

ECOPHYSIOLOGY OF SEED GERMINATION IN ENDEMIC LABIATES OF CRETE

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ABSTRACT

Ecophysiological aspects of seed germination were investigated in four aromatic labiate plants of Crete *Origanum dictamnus* (dittany), *Sideritis syriaca* L. ssp. *syriaca* (Cretan mountain tea), *Salvia pomifera* L. ssp. *pomifera* (gall-bearing sage), and *Salvia fruticosa* (three-lobed sage). Experiments were performed both at constant temperatures and darkness as well as under temperature and light conditions simulating those prevailing in nature during the main germination periods (i.e., start and middle of the rainy season, November and February-March, respectively). In three out of the four species, no particular dormancy was revealed and germination occurred rather promptly, although in a rather narrow range of cool temperatures and at a relatively slow rate; both characteristics determine and/or support an early, autumn seed germination and seedling establishment. In the fourth plant, *Sideritis syriaca*, germination was manifested at relatively warm temperatures and at a considerably faster rate, in accordance with its alpine distribution favoring spring seedling emergence and establishment. All four species tested showed an intermediate response towards light, as a result of their intermediate levels of active phytochrome maintained in darkness. Therefore seed germination was partially manifested in darkness but it was significantly enhanced (particularly at suboptimal temperatures) by white or red light; on the other hand, illumination with far-red light (simulating light conditions under a canopy) resulted in significant inhibition compared to dark controls.

INTRODUCTION

Aromatic and medicinal plants constitute a considerable portion of both the flora and the vegetation of the Mediterranean Rim. Particularly in the well-known cradle of civilization of the Eastern Mediterranean region, aromatic and medicinal plants have been collected from the wild and eventually cultivated for several millennia. Theophrastus (4th century BC) in his *Enquiry into Plants* has described a considerable number of aromatic plants (e.g., *HP* 6.2, *HP* 7), including three out of four of the taxa under investigation.

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Origanum dictamnus (dittany) is a white-lanate, dwarf shrub, a typical chasmophyte that grows on the steep limestone rocks of Crete, from sea level up to an altitude of 1500 m. The plant has been described as an endemic of Crete already by Theophrastus (HP 9.16.1.); he has further attributed the scarcity of the plant as well as its very narrow distribution to the fact that goats are fond of it and graze it out. *Sideritis syriaca* ssp. *syriaca* (Cretan mountain tea) is a grey- or white-lanate perennial shrub, up to 50 cm tall, growing on the mountain rocks of Crete, at altitudes higher than 1000 m. *Salvia pomifera* (gall-bearing sage) is an endemic species of Eastern Mediterranean, while its subspecies *S. pomifera* ssp. *pomifera* is a shrub up to 100 cm tall, growing in dry, sunny places with phryganic vegetation, in Crete and southern Greece. *Salvia fruticosa* (three-lobed sage) is a shrub up to 120 cm high, growing in dry, phryganic zones; it is endemic to the Mediterranean Rim with a wider distribution than the other plants (from Sicily to Israel).

All four plant species mentioned above belong to Labiatae, a very important plant family in Greece (Kokkini et al., 1988) with a particularly high percentage of endemism (around 30%, second family of the Greek flora in respect to endemic taxa). The aromatic plants under study are of significant economic importance; although *O. dictamnus* is cultivated as well, they are usually collected from the wild (leaves and apical parts of flowering stems, known to be richest in essential oil content) and they are used mainly as infusions and spices (e.g., Kokkini et al., 1989; Bosabalidis, 1990). In addition, *O. dictamnus* has been listed (WCMC, 1993) as a vulnerable plant for both Europe and the whole World and is among the protected plants of the Berne Convention (1992), European Union (1992), and Greece (1981).

Apart from certain general rules for laboratory germination (e.g., AOSA, 1981; Ellis et al., 1985; ISTA, 1993), there is only scarce information concerning aromatic plants in general and the taxa under study in particular (e.g., Macchia et al., 1983; Thanos, 1993). In the broad context of both conservation and the potential use of several selected aromatic plants as alternative crops in marginal lands, the ecophysiology of their seed germination (considered a key factor of reproductive biology) was investigated.

MATERIALS AND METHODS

PLANT MATERIAL

Seeds of *Origanum dictamnus* L. were collected in the summer of 1991 from plants cultivated in Crete. The seeds of the other aromatic plants were collected from the wild at various locations of the Prefecture of Chania, W. Crete: *Sideritis syriaca* L. ssp. *syriaca* (collected at an altitude of 1800 m, in Lefka Ori in August 1991); *Salvia pomifera* L. ssp. *pomifera* (two lots collected, in Agia Anna Gorge in August 1991 and at Kalathas in August 1993); and *Salvia fruticosa* Miller (two lots collected, at Mouzouras in June 1992 and at Georgioupolis in August 1993). Nomenclature and distribution data follow Greuter et al. (1986).

Seed weight was determined for each species: *O. dictamnus*, 0.13 mg; *S. syriaca* ssp. *syriaca*, 1.72 mg; *S. pomifera* ssp. *pomifera*, 7.35 and 8.58 mg (1st and 2nd lot,

respectively); and *S. fruticosa*, 5.54 mg. The percentage of unsound seeds (unfilled or infested by insects) was measured by dissection under a stereomicroscope and was found to be considerable in both *Salvia* species: in *S. pomifera* ssp. *pomifera*, 18% and 65%, for the 1st and 2nd lot, respectively; and in *S. fruticosa*: 21.4% and 23.8%, for the 1st and 2nd lot, respectively. Unsound seeds comprised a small fraction, ca. 10% in both *O. dictamnus* and *S. syriaca* ssp. *syriaca* seeds.

GERMINATION CONDITIONS

Seed germination was carried out with nutlets (hereafter called seeds for simplicity) manually; extracted from their calyces; the calyces were carefully rubbed and the seeds carefully cleaned from calyx fragments. Five replicates of 20 or 25 seeds were sown on moistened filter paper disks in Petri dishes. The criterion of germination was visible radicle protrusion. After each count, the germinated seeds were discarded, and the tests were considered finished when no additional seeds germinated. All manipulations of imbibed seeds were carried out under a dim, green safelight. T_{50} is the time needed for 50% of the final germination value, and it is calculated by linear interpolation from the two germination values closest to median germination. At the end of each experiment ungerminated seeds were dissected and inspected under a stereomicroscope; unfilled or insect-infested seeds (unsound seeds) were not counted and consequently all the results and graphs presented have been corrected for sound (germinable) seeds.

For germination experiments in darkness, seeds were incubated within light-proof, metal containers in cabinets (Model BK 5060 EL, W.C. Heraeus, Hanau, Germany) maintained at constant temperatures. Seed germination experiments under various light regimes were performed in temperature- and light-programmable growth benches (model GB48, Conviron, Controlled Environments, Winnipeg, Canada) equipped with a lamp canopy of 48 incandescent bulbs (Sylvania 50A19, 50 W, 277 V) and 10 fluorescent tubes (Sylvania Cool White FR96T12/CW/VHO-235/1). In order to simulate the natural conditions encountered during the two main germination periods (i.e., November and February-March), temperature and light programs were automatically changed according to the average climatic data (period 1955–1987) of a representative meteorological station (Ellinikon Airport, Athens) (Thanos et al., 1991). Temperature was programmed to change every 3 h and lights were turned on 30 min before official sunrise and off 30 min after official sunset, while both the quality and quantity of light were automatically adjusted several times during the “day” (Thanos et al., 1991). Photon flux densities were measured with a spectroradiometer (ISCO SR, Instrumentation Specialties Co., Lincoln, NE, USA); calculations of flux densities and values of ζ and ϕ were made with a previously described software application (Thanos et al., 1994). White light (W) had a value of ζ (660/730 nm photon ratio) equal to 1.119, while the phytochrome photostationary state ratio ($\phi = [Pfr]/[Ptot]$) was 0.641, and the total photon flux density at the visible range (400–800 nm) was $103.0 \mu\text{mol m}^{-2} \text{s}^{-1}$. Using blue and red Plexiglas filters, four types of light regimes were established: red (R), blue (B), and two types of “canopy simulating”, far-red-rich light (FR I and FR II). The spectral distribution curves for each of the five light sources are shown in Fig. 1. In

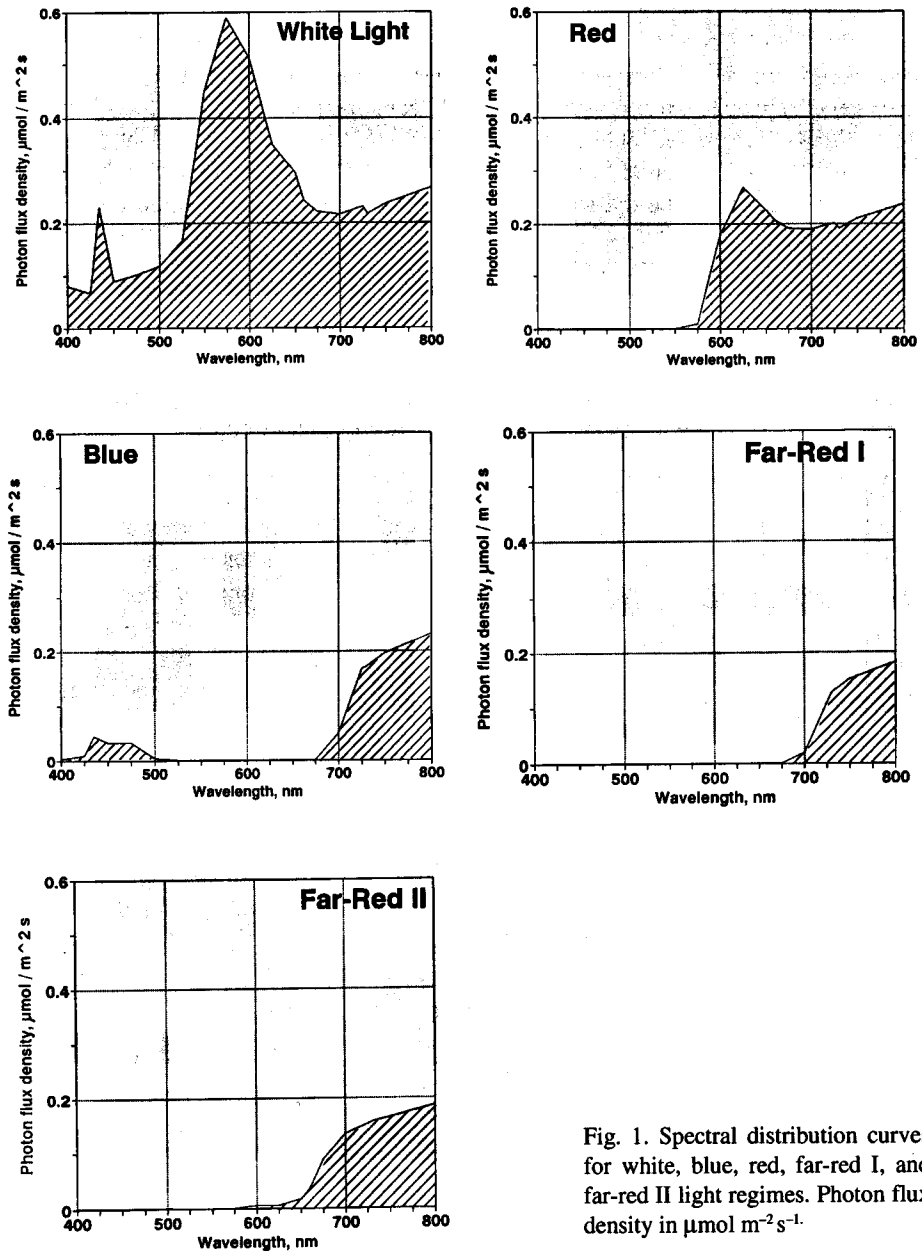


Fig. 1. Spectral distribution curves for white, blue, red, far-red I, and far-red II light regimes. Photon flux density in $\mu\text{mol m}^{-2} \text{s}^{-1}$.

addition, the values of ζ , ϕ , and total flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$), respectively, were the following; R: 1.081, 0.593, and 45.2; B: 0.001, 0.095, and 20.9; FR I: 0.001, 0.062, and 13.6; and FR II: 0.229, 0.320, and 20.7.

RESULTS

In *O. dictamnus* seeds, dark germination as a function of temperature (Fig. 2A) took place very slowly at a rather narrow range of "cool" temperatures (10–20 °C) while it was abruptly suppressed at 5 and 25 °C. However, having imbibed at inhibitory temperatures, ungerminated seeds were induced to germinate almost fully upon a subsequent transfer to the optimal temperature, 15 °C. Final germination levels of *O. dictamnus* seeds were always between 50 and 70%. Similarly, seeds of *S. syriaca* ssp. *syriaca* (Fig. 2B) germinated only partially (60–70%) in the dark, though at a warmer temperature range (20–25 °C); even at 30 °C, 40% germinated. Seed germination was fast, as illustrated by the very short T_{50} values. In *S. pomifera* ssp. *pomifera* (Fig. 2C), both temperature range and final levels of dark germination were similar to those of *O. dictamnus*. Nevertheless, the speed of germination was significantly increased and ungerminated seeds could be induced to germinate only partially upon a subsequent

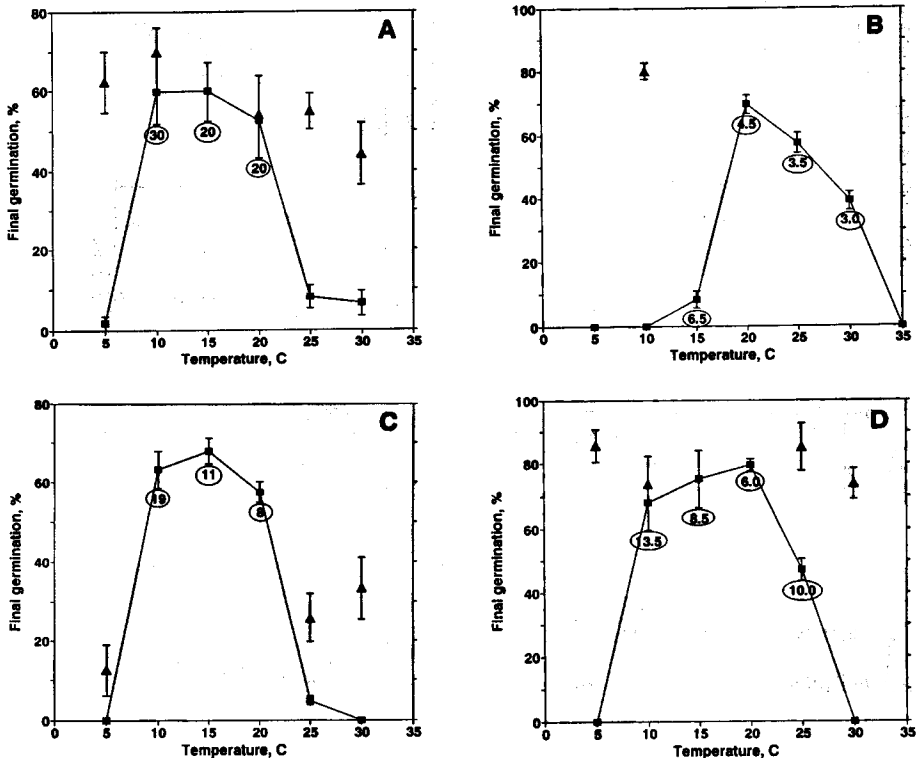


Fig. 2. Final seed germination, in darkness, as a function of constant temperature, for *Origanum dictamnus* (A), *Sideritis syriaca* ssp. *syriaca* (B), *Salvia pomifera* ssp. *pomifera* (C), and *Salvia fruticosa* (D). Final germination levels are shown by solid squares connected with straight lines; circled numbers are T_{50} values in days. The triangles represent final dark germination for the ungerminated seed samples (same samples shown in squares) after transfer to the optimal temperature (15 °C for A, C, and D; 20 °C for B). Vertical bars represent \pm standard error values.

transfer to 15 °C. In *S. fruticosa* (Fig. 2D), dark germination was optimal (70–80%) in the 10–20 °C range, although a significantly high level (nearly 50%) was scored at 25 °C. On the basis of germination speed, optimal temperature for *S. fruticosa* was 20 °C while the rate values were similar to those of *S. pomifera* ssp. *pomifera*. In contrast to the latter species, seeds unable to germinate at suboptimal temperatures could be induced to germinate after transfer to 15 °C.

Data on seed germination under “autumn” Mediterranean conditions are presented for *O. dictamnus*, *S. syriaca* ssp. *syriaca*, and the two *Salvia* species (Table 1). In *O. dictamnus*, germination was similar for seeds imbibing either in darkness or under diurnally alternating light and dark. In contrast, seeds of the other three species, imbibing under white light, were induced to germinate to levels higher than those of dark controls. This enhancement was significant for *Sideritis* and *Salvia pomifera* but statistically nonsignificant for *Salvia fruticosa*. In addition, under fluctuating temperature conditions and darkness, germination rate was considerably enhanced in *O. dictamnus* (13 days, in comparison to 20 days at constant 15 °C) while a significant increase of final germination was scored in *Sideritis* (55% in comparison to 10% at constant 15 °C).

A similar enhancement by light of either germination rate or final germination level was obtained in *O. dictamnus* and *S. syriaca* ssp. *syriaca*, respectively, under “winter” conditions (Table 2). These conditions simulated those of February and March and were significantly cooler than those of the previous experiment; as a result, the rates of

Table 1

Final seed germination (% ± standard error) under controlled conditions, simulating those prevailing during November (data from the meteorological station of Athens Airport)

Plant	Final germination, % ± SE (T ₅₀ in days)	
	W/D	D
<i>Origanum dictamnus</i>	59.2 ± 2.6 (12.3)	54.4 ± 9.5 (13.5)
<i>Sideritis syriaca</i> ssp. <i>syriaca</i>	85.6 ± 3.2 (5.4)	58.7 ± 4.1 (5.6)
<i>Salvia pomifera</i> ssp. <i>pomifera</i>	92.0 ± 3.8 (9.8)	71.3 ± 5.1 (10.1)
<i>Salvia fruticosa</i>	50.1 ± 4.5 (5.6)	36.8 ± 8.4 (5.5)

Germination experiments (in Petri dishes) lasted for 30 days either in complete darkness (D) or under diurnal alterations of white light and darkness (W/D); the daily length of the photoperiod being gradually decreased from 11.6 to 10.8 h during the experimentation period. The daily temperature fluctuation was between 15 and 20 °C at the beginning, being gradually shifted to 11–16 °C by the end of the 30-day period.

Table 2

Final seed germination (% \pm standard error) under controlled conditions, simulating those prevailing during February-March (data from the meteorological station of Athens Airport)

Plant	Final germination, % \pm SE (T_{50} in days)	
	W/D	D
<i>Origanum dictamnus</i>	60.0 \pm 4.6 (13.7)	56.8 \pm 6 (22.5)
<i>Sideritis syriaca</i> ssp. <i>syriaca</i>	50.4 \pm 2.8 (32)	0.8 \pm 0.8

Germination experiments (in Petri dishes) lasted for 60 days either in complete darkness (D) or under diurnal alterations of white light and darkness (W/D); the daily length of the photoperiod being gradually increased from 11.4 to 13.6 h during the experimentation period. The daily temperature fluctuation was between 8 and 12.5 °C at the beginning, being gradually shifted to 11.5–16.5 °C by the end of the 60-day period.

germination in both species tested were considerably slower in comparison to the conditions of Fig. 2 and Table 1.

Time courses of seed germination at 15 °C and under various, continuous light regimes were obtained with *O. dictamnus*, *S. syriaca* ssp. *syriaca*, and *S. pomifera* ssp. *pomifera* (Fig. 3). In *O. dictamnus* (Fig. 3A), final germinability was strongly suppressed by blue and far-red I light; on the other hand, an enhancement of germination rate (by nearly 5 days) was observed under red light in comparison to white, far-red II, and darkness. In the case of *Sideritis*, light was particularly important at the suboptimal temperature of 15 °C (Fig. 3B); red and blue light caused a significant induction of germination while the other illumination regimes, apart from a considerable enhancement of germination rate, had no significant effect on final germination. In *S. pomifera* ssp. *pomifera* (Fig. 3C), illuminations with low ϕ considerably inhibited final germination (irrespective of ϕ), while no differences were observed among darkness, white, or red light.

A similar enhancement of *Sideritis* germination by light was also obtained at 20 °C; germination under continuous R (fluorescent), B (fluorescent), and FR I illuminations was, respectively, 82.5 \pm 2.5, 88.4 \pm 4.3, and 54.0 \pm 4.5%. The latter value was only slightly lower (statistically nonsignificant) than in darkness (Fig. 2B). In addition, 30 days of prechilling at 5 °C resulted in only a slight enhancement of subsequent dark germination (18.2 \pm 2.8% and 80.4 \pm 2.6%, at 15 and 20 °C respectively). Prechilling was also ineffective in increasing subsequent dark germination at 20 °C in *S. fruticosus* seeds; 2 and 4 weeks of chilling resulted in 82.1 \pm 4.9% and 79.7 \pm 2.9%, respectively.

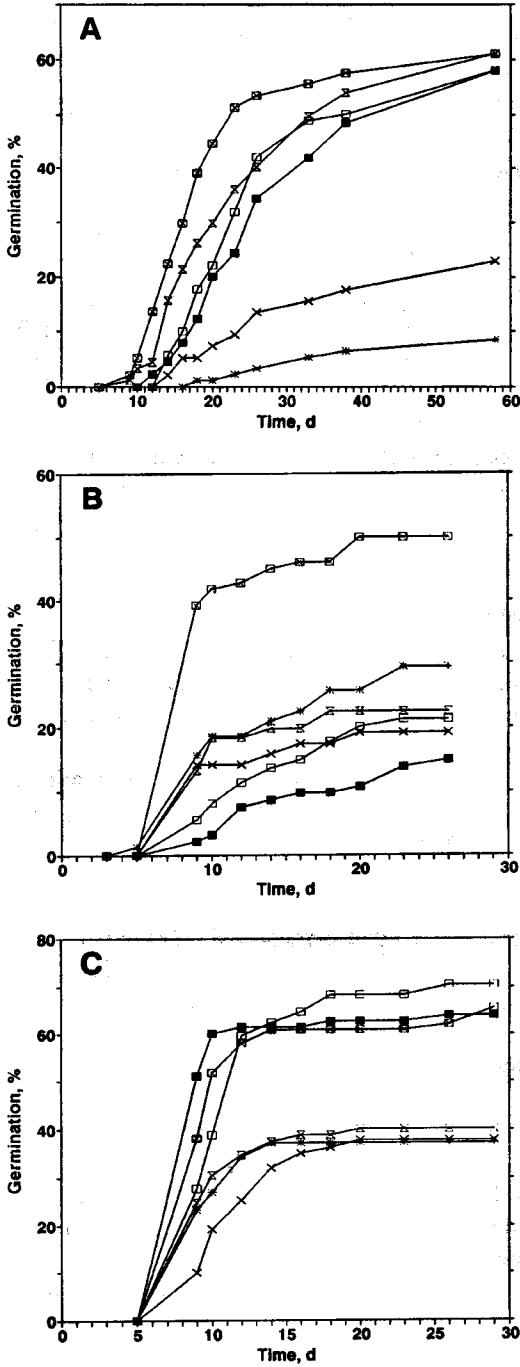


Fig. 3. Time course of seed germination at 15 °C and under various, continuous light regimes (White light: □; Red: ▣; Blue: *; Far-Red I: ×; Far-Red II: ⊠; Darkness: ■). (A) *Origanum dictamnus*, (B) *Sideritis syriaca ssp. syriaca*, and (C) *Salvia fruticosa*.

DISCUSSION

O. dictamnus seeds germinated in the dark under the relatively cool, "Mediterranean" temperature range, 10–20 °C. At suboptimal temperatures germination was simply inhibited without any induction of secondary dormancy. This species was additionally characterized by a slow germination rate, which could be significantly enhanced under fluctuating temperatures. White light also increased the speed of germination, especially under low temperature ("winter") conditions. A similar enhancement was realized under red light, while final germinability was strongly suppressed by blue and intense far-red (FR I, dense-canopy-like) light regimes.

In the endemic and endangered plant of Cyprus *Origanum cordifolium* (Cyprian dittany), the temperature range for optimum germination (80–100%) was also typically Mediterranean (5–20 °C); germination was notably slow, the fastest T_{50} values (12 days) being obtained for both 10 and 15 °C (Kadis and Georghiou, 1993). Continuous darkness or various light regimes had no effect on the germinability of the species.

In *Sideritis* seeds, dark germination as a function of temperature seems to be similar to plants of northern or alpine distribution (Thompson, 1970). Consistent with the species altitudinal distribution, germination took place at "warm" temperatures (above 15 °C), while a significant increase of final germination was scored under "autumn" (fluctuating) temperature conditions in comparison to constant 15 °C. This may be the result of the diurnal, partial presence of favorable temperatures. The particularly high rate of germination obtained (illustrated by low T_{50} values) also reflects the alpine habitat of the species. White light was found to increase both the final level and the rate of germination, the enhancement being most prominent under "winter" conditions. Light was also important at the suboptimal temperature of 15 °C; red and blue light resulted in a remarkable increase of the final germination level while a significant enhancement of germination rate was obtained under the other illumination regimes. Prechilling at 5 °C resulted in only a slight enhancement of subsequent dark germination, thus emphasizing the role of light as the principal agent of germination induction in *S. syriaca* ssp. *syriaca*.

In the two *Salvia* species studied, germinability was maximal (70–80%) in the range of 10–20 °C, but nearly 50% was obtained also at the rather warm 25 °C, in the case of *Salvia fruticosa*. The values of germination rate were intermediate between those of *O. dictamnus* and *S. syriaca* ssp. *syriaca*. White light increased final germination of *S. pomifera*, while no increase over darkness was obtained by white or red light in the case of *S. fruticosa*. Nevertheless, in the latter species, illuminations establishing a low ϕ considerably inhibited final germination. Prechilling, tested in the seeds of *S. fruticosa*, was ineffective in increasing subsequent dark germination.

Within the very large genus of *Salvia* (900 species), information or recommendations for seed germination exist for only about 20 species. According to Ellis et al. (1985), removing the nutlet covering tissues, prechilling, and treating with GA_3 are effective in overcoming seed dormancy. Similarly, the basic recommendation by ISTA (1993) is prechilling for all 8 species specified (including 4 that grow in the Mediterranean rim, as well). In *S. sclarea*, a southern European species, seed germination is reported to require

predrying or 6 months of afterripening to remove dormancy (Ellis et al., 1985). On the other hand, Macchia et al. (1983) found a quick germination (T_{50} 4–5 days) in a broad optimal temperature range (17–30 °C), and a considerably high, final dark germination (85–95%) in the range of 9–30 °C. White light was only slightly promotive at the “tails” of the temperature range, below 9 and above 30 °C. All these results generally conform to those of the present study, while both faster rate and wider range of germination in *S. sclarea* may be attributed to the more boreal character of the species in comparison to the entirely Mediterranean distribution of the species of the present study. Relief of seed dormancy in *S. glutinosa* (Thompson, 1969) was achieved by either prechilling, treatment with GA_3 , or removal of the nutlet covering tissues.

In a review on seed germination requirements of California native plants (Emery, 1988), no particular treatment was recommended for 6 out of 9 *Salvia* species, apart from sowing in the fall under the natural, Mediterranean-like conditions of California. In the well-studied Californian species *Salvia mellifera*, promotion of seed germination was obtained mainly by light but also by charate, ammonium nitrate, stratification, or presoaking in GA_3 (Keeley, 1986; Emery, 1988; Thanos and Rundel, 1995). In another California sage species, *S. columbariae*, germination was significantly enhanced by afterripening, heating the seeds at 50 °C for several weeks, soaking in GA_3 , or prechilling (Capon and Brecht, 1970; Hashemi and Estilai, 1994).

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