulgare*, *T. villosa* and *R. crispus* (Fig. 2). However, germination enhancement was basically observed at a pH of 6.7 (\approx neutral) and no increase in the rate of germination was observed in any species but *R. crispus*.

With respect to seed germination in the natural environment, most of the species studied are found in very poor soils, a scenario that differs from our experimental conditions. The increased levels of fertilizers in the media might cause a toxic effect, thus inhibiting seed germination. This will be consistent with the germination behaviour of other species found in similar environments in the Iberian Peninsula, for which an increase in the concentrations of exogenous nitrogen has been shown to inhibit germination (Pérez-Fernández and Rodríguez-Echeverría, 2003). Only *R. crispus* seems to have benefited from the exogenous addition of salts. This species is commonly found in ruderal areas, highly influenced by human and animal activities, where nitrogen accumulates (Devesa, 1995). High levels of nitrogen are thus beneficial for this species.

Pons (1992) suggested that germination in response to elevated NO₃⁻ can be interpreted as a gap or disturbance detection mechanism. Seedlings that rapidly establish in gaps have an advantage over plants later, when there is a greater competition for resources (Tilman, 1982). If we propose that the most common perturbation in the Mediterranean ecosystems is fire, after which there is an increase in pH and N, the germination of *M. arabica* and *E. hirsutum* substantiates the proposed mechanism of action. Germination of *F. vulgare*, *T.*

villosa and *R. crispus* (enhanced by N) is partly consistent with their establishment after fires and may explain their success as invasive species in several environments.

Our results, suggest a clear species effect in the germination responses at given concentrations of nitrogen. The stimulatory effect of nitrates and other nitrogenous compounds on seed germination of weedy plant species is well documented (Thanos and Rundel, 1995; ISTA, 2003).

However, the positive effects on germination do not occur at the same concentration of nitrogenous compounds in all the species. Promotion by nitrates is obtained in the range of 0 to 50 mM in *E. pendulifolia* (Thanos and Rundel, 1995) as well as in other species from Western Australia (Bell *et al* 1999) and from the Iberian Peninsula (Pérez-Fernández and Rodríguez-Echeverría, 2003), with higher concentrations resulting in a toxic effect. In our study, only the highest concentration (50 mM) had a positive effect on germination indicating that these species might require still higher concentrations to trigger the germination process.

The lack of differences in germination of seeds under different values of pH in the solutions indicates that this treatment does not effect germination in the species studied, contrary to earlier observations on other species (Keeley and Fotheringham, 1998). Similarly equality of germination in the eight species at any value of pH in nutritious solutions with and without nitrogen suggests that the germination of these species is not associated with the concentration of H⁺ with or without the nitrate ion. Isolated or in combination with nutritious compounds, pH seemed not to affect germination, thus indicating that the mechanisms involved in the germination of these species are not related to pH. Given that the nitrogenous compounds did not

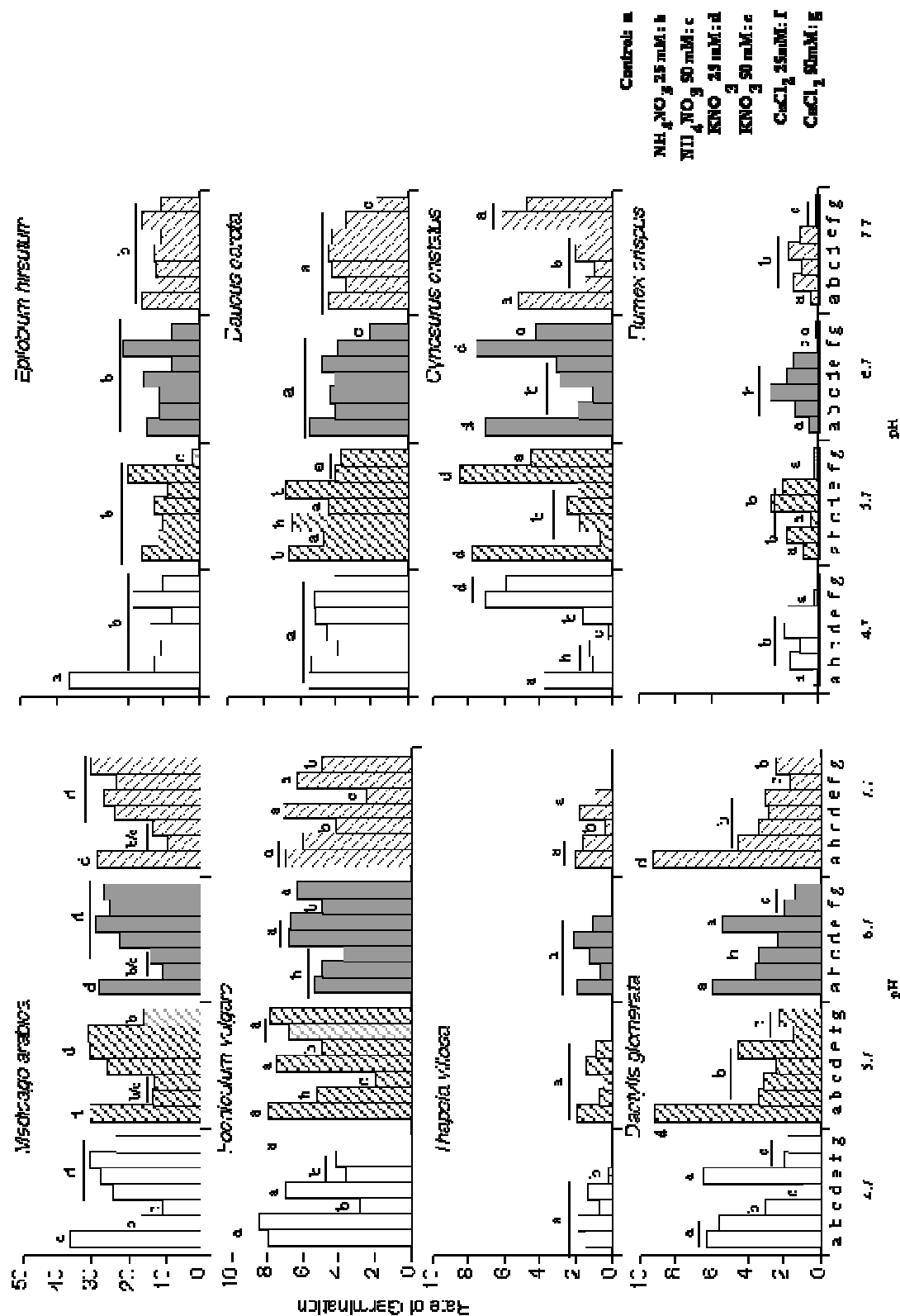


Fig. 4: Rate of germination expressed as Timson's Index, of *M. arabica*, *E. hirsutum*, *F. vulgare*, *D. carota*, *T. villosa*, *C. cristatus*, *D. glomerata* and *R. crispus* (after 32 days incubation) treated with three nitrogenous solutions (NH_4NO_3 , KNO_3 and CaCl_2) at two concentrations (25 and 50 mM) and adjusted at four values of pH (4.7, 5.7, 6.7 and 7.7). Different letters indicate significant differences (Fisher's $P \leq 0.05$). Control consisted in distilled water adjusted at the same pH values. Note different scale.

have strong triggering effects on germination, we assume that there are complex mechanisms regulating germination, and never limited to a restricted number of variables.

Acknowledgements

This work was supported by the project REN2001-0749/GLO 'Elaboración de modelos predictivos para el uso de especies pioneras en proyectos de recuperación de suelos degradados. Aplicación a *Retama sphaerocarpa* y *Cytisus striatus*'.

References

- Bell, D.T., L.A. King and J. Plummer: Ecophysiological effects of light quality and nitrate on seed germination in species from Western Australia. *Aust. J. Ecol.*, **24**, 2-10 (1999).
- Bewley, J.D. and M. Black: Physiology and biochemistry of seeds in relation to germination. Springer-Verlag, New York, pp: 294-307 (1982).
- Bungard, R.A., C. McNeil and J.D. Mortons: Effects of chilling, light and nitrogen-containing compounds on germination, rate of germination and seed imbibition of *Clematis vitalba* L. *Annals Bot.*, **79**, 643-650 (1997).
- Cochran, W.G.: The distribution of the largest of a set of estimated variances as a fraction of their total. *Ann. Eugen.*, **11**, 47-61 (1941).
- Cochrane, A., K. Brown, N. Meeson and C. Harding: The germination requirements of *Hemigenia exilis* (Lamiaceae) – seed plug removal and gibberellic acid as a successful technique to break dormancy in an arid zone shrub from Western Australia. *CALMScience* **3**, 21-30 (1999).
- David, H.A., H.O. Hartley and E.S. Pearson: The distribution of the ratio in a single normal sample of range to standard deviation. *Biometrika*, **41**, 482-493 (1954).
- Devesa, J.A.: Vegetación y flora de Extremadura. Editorial Universitas. Badajoz (1995).
- Eshel, A., N. Henig-Sever and G. Ne'eman: Spatial variation of seedling distribution in an east Mediterranean pine woodland at the beginning of post-fire succession. *Plant Ecol.*, **148**, 175-182 (2000).
- Franco-Vizcaino, E. and J. Sosa-Ramírez: Soil properties and nutrient relations in burned and unburned Mediterranean-climate shrublands of Baja California, México. *Acta Oecol.*, **18**, 503-517 (1997).
- Freijesen, A.H.J., S.R. Troelstra and M.J. Van Kats: The effect of soil nitrate on the germination of *Cynoglossum officinale* L. (Borraginaceae) and its ecological significance. *Acta Oecol./Oecol. Plant.*, **1**, 71-79 (1980).
- García-Fayos, P., B. García-Ventoso and A. Cerdá: Limitations to plant establishment on eroded slopes in southeastern Spain. *J.f. Veget. Sci.*, **11**, 77-86 (2001).
- González-Rabanal, F. and M. Casal: Effect of high temperature and ash on germination of ten species from gorse shrubland. *Vegetatio* **116**, 123-131 (1995).
- Goubitz, S., M.J.A. Werger and G. Ne'eman: Germination response to fire-related factors of seeds from non-serotinous and serotinous cones. *Plant Ecol.*, **169**, 195-204 (2003).
- ISTA. International Rules for Seed Testing. Zurich, Switzerland: ISTA (2003).
- Keeley, J.E.: Seed-germination patterns in fire-prone Mediterranean-climate regions. In: Ecology and biogeography of mediterranean ecosystems in chile, california and Australia, (Eds: M.T.K. Arroyo, P.H. Zedle and M.D. Fox), Springer-Verlag, New York, pp. 239-273 (1994).
- Keeley, J.E. and C.J. Fotheringham: Mechanisms of smoke-induced seed germination in a post-fire chaparral annual. *J. Ecol.*, **86**, 27-36 (1998).
- Khan, M.A. and Y. Rizvi: Effect of salinity, temperature, and growth regulators on the germination and early seedling growth of *Atriplex griffithii* var. *stocksii*. *Canadian J. Bot.*, **72**, 475-479 (1994).
- Killi, F.: Effects of potassium humate solution and soaking periods on germination characteristics of undelimited cotton seeds (*Gossypium hirsutum* L.). *J. Environ. Biol.*, **25** (4), 395-398 (2004).
- Lamont, B.B., E.T.F. Witkowski and N.J. Eright: Postfire liter microsites-safe for seeds, unsafe for seedlings. *Ecology* **74**, 501-512 (1993).
- Lyman, O.R.: An introduction to statistical methods and data analysis, 4th edition. Duxbury Press. United States, pp: 12-19 (1998).
- Mayer, A.M. and A. Poljakoff-Mayber: The Germination of Seeds. Pergamon Press, Oxford (1989).
- Ne'eman, G., N. Henig-Sever and A. Eshel: Regulation of the germination of *Rhus coriaria*, a post-fire pioneer, by heat, ash, pH and ethylene. *Physiol. Plant.*, **106**, 47-52 (1999).
- Núñez, M.R. and L. Calvo: Effect of high temperatures on seed germination of *Pinus sylvestris* and *Pinus halepensis*. *Forest Ecol. Manag.*, **131**, 183-190 (2000).
- Pérez-Fernández, M.A., B.B. Lamont, A.J. Marwick and W.G. Lamont: Germination of seven exotic weeds and seven native species in south-western Australia under steady and fluctuating water supply. *Acta Oecol.*, **21**, 323-336 (2000).
- Pérez-Fernández, M.A. and S. Rodríguez-Echeverría: Effect of smoke, charred wood and nitrogenous compounds on seed germination of ten species from woodland in Central-Western Spain. *J. Chem. Ecol.*, **29**, 237-251 (2003).
- Pons, T.L.: Breaking of seed dormancy by nitrate as a gap detection mechanism. *Ann. Bot.*, **63**, 139-143 (1989).
- Pons, T.L.: Seed responses to light. In: The ecology of regeneration in plant communities, (Ed: M. Fenner), Wallingford, IK, BAC International pp: 259-284 (1992).
- Reyes, O. and M. Casal: Germination of *Pinus pinaster*, *P. radiata* and *Eucalyptus globulus* in relation to the amount of ash produced in forest fires. *Ann. des Sciences Forestiers*, **55**, 837-845 (1998).
- Roberts, E.H. and S.K. Benjamin: The interaction of light, nitrate and alternating temperature on the germination of *Chenopodium album*, *Capsella bursapastoris* and *Poa annua* before and after chilling. *Seed Sci. and Technol.*, **7**, 379-392 (1979).
- Thanos, C.A. and P.W. Rundel: Fire-followers in chaparral: nitrogenous compounds trigger seed germination. *J. Ecol.*, **83**, 207-216 (1995).
- Tilman, D.: Resource competition and community structure. University Press, Princeton, New Jersey, USA (1982).
- Vleeshouwers, L.M., H.J. Bowmeester and C.M. Karssen: Redefining seed dormancy: an attempt to integrate physiology and ecology. *J. Ecol.*, **83**, 1031-1037 (1995).
- Whelan, R.J.: The ecology of fire. Cambridge University Press, Cambridge (1995).

Correspondence to :

Dr. María A. Pérez-Fernández

Ecology Area, University Pablo de Olavide

Corretera a Ulrera, km1,

41013 Sevilla. Spain.

E-mail: maperfer@upo.es

Tel.: +34 954977935; Fax: +34 954349151