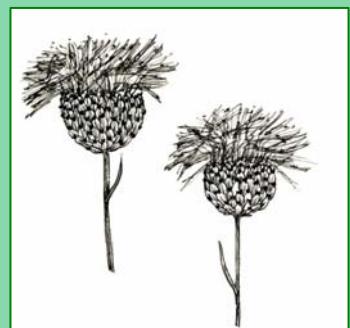




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Germination trials in mediterranean sages

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Summary

We studied the germination process of the seeds of four Mediterranean species of the genus *Salvia*. Three (*S. argentea* L., *S. sclarea* L., *S. verbenaca* L.) were collected from natural populations in the SW of the Iberian Peninsula (Extremadura, Spain), and the fourth (*S. officinalis* L.) from a commercially grown crop in the NE (Lérida, Spain). Germination trials were performed with 4 replicates of 50 seeds for each population under conditions of alternating temperatures of 6 hours at 20°C and 18 hours at 30°C, in darkness, and at 40-60% humidity. Cumulative germination curves were determined, and the germinability (in percentage terms) and germination rate (in terms of the vigour index, Iv) were determined. The germinability and germination rate were, respectively, high and rapid for *S. officinalis* (70.5%-84%; Iv = 14.51-19.09), low and slow for *S. sclarea* (2.5% 4%; Iv = 0.33-1.17), low to moderate and slow to rapid for *S. argentea* (10%-50.5%; Iv = 2.06-20.97), and high and very rapid for some populations of *S. verbenaca* (93%; Iv = 42.64). There were interspecific and interpopulational differences in germination capacity. Pre-cooling the seeds for 7 days at 10-12°C prior to sowing produced no significant changes in the results.

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Keywords: Germination, Iberian-Peninsula, Lamiaceae, Seed, *Salvia-argentea*, *Salvia-officinalis*, *Salvia-sclarea*, *Salvia-verbenaca*, Vigour, Wild-sages.

Resumen

Se ha estudiado el proceso de germinación de las semillas de cuatro especies mediterráneas del género *Salvia*. Tres de ellas (*S. argentea* L., *S. sclarea* L., *S. verbenaca* L.) fueron recolectadas en poblaciones naturales del SW ibérico (Extremadura, España). La cuarta (*S. officinalis* L.) procedía de un cultivo comercial en el NE (Lérida, España). Se realizaron experimentos de germinación en 4 réplicas de 50 semillas para distintas poblaciones donde las muestras fueron inducidas a germinar a temperaturas alternas 6 horas a 20°C, y 18 horas a 30°C en oscuridad y 40-60 % de humedad. Se realizaron curvas de porcentajes acumulados de germinación y se evaluó la germinabilidad (en términos de porcentaje) y la velocidad germinativa (en términos de índice de vigor Iv). Para *S. officinalis* se obtuvieron germinaciones altas y rápidas (70.5%-84%; Iv = 14.51-19.09), para *S. sclarea* bajas y lentas (2.5%-4%; Iv = 0.33 -1.17), para *S. argentea* bajas o moderadas y de velocidad lenta a rápidas (10% - 50.5%; Iv = 2.06 - 20.97). Para *S. verbenaca* algunas poblaciones llegaron a alcanzar germinaciones altas (93 %) y muy rápidas (Iv = 42.64). Se pusieron en evidencia diferencias en cuanto a capacidad germinativa, tanto a nivel interespecífico como interpoblacional. La aplicación de un preenfriamiento previo a la siembra de 7 días a 10-12 °C, no produjo cambios significativos en los resultados.

Blanco, J.; Ruiz, T. & Vázquez, F. M. 2009. Ensayos de germinación en salviás mediterráneas. *Folia Bot. Extremadurensis* 4: 33-43.

Palabras clave: Germinación, Península Ibérica, Lamiaceae, Semilla, *Salvia-argentea*, *Salvia-officinalis*, *Salvia-sclarea*, *Salvia-verbenaca*, Vigor, *Salvia-silvestre*.

Introduction

The genus *Salvia* L., belonging to the Lamiaceae family, includes some 900 species distributed over five continents. Its origin is in SW and C Asia. In the Mediterranean region, where it is very well represented, 131 species are known, of which 16 have a distribution in the Iberian Peninsula (Rosua & Blanca, 1989; Morales, 2000). It is an interesting genus from the points of view of reproduction, taxonomy, ecology, chorology, and applications. From any of these perspectives, information on germination capacity would be very useful in understanding the genus.

Most work published on this topic has focused on sages that are grown as ornamentals or as medicinal and culinary herbs. For example, of the garden species, there have been germination studies on *S. splendens* Sellow (Finch-savage & al., 1991a, b) and *S. farinacea* Benth. (Magnani & al., 1994) and of the medicinal species, on *S. officinalis* L. (Oberczian & Bernath, 1988; Kretschmer, 1989; Macchia & al. 1988; Takano & al., 1993) and *S. sclarea* L. (Oberczian & Bernath, 1988; Takano & al., 1993). There have also been data published on the germination of wild sages from Greece (*S. fruticosa* Miller, *S. pomifera* L., (Thanos, 1993; Thanos & Doussi, 1995)), Central Europe (*S. pratensis* L. (Ourbor & Van Treuren, 1995)), and the W of the United States (*Salvia dorrii* Kellogg) Abrams (Love & al., 1994)).

Overall, however, information with respect to the biology of germination in the genus is very sparse, representing hardly 2% of the species (Thanos & Doussi, 1995), and is practically non-existent for species from the W of the Iberian Peninsula. Nevertheless, these species are of interest because they can be used in pharmacy and horticulture, and because they must be incorporated into germ-plasm banks and kept in collections *ex situ*. It is hence necessary to evaluate their germination capacities. This is the context of the present study's objectives to contribute data on the germination conditions of some Mediterranean sages with an Iberian Peninsula distribution, and with potential applications in the field of new commercial crops. In particular, these objectives are:

- To study the germination processes in wild *Salvia* populations of the W Iberian Peninsula, and compare them with that of seeds from commercially grown *Salvia officinalis*.
- To evaluate the influence of a cooling pretreatment on those processes.

Materials and methods

Characteristics of the study material

S. argentea L. is a basophilic hemicryptophyte with a strongly thermophile character that naturally inhabits the southern half of the Iberian Peninsula (Alentejo, Algarve, Extremadura, Castilla la Mancha, Madrid, and Andalucia, but scarce or absent in Valencia and Murcia). It is found on roadsides and on the edges of fields of crops. It grows at 800m a.s.l (Bolos & Vigo, 1995). Its range of distribution is S Europe (Portugal, Southern Spain, Southern Italy, Southern Yugoslavia, Albania, Bulgaria, and Greece) and N Africa (Morocco, Algeria, and Tunisia). It presents nutlets of 3 x 2 mm, trigonal, rounded, greenish, veined (Rosua & Blanca, 1989). It is a plant of ornamental interest.

S. sclarea L. is an hemicryptophyte typical of roadsides near human population centres. It is distributed over S Europe, SW and C Asia, and N Africa (Algeria, Tunisia) (Rosua & Blanca, 1989). It is cultivated and more or less naturalized in much of the territory of the Iberian Peninsula, and is found between 0 and 1200 m a.s.l. (Bolos & Vigo, 1995). It presents nutlets of 3 x 2 mm, cream coloured, veined, broadly elliptic, smooth (Rosua & Blanca, 1989; Valdes & al., 1987). It has been used by herbalists, and recent studies have shown it to possess interesting, and previously unknown, pharmacological properties (Peana & al., 1999; Then & al., 2003; Dimmas & al., 1999; Hudaib & al., 2001).

S. verbenaca L. is an hemicryptophyte that colonizes much of the territory of the Iberian Peninsula, preferring fairly deep, neutral soils between 0 and 1000 (-1700) m a.s.l. (Bolos & Vigo, 1995). It is distributed over W and S Europe, N Africa, SW Asia, and Macaronesia (Madeira, Canary Islands), and is naturalized in S Africa, N America, and Australia. It presents nutlets of 1.5 x 2 mm, obovoid, smooth (Valdés & al., 1987). It has been used by herbalists, and is currently the subject of pharmacological research (Al-Howiriny, 2002).

S. officinalis L. is an hemicryptophyte whose native distribution is restricted to the western part of the Balkan Peninsula, i.e., Albania, the former Yugoslavia, Greece, and N Italy. It is naturalized in S Europe (Pignati, 1982; Greuter & al., 1986). It is also grown as a culinary herb and an ornamental plant all over Europe, where it was possibly introduced by the Romans in antiquity or by monks in the Middle Ages (Gams, 1927). It presents nutlets of 2.6 x 2.3 mm., obovoid, smooth. It is currently finding new applications in pharmacology (Wake & al., 2000).

Collection of the material

We collected nutlets (henceforward, denominated seeds for the sake of simplicity) of fruiting individuals of *S. argentea*, *S. sclarea*, and *S. verbenaca* from the natural populations listed in Appendix 1. For *S. officinalis*, the seeds came from a commercial crop (Appendix 1).

The seeds were placed in closed, labeled, paper bags after being cleaned of impurities and contaminating elements, and inspected under a stereoscopic microscope (20x) in order to eliminate those suspected to be inviable. These represented a proportion of 2% in *S. officinalis*, 6% in *S. sclarea*, 14.5-30.5% in *S. argentea*, and 0.5-25% in *S. verbenaca* (see Appendix 1). They were conserved for 4-6 months in darkness at the ambient temperature of the laboratory (22-28°C).

Germination trials

Germination trials were performed under controlled conditions of temperature, relative humidity, and lighting. The actual conditions were chosen on the basis of the available literature on affine species (Macchia & al., 1988; Finch-Savage & al., 1991a, b; Magnani & al., 1994; Love & al., 1994; Thanos & Doussi, 1995).

The experiments were carried out in a P SELECTA (Model Hot-Cold-S) precision refrigerated incubator in which the seeds were placed on Albert filter paper wetted to saturation with distilled water in 8.5 cm diameter Petri dishes. As many populations as possible were studied per taxon. Following the Ista (1999) norm, 4 replicates of 50 seeds each were run for every population, taking 21 days as the duration of the trial. Seeds were considered to have germinated if there was a radicle of at least 0.75 mm in length. Counts were made daily, and germinated seeds were removed.

The samples of seeds used as controls (C) were induced to germinate under alternating temperature conditions of 6 hours at 20°C and 18 hours at 30°C. In parallel, identical batches of seeds were prepared, and subjected to a thermal pretreatment (T) that consisted of keeping them for 7 days cooled at 10-12°C. All the experiments were carried out in darkness and at a relative humidity of 40-60%.

Evaluation of the germination

The data were used to construct the corresponding germination curves, and to calculate two indices of germination – the percentage germination, and the vigour index (Iv). The latter parameter quantifies the germination rate, and is calculated according to the expression: $Iv = (a/1 + b/2 + c/3... + z/n) \times 100/s$, where a, b, c, ... z are the numbers of seeds that germinate each day, n is the duration of the experiment in days, and s the number of seeds sown (Jain & Saha, 1971).

With the germination percentages (%), we evaluated the germinability using the categories of Devesa & al. (1998): null (0%), low (0<%<30), moderate (30≤%≤70), high (70<%<100), and maximal (100%).

With the Iv, we evaluated the germination rate using the categories of Cabello & al. (1998): slow ($0 \leq Iv < 5$), medium ($5 \leq Iv \leq 11.11$), fast or 'quick' ($11.11 < Iv \leq 33.33$), and very fast or 'rushy' ($33.33 < Iv \leq 100$).

Statistical analysis

We used the statistical package SPSS (11.0 for Window) to evaluate the statistically significant differences in both the germination percentages and the vigour indices (Iv). Differences between species and between populations of a given species were analysed using the Kruskal-Wallis and Mann-Whitney tests. Possible differences between the control (C) and the pretreated (T) seeds were analysed using the Wilcoxon test (Zar, 1996).

Results

Germination trials

Table 2 lists the results. The percentage germination in *S. officinalis* was 70.5% in the control (C) and 84% in the pretreated (T) seeds, i.e., a high germinability in the classification of Devesa & al. (1998); in *S. sclarea* it was 2.5% in the control (C) and 4% in the pretreated (T) seeds, both cases corresponding to a low germinability; in *S. argentea*, the germination percentages varied from 11.5% to 41.5% in the control (C) and from 10% to 50% in the pretreated (T) seeds, implying low to moderate germinability in both cases; and in *S. verbenaca* the germination percentages varied from 0% to 93% in both cases, corresponding to all the classes of germinability except the maximum.

With respect to the vigour index, *S. officinalis* presented values of 14.51 in the control (C) and 19.09 in the pretreated (T) seeds, corresponding to a 'quick' germination rate according to the classification of Cabello & al. (1998); *S. sclarea* presented 0.33 in the control (C) and 1.17 in the pretreated (T) seeds, i.e., slow germination rates; *S. argentea* had values from 5.04 to 17.35 in the control (C) and 2.06 to 20.97 in the pretreated (T) seeds, i.e., slow to 'quick' germination rates; and *S. verbenaca* from 0 to 41.40 in the control (C) and from 0 to 42.64 in the pretreated (T) seeds, corresponding to germination rates from slow to 'rushy', according to the case.

The data for the germination over the course of the experimental period was used to construct the cumulative percentage germination curves shown in Figure 1.

Statistical analysis

The Wilcoxon test showed no statistically significant differences between the respective results of the control (C) and the pretreated (T) seeds in either the percentage germination ($p = 0.306$) or in the vigour index ($p = 0.306$).

With respect to differences between species, the Kruskal-Wallis test showed statistically significant differences in the germination percentages in both the control (C) (* $p = 0.011$) and the pretreated (T) (* $p = 0.019$) seeds, and in the vigour indices (** $p = 0.010$, for C; ** $p = 0.008$ for T).

		<i>S. officinalis</i>		<i>S. sclarea</i>		<i>S. argentea</i>							
		Lérida		Badajoz		La Haba		Los Santos		Alconera		La Albueria	
Replicate		C	T	C	T	C	T	C	T	C	T	C	T
1		50 (10.19)	78 (17.40)	4 (0.65)	8 (2.33)	24 (10.50)	64 (28.00)	12 (5.00)	10 (4.17)	4 (1.33)	36 (3.67)	30 (11.61)	54 (23.00)
2		74 (15.98)	86 (19.36)	4 (0.40)	2 (0.50)	26 (11.17)	60 (24.83)	12 (5.33)	22 (8.52)	6 (1.80)	6 (0.50)	44 (18.28)	48 (20.83)
3		72 (14.46)	98 (22.21)	0 (0.00)	4 (1.33)	56 (22.50)	54 (21.23)	8 (4.00)	2 (1.00)	20 (7.02)	16 (1.63)	36 (15.17)	58 (24.17)
4		86 (17.43)	74 (17.39)	2 (0.25)	2 (0.50)	48 (21.83)	24 (8.83)	14 (5.83)	6 (2.50)	24 (7.35)	26 (2.46)	56 (24.33)	26 (11.00)
Mean		70.50 (14.51)	84 (19.0)	2.50 (0.33)	4.00 (1.17)	38.50 (16.5)	50.50 (20.9)	11.50 (5.04)	10 (4.05)	13.50 (4.38)	21 (2.06)	41.50 (17.35)	46.50 (19.75)

<i>S. verbenaza</i>													
		Valle Sta. Ana (a)		La Haba		Magacela		Valle Sta. Ana (b)		Cáceres (Portanchito)		Aliseda	
Replicate		C	T	C	T	C	T	C	T	C	T	C	T
1		4 (0.47)	2 (0.50)	12 (3.67)	12 (3.17)	22 (6.33)	46 (14.19)	2 (0.12)	2 (0.14)	0 (0.00)	0 (0.00)	40 (10.06)	22 (5.19)
2		0 (0.00)	0 (0.00)	26 (6.56)	28 (6.56)	30 (6.74)	12 (4.83)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	24 (5.10)	36 (9.07)
3		0 (0.00)	4 (1.33)	10 (2.15)	24 (5.37)	18 (4.83)	44 (13.53)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	40 (9.35)	28 (6.75)
4		0 (0.00)	0 (0.00)	16 (4.02)	8 (1.70)	26 (7.22)	32 (9.17)	0 (0.00)	0 (0.00)	2 (0.67)	0 (0.00)	22 (4.70)	26 (5.07)
Mean		1.00 (0.12)	1.50 (0.46)	16 (4.10)	18 (4.20)	24 (6.28)	33.50 (10.4)	0.50 (0.03)	0.50 (0.04)	0.50 (0.17)	0.00 (0.00)	31.50 (7.30)	28.00 (6.52)

		Guadajira		Lobón		Ribera del Fresno		Solana		La Parra		Bienvenida	
Replicate		C	T	C	T	C	T	C	T	C	T	C	T
1		24 (5.20)	30 (8.03)	0 (0.00)	0 (0.00)	48 (9.86)	16 (4.17)	34 (11.67)	0 (0.00)	94 (42.54)	90 (42.88)	10 (3.00)	12 (4.00)
2		26 (6.69)	34 (9.76)	0 (0.00)	0 (0.00)	62 (16.14)	20 (4.74)	26 (9.73)	10 (4.33)	90 (39.73)	92 (41.98)	18 (5.33)	14 (4.50)
3		46 (10.82)	52 (15.40)	0 (0.00)	0 (0.00)	40 (9.02)	30 (8.73)	16 (6.67)	4 (1.07)	94 (41.03)	94 (43.00)	12 (3.50)	10 (3.33)
4		48 (13.92)	40 (9.50)	0 (0.00)	2 (0.25)	22 (5.44)	28 (7.36)	18 (7.07)	32 (12.98)	94 (42.30)	96 (42.70)	14 (4.17)	18 (5.83)
Mean		36.00 (9.16)	39.00 (10.3)	0 (7)	0.50 (0.06)	43.00 (11.11)	23.50 (6.25)	23.50 (8.78)	11.50 (4.60)	93.00 (41.4)	93.00 (42.6)	13.50 (4.00)	13.50 (4.42)

Table 1. Germination percentages and vigour indices (Iv, in parentheses) obtained for the different replicates of the trials with control (C) and pretreated (T) seeds.

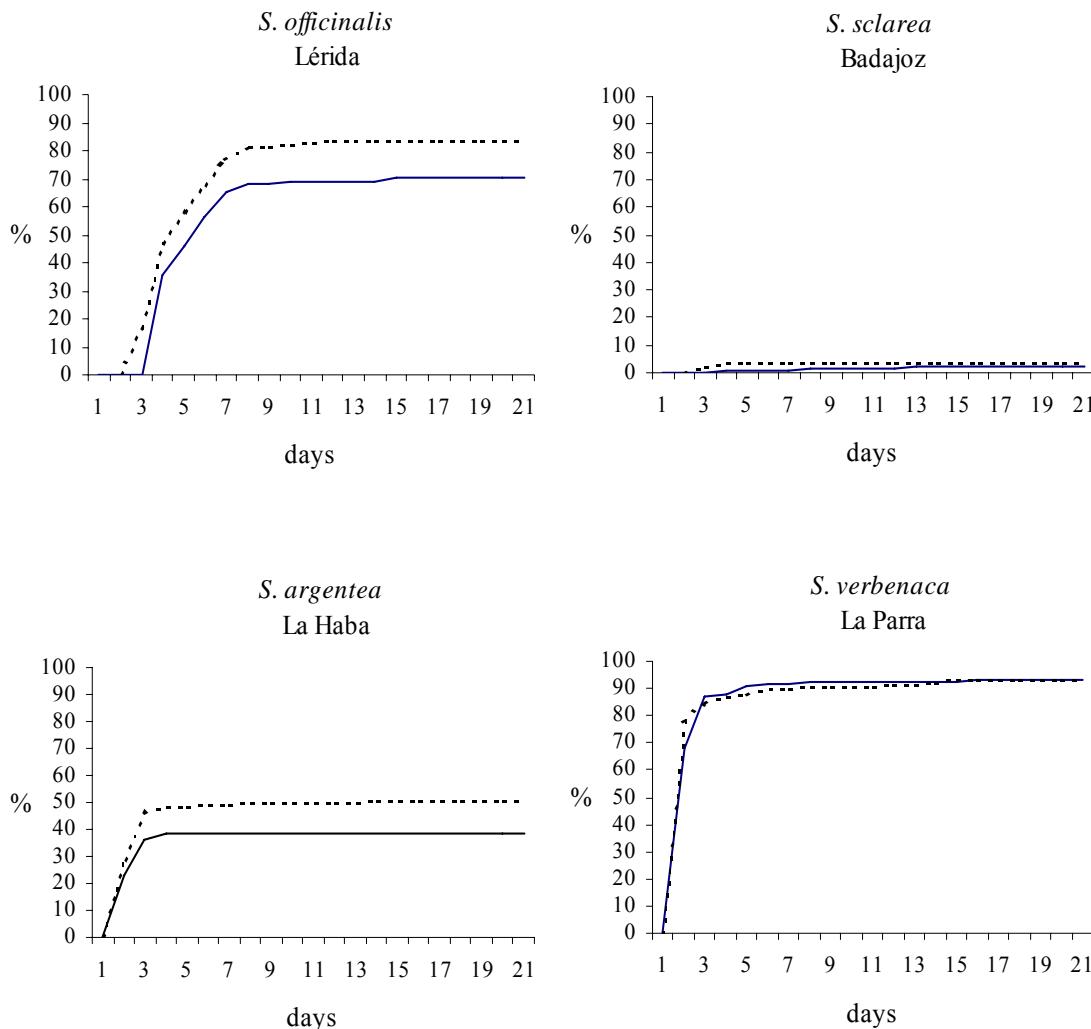


Figure 1. Cumulative germination percentages obtained over an experimental period of 21 days. For the taxa with more than one population available, that which attained the greatest percentage is shown. The values represented are the means of 4 replicates of the samples of control (C, continuous line) and pretreated (T, dashed line) seeds for *S. officinalis* (Lérida), *S. sclarea* (Badajoz), *S. argentea* (La Haba), and *S. verbenaca* (La Parra).

The Mann-Whitney test confirmed the differences between species except for those between *S. verbenaca* and *S. sclarea*, between the vigour indices of *S. officinalis* and *S. argentea*, and between the germination percentages of the control seeds (C) of *S. verbenaca* and *S. argentea* (Table 2).

The results of the Kruskal-Wallis test used to determine whether differences existed between populations for the two species – *S. verbenaca* and *S. argentea* – that were represented by various populations showed significant differences in both the percentage germination and the vigour index in control (C) and in pretreated (T) seeds (Table 3).

The Mann-Whitney test (Tables 5a and 5b) was used to show which pairs of populations were significantly different in percentage germination and in vigour index. In the case of *S. argentea* (Table 4), the most noticeable finding was the appearance of two groups of populations – La Haba/La Albuera and Los Santos/Alconera. Between these two groups there were statistical differences in germination behaviour, but not within each group. The germinability of the La Haba/La Albuera seeds was moderate and their germination rate was 'quick', whereas the Los Santos/Alconera seeds had a low germinability and slow germination rate.

In the case of *S. verbenaca*, where the number of pairs of populations compared was far greater (Table 5a and 5b), the most noteworthy finding was the significant differences between La Parra and the other populations. One observes in the table that there are three groups of populations that differ significantly from each other, but not within each group. The first consists of 4 populations (Lobón, Santa Ana (b), Cáceres, and Santa Ana (a)), the second of 7 populations (Ribera Fresno, Guadajira, Aliseda, Magacela, Solana, La Haba, and Bienvenida, with this last population being a little more deviant), and the third exclusively of the La Parra population. The behaviour is basically similar for the percentage

germination and the vigour index, as well as for the control and the pretreated seeds. The first group stands out as having a null or very low germinability, not surpassing a germination percentage of 1.5%. The second has generally low germinabilities, occasionally moderate (with germination percentages surpassing 30%), together with slow or occasionally medium germination rates. The La Parra population, which is different from the rest, has germination percentages of more than 90% (high germinability) and 'rushy' germination rates.

Control C	<i>S. officinalis</i> 1	<i>S. sclarea</i> 2	<i>S. argentea</i> 3	<i>S. verbenaca</i> 4
1	-----			
2	* 0.029 (* 0.029)	-----		
3	** 0.002 (ns 0.335)	*** 0.001 (*** 0.000)	-----	
4	** 0.003 (** 0.007)	ns 0.130 (ns 0.113)	ns 0.204 (* 0.015)	-----

Pretreatment T	<i>S. officinalis</i> 1	<i>S. sclarea</i> 2	<i>S. argentea</i> 3	<i>S. verbenaca</i> 4
1	-----			
2	* 0.029 (* 0.029)	-----		
3	*** 0.000 (ns 0.385)	** 0.007 (* 0.011)	-----	
4	*** 0.001 (** 0.003)	ns 0.292 (ns 0.341)	* 0.038 (* 0.050)	-----

Table 2. Mann-Whitney test significance levels for the comparison of the germination and vigour index (Iv, in parentheses) results between pairs of species, on samples of control (C) and pretreated (T) seeds: ns, not significant; *, p < 0.05; ** p < 0.01; *** p < 0.001.

	Control (C)	Pretreatment (T)
<i>S. argentea</i>	* 0.011 (** 0.010)	* 0.019 (** 0.008)
<i>S. verbenaca</i>	*** 0.000 (*** 0.000)	*** 0.000 (* 0.029)

Table 3. Kruskal-Wallis test significance levels for the comparison of the germination and vigour index (Iv, in parentheses) results of the different populations of *Salvia argentea* (n = 4) and *Salvia verbenaca* (n = 12), on samples of control (C) and pretreated (T) seeds: ns, not significant; *, p < 0.05; ** p < 0.01; *** p < 0.001.

Control C	La Haba 1	La Albuera 2	Los Santos 3	Alconera 4
1	-----			
2	ns 0.686 (ns 0.686)	-----		
3	* 0.020 (* 0.029)	* 0.029 (* 0.029)	-----	
4	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 1.000 (ns 1.000)	-----

Pretreatment T	La Haba 1	La Albuera 2	Los Santos 3	Alconera 4
1	-----			
2	ns 0.486 (ns 0.686)	-----		
3	ns 0.114 (* 0.029)	* 0.029 (* 0.029)	-----	
4	ns 0.114 (* 0.029)	ns 0.057 (* 0.029)	ns 0.200 (ns 0.343)	-----

Table 4. Mann-Whitney test significance levels for the comparison of the germination and vigour index (Iv, in parentheses) results between pairs of populations of *S. argentea*, on samples of control (C) and pretreated (T) seeds: ns, not significant; *, p < 0.05; ** p < 0.01; *** p < 0.001.

C	Lobón 1	St. Ana b 2	Cáceres 3	St. Ana a 4	Bienven. 5	La Haba 6	Solana 7	Magacel a 8	Aliseda 9	Guadajir a 10	R. Fresno 11	La Parra 12
1	-----											
2	ns 0.686 (ns 0.686)	-----										
3	ns 0.686 (ns 0.686)	ns 1.000 (ns 0.886)	-----									
4	ns 0.686 (ns 0.686)	ns 0.886 (ns 0.886)	ns 0.886 (ns 0.886)	-----								
5	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	-----							
6	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.886 (ns 1.000)	-----						
7	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.057 (* 0.029)	ns 0.200 (* 0.029)	-----					
8	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.114 (ns 0.057)	ns 0.886 (ns 0.114)	-----				
9	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.114 (ns 0.114)	ns 0.343 (ns 0.486)	ns 0.343 (ns 0.886)	-----			
10	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.057 (ns 0.057)	ns 0.200 (ns 0.057)	ns 0.200 (ns 1.000)	ns 0.343 (ns 0.343)	-----		
11	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.057 (* 0.057)	ns 0.114 (* 0.057)	ns 0.114 (* 0.886)	ns 0.343 (* 0.486)	ns 0.686 (* 0.886)	-----	
12	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	-----

Table 5a. Mann-Whitney test significance levels for the comparison of the germination and vigour index (Iv, in parentheses) results between pairs of populations of *S. verbenaca*, on samples of control (C) seeds: ns, not significant; *, p < 0.05; ** p < 0.01; *** p < 0.001.

T	Lobón 1	St. Ana b 2	Cáceres 3	St. Ana a 4	Bienven. 5	La Haba 6	Solana 7	Magacel a 8	Aliseda 9	Guadajir a 10	R. Fresno 11	La Parra 12
1	-----											
2	ns 1.000 (ns 0.886)	-----										
3	ns 0.686 (ns 0.686)	ns 0.686 (ns 0.686)	-----									
4	ns 0.486 (ns 0.486)	ns 0.486 (ns 0.486)	ns 0.343 (ns 0.343)	-----								
5	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	-----							
6	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.686 (ns 0.886)	-----						
7	ns 0.114 (ns 0.114)	ns 0.114 (ns 0.114)	ns 0.114 (ns 0.114)	ns 0.200 (ns 0.343)	ns 0.343 (ns 0.586)	ns 0.486 (ns 0.686)	-----					
8	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.114 (ns 0.057)	ns 0.114 (ns 0.114)	ns 0.057 (ns 0.114)	-----				
9	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (ns 0.114)	ns 0.200 (ns 0.343)	ns 0.200 (ns 0.343)	ns 0.486 (ns 0.343)	-----			
10	* 0.029 (* 0.029)	ns 0.057 (* 0.029)	ns 0.886 (ns 0.200)	ns 0.114 (ns 0.057)	-----							
11	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	ns 0.057 (ns 0.200)	ns 0.343 (ns 0.343)	ns 0.343 (ns 0.486)	ns 0.343 (ns 0.114)	ns 0.486 (ns 0.686)	* 0.029 (* 0.057)	-----	
12	* 0.029 (* 0.029)	* 0.029 (* 0.029)	* 0.029 (* 0.029)	-----								

Table 5b. Mann-Whitney test significance levels for the comparison of the germination and vigour index (Iv, in parentheses) results between pairs of populations of *S. verbenaca*, on samples of pretreated (T) seeds: ns, not significant; *, p < 0.05; ** p < 0.01; *** p < 0.001.

Discussion

In germination biology, seeds from wild populations have the characteristics of dormancy, a wide range of stages of maturity of the collected material, and a limited range of temperatures and conditions under which they can germinate rapidly (Finch-Savage & al., 1991a). These are limiting factors in the practical cultivation of wild species which have to be resolved through seed technology (Finch-Savage & al., 1991b) and the optimization of the germination ranges.

There is little published data on the process of germination in the genus *Salvia* (Thanos & Doussi, 1995), although some workers have studied the influence on some of the species of such factors as fixed temperatures (Finch-Savage & al., 1991a; Magnani & al., 1994), alternating temperatures (Love & al., 1994), light (Thanos & Doussi, 1995), the combination of temperature and light (Macchia & al., 1988), and the action of chemical or hormonal products (Thanos & Doussi, 1995).

Slow germination is a typical strategy of Mediterranean plants to protect them from the frequent short dry spells at the beginning of the rainy season (Thanos, 1993). Indeed, species with a more northerly distribution area also present faster germination rates and a broader range of germination (Thanos & Doussi, 1995).

Another typical strategy of Mediterranean plants is for dormancy to be broken by the effect of low temperatures. This is interpreted as a response to what occurs in nature in the period prior to the germination of seeds in spring. Experimental thermal pretreatment often leads to an increase in germination.: In the case of *Salvia*, pre-cooling by 4°C during 5 days lead to differences in *S. officinalis* when they were germinated at 10°C/20°C alternating temperatures, although not in the case of similar experiments performed at 20°C/30°C (Oberczian & Bernath, 1988).

For the species of the present study, there were statistically significant differences in the results for the seeds with a commercial origin and those from wild populations. Overall, for the conditions that we tested, one can state that the best results were obtained for the seeds of commercial origin – *S. officinalis*. Because of its economic interest, there is a fair amount of data available concerning the transplant cultivation of this perimediterranean species (Bezzi & al., 1992). The application of temperatures between 20°C and 25°C results in a high germinability (90% germination percentage) according to Macchia & al.

(1988) and Kretschmer (1989). Other workers (Obercian & Bernath, 1988) have found similar results (84-94%) for slightly broader temperature intervals (20°-30°C).

Our experiments with alternating temperatures of 20°C/30°C gave high levels of germinability – 70.5% (C) and 84% (T) – and a 'quick' germination rate (14.51-19.09), confirming in general terms the results reported in the literature. The advantage that, according to Love & al. (1994), alternating temperatures give to the germination of some *Salvias* was not clearly manifest in our trials, since any germination percentages reached the maximum level (100%).

Salvia sclarea, *S. argentea*, and *S. verbenaca* are more eumediterranean species, with statistically significant differences in their germination processes. Their wild origin must have conditioned the results in comparison with *S. officinalis*. In the case of *S. sclarea*, we found low germinabilities (2.5% C and 4% T), and slow germination rates (from 0.33 to 1.17). Hence one deduces that, in principle, neither the temperatures (20°-30°C) nor the pretreatment applied are especially recommendable for this taxon, although it must be borne in mind that we were working with a single population in this case. It would seem interesting to investigate further into the germination process of this species, since it may be that the present results are only the reflection of induced dormancy, since, as indicated by Gray (1993), some species that do not have true dormancy may be induced to enter a dormant state by high temperatures. On the other hand, various workers have reported good germination results with seeds of this species using temperature intervals between (10-) 20°C and 30 °C, although the origin of the material was in locations of C and E Europe (Obercian & Bernath, 1988; Takano, 1988).

Something similar could be said of the results for *S. argentea*, although here the sample size was larger. We could find no published data about the germination requirements of this species, but the experimental conditions that we applied led to only low or moderate germination percentages (10%-50.5%) and slow or 'quick' germination rates (2.06 to 20.97), depending on the origin of the material. The seeds collected at Los Santos and Alconera, whose populations have in common the shallowness of the soils of rubbish dumps and limestone quarries, had the lower germination success under the trial conditions (low germinability and slow germination rate), while the La Haba and La Albuera seeds, collected from the edges of fields of crops and with generally deeper soils, had greater germinability (moderate) and germination rate ('quick').

Neither was there any previous information available on germination conditions for the case of *S. verbenaca*. For this species we studied a greater number of populations, and the results with the experimental conditions tested varied according to the population. The seeds from La Parra were significantly different from the rest, showing a very positive response to the experimental conditions: germination percentages greater than 90% and high germination rates. This population formed part of communities of weeds, and was collected in a different year, so that there could have been an influence in the result of climatic variations from one year to another.

At the opposite extreme were the results for the material collected from the populations of Cáceres, Lobón, Santa Ana (a), and Santa Ana (b). These seeds had a very poor response to the applied treatment. They all came from natural populations with the same climate, and all inhabited fairly deep and moist soils, which may have been the reason that the plants of these populations were tall in bearing. The Santa Ana populations were from the same site, but were phenologically different – one of a pre-spring period (January) and the other of mid-spring (April) – although no significant differences in germination were found between them.

The results for the other seven populations – Ribera Fresno, Guadajira, Aliseda, Magacela, Solana, La Haba, and Bienvenida – occupied an intermediate situation, with germination percentages seldom greater than 30% and slow or occasionally medium germination rates. Although the Bienvenida population was included in this group, it presents a certain degree of deviation from the rest, possibly enhanced because, like the La Parra population, it had been collected in a different year from the rest. In these cases, the results can not be related to the season when the seeds were collected (i.e., spring or summer), to the type of substrate or microhabitat of the different populations, or to the vulnerability of the seeds to insect attack as quantified in the percentages of attack given in Table 1. Recent studies on affine species (Buchwald and Kitkowska, 2001) have shown the importance of other complex factors such as the weather conditions during flower formation and seed ripening.

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Appendix 1

Material studied*

Salvia officinalis

- Lérida: Crop. 12/07/2002. J. Blanco, F.M. Vázquez nº6/02. 2%

Salvia sclarea

-Badajoz: Badajoz. 29SPD70. Freeway ditch. Mesomediterranean bioclimate. 13/06/2002. J. Blanco, D. Martín, F.M. Vázquez nº5/02. 6%

Salvia argentea

-Badajoz: La Albuera. 29SPC88. Edge of field of crops. Mesomediterranean bioclimate. 21/06/2002. J. Blanco, D. Martín nº32/02. 21.75%

-Badajoz: Alconera. 29SQC15. Proximity of limestone quarry. Mesomediterranean bioclimate. 26/06/2002. J. Blanco, D. Martín nº31/02. 30.5%

-Badajoz: Los Santos de Maimona. 29SQC25. Rubbish dump. Mesomediterranean bioclimate. 14/06/2002. J. Blanco, D. Martín nº30/02. 14.5%

-Badajoz: La Haba. 30STJ51. Edge of olive grove. Mesomediterranean bioclimate. 18/06/2002. J. Blanco, D. Martín nº29/02. 15.5%

Salvia verbenaca

-Badajoz: Ribera del Fresno. 29SQC37. Edges of fields of crops and roadsides. Mesomediterranean bioclimate. 18/04/2002. J. Blanco, D. Martín nº28/02. 7.5%

-Badajoz: Guadajira. 29SPD90. Edges of fields of crops. Mesomediterranean bioclimate. 19/04/2002. J. Blanco, F.M. Vázquez nº26/02. 7.5%

-Badajoz: Lobón. 29SQC09. Edges of fields of crops. Mesomediterranean bioclimate. 19/04/2002. J. Blanco, F.M. Vázquez nº27/02. 1%

-Cáceres: Aliseda. 29SPD96. Roadside ditches. Mesomediterranean bioclimate. 16/04/2002. J. Blanco, D. Martín nº25/02. 2.5%

-Cáceres: Cáceres. El Portanchito. 29SQD27. Edges of olive groves. Mesomediterranean bioclimate. 27/05/2002. J. Blanco, D. Martín nº24/02. 4%

-Badajoz: Valle de Santa Ana (b). 29SPC94. Dehesa (open evergreen-oak woodland) next to stream. Mesomediterranean bioclimate. 7/05/2002. J. Blanco, D. Martín nº23/02. 0.5%

-Badajoz: Magacela. 30STJ51. Castle of Magacela, on stony substrate. Mesomediterranean bioclimate. 14/04/2002. J. Blanco, P. Escobar nº4/02. 5%

-Badajoz: La Haba-Quintana de la Serena. 30STJ51. Edges of olive groves. Mesomediterranean bioclimate. 14/04/2002. J. Blanco, P. Escobar nº3/02. 18.5%

-Badajoz: Valle de Santa Ana (a). 29SPC94. Dehesa next to stream. Mesomediterranean bioclimate. 21/01/2002. J. Blanco, S. Ramos, F.M. Vázquez. nº1/02. 3%

-Badajoz: Solana de los Barros. 29SQC09. Edges of fields of crops. Mesomediterranean bioclimate. 9/08/2001. J. Blanco, D. Martín, F.M. Vázquez nº5/01. 25%

-Badajoz: La Parra. 29SQC06. Weed. Mesomediterranean bioclimate. 8/08/2000. NC-074184. 2.5%

-Badajoz: Bienvenida. 29SQC44. Roadside. Mesomediterranean bioclimate. 9/08/2000. NC-074229. 3.5%

* Provence of the study material, indicating province, locality, UTM coodenates, habitat type, climate (see Tormo & al., 1995), collection date, legit, collection number, and percentage of inviable seeds calculated from a sample of 4 replicates, of 50 seeds each. Voucher specimens in the HSS Herbarium (Badajoz, Spain).

ÍNDICE

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