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Influence of temperature on seed germination in seven Mediterranean grassland species from SE Portugal

Abstract

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The aim of this work was to analyze seed germination characteristics between species that share the same habitat. We studied the influence of temperature on the germination of seven species that grow in grassland areas of SE Portugal: *Bellardia trixago* (L.) All. (*Scrophulariaceae*), *Daucus carota* L. (*Apiaceae*), *Petrorhagia nanteuilii* (Burnat) P.W. Ball & Heywood (*Caryophyllaceae*), *Scabiosa atropurpurea* L., *S. simplex* Desf. (*Dipsacaceae*), *Silene colorata* Poiret and *S. gallica* L. (*Caryophyllaceae*). Seeds were incubated under 16/8 hours light/dark photoperiod, constant 15ºC, 20ºC or 25ºC and alternating 25/15ºC temperature conditions. Final germination percentage, germination rate (T50), uniformity (T75-T25) and skewness (T50/Mean Germination Time) were obtained. Seed germinability varied among the tested temperatures. *B. trixago, D. carota, P. nanteuilii, Scabiosa atropurpurea* and *S. simplex* presented the highest results at the lowest temperatures although *D. carota* seemed indifferent to the tested temperatures. *S. colorata* and *S. gallica* only germinated at 25ºC and 15/25ºC but the very low results suggest that seeds may present dormancy. All the species presented a rapid and constant germination with most seeds germinating in the first days. In general, the seven species showed different behavior among the assayed temperatures, which allow these species to explore different ecological conditions in the same habitat.

Introduction

Grassland communities occurring in meso- and thermo-Mediterranean xerophile habitats that are mostly open are mainly dominated by therophyte species. They often grow in oligotrophic soils with calcareous substrates. In more continental areas, these xerothermic communities are mainly dominated by tussock-grasses, chamaephytes and perennials species. These communities are partially of natural and anthropogenic origin. Germination may be considered the major and the first constraint to plant establishment in these communities. Comparative studies of species sharing the same habitat (Smith-Ramírez 1998; Albert & al. 2002; Valbuena & Vera 2002) and analyses of species functional traits can help to understand biological systems in ecological terms.

In this study, we selected seven herbaceous species: *Bellardia trixago* (L.) All. (*Scrophulariaceae*), *Daucus carota* L. (*Apiaceae*), *Petrorhagia nanteuilii* (Burnat) P. W. Ball & Heywood (*Caryophyllaceae*), *Scabiosa atropurpurea* L., *S. simplex* Desf. (*Dipsacaceae*), *Silene colorata* Poiret and *S. gallica* L. (*Caryophyllaceae*). All of these species are annual except *S. atropurpurea* that may be biennial. These taxa are common in open grasslands of the Western Mediterranean region where they frequently coexist. The flowering period is mainly in spring and seeds are released during summer at the end of their life cycle.

The main objectives of this work were to identify the optimal seed germination temperature for each species and to compare their germination physiological behavior. Germination profile, timing and pace will help us to recognize whether the species sharing the same habitat present the same degree of fitness and, thus, belong to similar functional groups.

Material and Methods

Plant material

Seeds of *B. trixago*, *D. carota*, *P. nanteuilli*, *S. atropurpurea*, *S. simplex*, *S. colorata* and *S. gallica* were collected in July-August 2003 from open grasslands in Evora District (Alentejo, Portugal). All the seeds presented the same degree of ripeness. Following the classification of Rivas-Martínez & al. (1990), climate is Mediterranean subhumid with an annual mean temperature of 16ºC and a 3-4 month dry period. Seeds were kept in paper bags under laboratory conditions (darkness, 21 ± 1ºC) until they were used.

Germination experiments

One-month-old stored seeds were placed in Petri dishes (7 cm in diameter) lined with two filter paper discs and moistened with 3 ml of distilled water. Seeds were incubated at 15ºC, 20ºC, 25ºC or 25/15 ºC under a 16-h light/ 8-h dark photoperiod. Light was provided by cool white fluorescent tubes with an irradiance of $35 \mu mol m^{-2} s^{-1}$ (OSRAM LUMILUX Cool white). When the alternating temperature was used, the lower temperature coincided with the dark period. The criterion of germination was radicle emergence. Seeds were monitored every two days for a period of 30 days and germinated seeds were removed. Each value is the mean of four replicates of 25 seeds ± standard deviation (sd). Non-germinated seeds were checked for viability using the tetrazolium test.

Germination parameters

The following parameters were used to compare germination behaviour among treatments: 1) final germination percentage; 2) germination rate defined by T_{50}, time needed to reach 50% final germination value; 3) uniformity (T_{75}- T_{25}) defined as the time between 25% and 75% final germination value and 4) skewness (considered as T_{50}/Mean Germination Time) a value measuring the asymmetry of germination distribution. A value of T_{50}/MGT near 1 means that the germination before T_{50} is faster than germination after T_{50}, which is normally the case. However, in some cases values greater than 1 can occur, when germination before T_{50} is lower than after T_{50}. Mean Germination Time was calculated as follows: Σ ni x di / N, where Ni is the number of seeds germinated in each day, Di is the duration of the study and N the total number of seeds germinated. The parameters T_{50}, uniformity and skewness were calculated using the mathematic expression that expressed each germination curve. Expressions were determined using CurveExpert 1.3 considering a sigmoid model. Correlation coefficients were always superior to 0.95.

Statistical analysis

For each species the selected parameters were submitted to a one-way ANOVA with temperature as factor. For comparison of means, Scheffe post hoc multiple comparison test was used ($p < 0.05$). Germination percentages were arcsin transformed. All statistical analyses were performed with SPSS package 11.0.

Results

Tables 1 and 2 show the effect of temperature on the selected germination parameters of the seven studied species. Seed germinability varied among the tested temperatures but 25°C was generally too high to promote germination except in *D. carota*, which attained high germination percentages at all, temperatures tested (Tab. 1). When alternating temperatures were assayed, some of the species (*P. nanteuilli* and *S. simplex*) showed a similar germination response to that at low incubation temperatures (Tab. 1). *P. nanteuilli* presented high results at 15-20ºC and 25/15ºC temperatures followed by *B. trixago* and the *Scabiosa* species. These five species generally achieved higher germination results at lower temperatures (15ºC and 20ºC). On the contrary, in *Silene colorata* and *S. gallica* no germination occurred at 15ºC or 20ºC and at the remaining temperatures germination percentages were always very low (Tab. 1). For each species, T_{50} also varied among temperatures but the values obtained were always very low, indicating rapid germination usually reaching 50% of the final germination percentage within one week (Tab. 2). These results are also supported by the low values of uniformity, which are inferior to four days (Tab. 2). Skewness values indicate that germination was usually faster before rather than after T_{50}, with some exceptions at higher temperatures (Tab. 2).

Discussion and Conclusion

Interspecific variation in germination behaviour has been commonly found in seeds of species from the same habitat (Herranz & al. 1998; Doussi & Thanos 2002). Relatively low temperatures (between 15ºC and 20ºC) are usually recorded as optimal germination temperatures among Mediterranean species (Thanos & Doussi 1995; Baskin & Baskin 1998; Doussi & Thanos 2002). This is in agreement with the results obtained for *B. trixago*, *D. carota*, *P. nanteuilli*, *S. atropurpurea* and *S. simplex*. Temperature seems to play an important ecological role in controlling the germination of these species. In this sense and considering that these species disperse their seeds during summer, soil conditions would be favorable for germination in early autumn or early spring when temperatures and rainfall would meet germination requirements. The positive influence of alternating temperatures on germination has been observed by several authors (Baskin & Baskin 1998; Albert & al. 2002) and has been interpreted as an adaptive mechanism of seeds in upper soil layers.

Table 1. Effect of incubation temperature on final germination percentage (mean values ± standard deviation). F: Anova F ratio value (** $p < 0.01$; *** $p < 0.001$ n. s.) for each species. Superscripts with different letters indicate significant differences ($p < 0.05$) within each species.

Species	15°C	20°C	25°C	25/15 °C	F
B. trixago	55 ± 6^{b}	53 ± 5^{b}	0	14 ± 13^{a}	14.52**
D. carota	88 ± 5^{a}	91 ± 6^{b}	70 ± 7^{a}	68 ± 7^{a}	18.56***
P. nanteuilii	100 ± 0^{b}	99 ± 2^{b}	2 ± 4^{a}	100 ± 0^{b}	286.64***
S. atropurpurea	$54 \pm 8^{a,b}$	64 ± 11^{b}	37 ± 7^{a}	31 ± 3^{a}	5.35**
S. simplex	58 ± 10^{b}	54 ± 10^{b}	11 ± 5^{a}	38 ± 6^{b}	26.81***
S. colorata	0	0	28 ± 23	15 ± 6	5.77 (n.s.)
S. gallica	0	0	14 ± 1	20 ± 5	1.11 (n.s.)

Table 2. Effect of incubation temperature on germination rate (T_{50}), uniformity (T_{75}-T_{25}) and skewness (T_{50}/MGT). Mean values ± standard deviation. F: Anova F ratio value (** $p < 0.01$; *** $p < 0.001$n. s.) for each species. Superscripts with different letters indicate significant differences ($p < 0.05$) within each species. Blanks correspond to absence of germination in a species at that particular temperature.

	Species	15°C	20°C	25°C	25/15°C	F
T_{50}	*B. trixago*	$5{\pm}0^{a,b}$	$7{\pm}1^{b}$	-	$4{\pm}3^{a}$	7.35**
	D. carota	$7{\pm}1^{b}$	$8{\pm}0^{b}$	$7{\pm}1^{b}$	$5{\pm}0^{a}$	11.69***
	P. nanteuilli	3±0	3±1	3±7	4±2	0.09 (n.s.)
	S. atropurpurea	6±1	6±0	5±0	5±0	2.68 (n.s.)
	S. simplex	3±0	4±1	3±1	2±0	2.29 (n.s.)
	S. colorata	-	-	5±1	13±1	45.50 (n.s.)
	S. gallica	-	-	6±1	5±1	0.17 (n.s.)
T_{75}-T_{25}	*B. trixago*	$4{\pm}1^{b}$	$4{\pm}1^{b}$	-	$1{\pm}1^{a}$	13.45**
	D. carota	4±0	4±0	3±1	4±0	3.23 (n.s.)
	P. nanteuilli	1±0	1±0	1±2	1±0	0.24 (n.s.)
	S. atropurpurea	$3{\pm}1^{b}$	$4{\pm}0^{b}$	$3{\pm}1^{b}$	$1{\pm}1^{a}$	14.93***
	S. simplex	$2{\pm}1^{a,b}$	$3{\pm}1^{b}$	$0{\pm}0^{a}$	$1{\pm}0^{a}$	12.54***
	S. colorata	-	-	3±1	4±1	2.18 (n.s.)
	S. gallica	-	-	1±0	4±3	3.40 (n.s.)
T_{50}/MGT	*B. trixago*	0.88±0.01	0.95±0.03	-	0.88±0.05	3.99**
	D. carota	$0.95{\pm}0.02^{b}$	$0.97{\pm}0.02^{b}$	$0.95{\pm}0.06^{b}$	$0.78{\pm}0.03^{a}$	18.66***
	P. nanteuilli	0.91±0.03	0.83±0.08	0.27±0.55	0.92±0.04	4.78 (n.s.)
	S. atropurpurea	0.84±0.08	0.86±0.02	0.82±0.05	0.92±0.02	2.57 (n.s.)
	S. simplex	$0.77{\pm}0.05^{a}$	$0.74{\pm}0.05^{a}$	$0.90{\pm}0.07^{b}$	$0.86{\pm}0.01^{a,b}$	7.84**
	S. colorata	-	-	0.80±0.04	1.17±0.05	7.68 (n.s.)
	S. gallica	-	-	0.96±0.01	0.86±0.12	2.52 (n.s.)

This strategy seems particularly important for these five species that might be able to germinate when daily temperatures have already risen but night temperatures are still low. *D. carota* with high germination percentages at all tested temperatures (Tab. 1) seems to present an opportunistic behaviour, independent of its longer germination time (Tab. 2).

The first five selected species produce a substantial fraction of soft-coated seeds, which germinated without the necessity of any particular pre-treatment. This seed pool is responsible for the maintenance of population levels in a variety of conditions and explains their colonizing role in disturbed areas. Uniformity and skewness values indicate that almost all of these soft-coated seeds can germinate at the same time and usually in the first few days with optimum temperatures. This strategy can be useful for the rapid colonization of new areas but it also implies that they must have high reproductive fitness and low intraspecific competition. If not, the soil seed pool would quickly diminish, and although most of the seeds germinate well, they would not be able to complete the life cycle.

Contrary to this previous group of five species, *Silene colorata* and *S. gallica* only germinated at 25ºC and 25/15ºC. These species seem to produce a major fraction of hard-coated seeds. The hardness and impermeability of the seed coat might be the cause of dormancy in these species as in other *Silene* species (Thompson 1970). The results of the tetrazolium test show that all non-germinated seeds were viable, thus supporting the hypothesis of seed dormancy. It would be interesting to observe the response of these species after pretreatments like soaking in a gibberelic acid solution or dry heat. The non-dormant fraction of these seeds can germinate just after dispersion if soil and temperature conditions are favorable whereas dormancy allows the rest of seeds to stay dormant during the hot and dry summer. The obtained results seem to indicate that these *Silene* species are able to control their population effectives under natural conditions through dormancy, thus maintaining a permanent soil seed bank in the field.

In conclusion, the seven grassland species do not present similar germination behaviours, which should be expected since they belong to different families. Even though they share the same type of habitat, they seem to explore different ecological niches dependent on seasonal characteristics, allowing their coexistence. Three different patterns were observed: 1) species with an opportunistic behaviour, *D. carota*, 2) species adapted for early spring or early autumn germination in Mediterranean climate, *B. trixago*, *P. nanteuilli*, *Scabiosa atropurpurea* and *S. simplex* and 3) species with seed dormancy, *Silene colorata* and *S. gallica*. Thus, with the exception of *D. carota*, temperature is an important driving force for germination to occur. This different behaviour can be interpreted as an important ecological strategy since the species could explore environmental conditions and available resources in different ways.

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