Web Ecol., 26, 61–70, 2026
https://doi.org/10.5194/we-26-61-2026

Seedless fruits mask germination response to key environmental factors

Patricio Garcia-Fayos[1,☆]

[1]Centro de Investigaciones sobre Desertificación (CSIC-UV-GV), Carretera CV-315 Km 10,7,
Ant. Carretera Moncada-Náquera, km 4.5, Valencia, Spain
[☆]retired

Correspondence: Patricio Garcia-Fayos (patricio.garcia-fayos@ext.uv.es)

Received: 14 November 2025 – Revised: 24 March 2026 – Accepted: 9 April 2026 – Published: 5 May 2026

Abstract. In studies on the reproduction and germination of species with indehiscent fruits containing a single seed, it is often assumed that all fruits that reach maturity contain a seed capable of generating a new plant. This is not always the case, and failing to take this into account can lead to erroneous conclusions regarding the factors affecting reproduction or germination in plants.

The research team illustrates this issue by analysing the effects of temperature, soil water potential and light exposure on the germination of *Salvia rosmarinus* fruits from four different populations, which vary in the proportion of empty fruits. When considering all fruits, we detected a negative effect of water potential in three of the four populations but no effect of temperature. However, when considering only the fruits with seeds, we detected a negative effect of water potential in all populations, as well as a positive response to increased temperature in three of them. In the temperature and light experiment, we observed that darkness reduced germination in three of the four populations when using all the fruits, but, when using only the fruits with seeds, we observed this negative effect in only one population. Despite this greater consistency of results across populations, differences persisted between populations regarding the effect of various factors on germination. These differences appear to be more a consequence of the reduced number of replicates remaining after removing empty fruits from the analyses than of any possible local adaptation of the populations. All these inconsistencies and incoherences found in this study highlight the danger of drawing conclusions from experiments and observations that do not account for the presence of seedless fruits in the samples.

1 Introduction

In plants whose reproductive unit is an indehiscent fruit with a single seed inside – namely drupes, nuts, achenes, etc. – the term “seed” is often used to refer to them, not only for convenience when writing a report but also because it is also often assumed that if the plant has invested resources in the tissues of the fruit, it is because it contains a viable seed. However, this is not always the case, and seedless fruits have been described in many plant families (Picarella and Mazzucato, 2018), which, in most cases, are morphologically indistinguishable from fruits with seeds.

The most classical causes of seedless fruits are attributed to self-incompatibility, male sterility, degradation of mother pollen cells, chromosomal irregularities during meiosis and a shortage of resources (Picarella and Mazzucato, 2018; Stephenson, 1981; Varoquaux et al., 2000). In any case, one would expect selection to favour early abortion of inbred or incompatible zygotes to minimise resource loss. However, it has been suggested that the retention of inbred and aborted seeds until the end of maturation may be adaptive (Ghazoul and Satake, 2009), often invoking escape from predation as the ultimate cause. In such cases, the plants benefit simply because indiscriminate egg-lying by pre-dispersal predators means that some of the potential predation falls on fruits containing infertile seeds rather than solely on those containing viable seeds, thereby increasing the chances that the remaining fruits will escape predation (Fuentes and Schupp, 1998;

Perea et al., 2013; Traveset, 1993; Verdú and Garcia-Fayos, 2001).

Although scientific literature has repeatedly warned about the consequences of not taking seed viability into account in experiments (Baskin et al., 2006; Bezerra et al., 2023), it is not difficult to find studies on germination in which the authors had failed to take this into account, including species with indehiscent single-seeded fruits. It could be argued that this is unnecessary, as one might expect the number of potentially empty fruits and non-viable seeds to be evenly distributed across the experimental replicates, but this assumption has rarely been explicitly tested and, when it has been, it has not been confirmed (van Hintum and van Treuren, 2012). It could also be argued that the assumption of uniformity approximates reality when a large number of replicates are used, but it does not hold when there are few replicates, which is often the case in studies on endangered and protected species, or when populations have a small number of individuals and plants produce few fruits and seeds.

In this study, our aim is to demonstrate that different conclusions are reached regarding the effects of various environmental factors on germination when the presence of seeds within the fruits is not taken into account, using *Salvia rosmarinus* Spenn as a model plant.

Based on the scientific literature on the germination of *S. rosmarinus*, we found that, in around 40 % of the studies, germination under controlled conditions did not exceed 10 % and that it was even difficult to find any study reporting a germination rate of 50 % or higher. Furthermore, with regard to the effects of treatments influencing the germination rate and speed, we found divergent and even contradictory conclusions across the various studies. Thus, while one scientific article concluded that stratification affects the germination of rosemary seeds (Madeiras et al., 2009), another found no effect (Salvador and Lloret, 1995). Similarly, some manuals on forest seed germination recommend seed stratification (Navarro-Cerrillo and Gálvez, 2001; Piotto and Di Noi, 2003), while others indicate that it does not affect germination (Navarro-Cerrillo et al., 2012). Similarly, some authors noted that darkness significantly reduces germination (Takano, 1993), while others observed no effect of darkness (Mehalaine et al., 2023), and some authors even found differences in germination depending on the light quality (Sanoubar et al., 2018). The addition of gibberellic acid (GA3) at different doses (10–500 $\mathrm{mg\,L^{-1}}$) also produced very different results; in some cases, no effect was observed (Mehalaine et al., 2017, 2023); in others, a reduction in final germination and velocity was found (Liopa-Tsakalidi et al., 2011); nevertheless, in others, an increase was observed (Madeiras et al., 2009; Nazir et al., 2024). Similar inconsistencies were found regarding the effect of heat and smoke (Moreira et al., 2010; Reyes and Trabaud, 2009; Salvador and Lloret, 1995; Trabaud and Casal, 1989) and that of soil chemicals (Cañadas et al., 2014; Fung-Boix et al., 2010; Sharma et al., 2020). Madeiras et al. (2009) suggested that these inconsistencies could be explained by differences in the percentage of seeded fruits used in the experiments, as this species produces many seedless fruits following self-pollination and geitonogamy, the proportion of which varies between populations and years (Garcia-Fayos et al., 2018). Of the studies we reviewed on *S. rosmarinus* germination, only two determined the percentage of seedless fruits in their samples (Madeiras et al., 2009; Moreira et al., 2010), and only one of them (Moreira et al., 2010) excluded empty fruits from Petri dishes from their calculations.

To shed light on this issue, we present the results of experiments on the effect of temperature regime, water potential and light on the germination of *S. rosmarinus*, checking whether the fruits remaining at the end of the experiments contained seeds and comparing the models fitted with the total number of fruits with the models fitted using only those fruits that contained seeds. We carried out the experiments using fruits from four different populations to investigate whether the differing conclusions reached by researchers might be due to differences in the origin of the fruits. At the same time, we selected these four populations along an environmental gradient of temperature and precipitation in order to explore whether, should differences between the populations exist, these could be interpreted as evidence of local adaptation.

2 Material and methods

Rosemary (*Salvia rosmarinus* Spenn., formerly known as *Rosmarinus officinalis* L.), is an aromatic perennial shrub up to 2 m tall, belonging to the family Lamiaceae. It is native to dry, sunny areas of the Mediterranean region, where it is one of the most common and widely distributed shrub species (Morales, 2010). Plants grow in dense populations and can produce hundreds of flowers simultaneously throughout the reproductive season. The flowers are self-compatible and, after pollination, can produce up to four free dry indehiscent fruits, each of which can contain a seed. Less than a third of the flowers on wild rosemary plants produce apparently healthy fruits (Herrera, 1986, 1987; Hidalgo-Fernández and Ubera, 2001), and many of these fruits contain no seed or the seed has aborted. Experimental studies carried out by our group showed that the purging of inbreeding derived from the pollination system of *S. rosmarinus* would explain much of the proportion of seedless fruits, although water scarcity could also play a role on it (Garcia-Fayos et al., 2018; Segarra-Moragues et al., 2016).

On 22 October 2025, we conducted a search of the scientific literature on the germination characteristics of rosemary using Google Scholar (https://scholar.google.com/, last access: 24 March 2026), including the words “rosmarinus” and “germination”. Of the approximately 13 700 articles obtained, we selected only 17 that reported the results of laboratory experiments which included a control treatment. In total, there were 17 articles, which are referred to in the Introduction.

In the spring of 2004, we collected fruits from four populations of *S. rosmarinus*, each with more than 5000 individuals, capturing the entire gradient of average annual temperatures and rainfall for this species in the east of the Iberian Peninsula (see Table 1 for a description of the populations).

In each population, we collected calyxes with fruits from 15 adult individuals separated by at least 2 m from the nearest sampled individual, placed them in paper bags and transferred them to the laboratory. A week later, after shaking the bags to release the mature fruits from the calyxes, we collected all the loose fruits, transferred them to new paper bags and stored them in the laboratory until use. In our experience, ripe fruits are brownish-grey in colour, compared to the lighter even green colour of unripe fruits, and most of them come loose from the calyxes after being air-dried for a few days and then shaken in a container. Waiting many days to extract them has the disadvantage that many unripe fruits also fall off, which soon turn greyish-brown, as well as increasing losses due to predation.

We conducted a germination experiment using Petri dishes, in which we crossed a temperature treatment with two other treatments: one relating to water availability and the other to light. As for the temperatures, we used three levels (5–15, 10–20 and 15–25 °C), applying each temperature consecutively for 12 h periods, so that the highest temperature coincided with light exposure and the lowest with darkness. We used three levels of soil water potential (0.00, −0.01 and −0.05 MPa) as a proxy of water availability. We simulated soil water potentials by adjusting polyethylene glycol (PEG 6000) concentrations to the highest temperature of each thermal regime, following the standard equations by Michel et al. (1983). PEG is a polymer that increases water density and allows the researcher to mimic different water availabilities during the germination. Distilled water (treatment 0.00 MPa) served as a control. We selected these temperature and water potential levels based on information on air temperature and soil drying curves following rainfalls that triggered the germination of wild plant species in the region where the fruits were collected (Garcia-Fayos and Verdú, 1998; Garcia-Fayos et al., 2000; Garcia-Fayos and Gasque, 2006; Verdú and Garcia-Fayos, 1996). The light treatment was designed to determine the effect of continuous darkness on germination in relation to the alternating light conditions between day and night. We applied this treatment only under control water potential conditions, as it was not possible to maintain constant water potential values throughout the germination experiment without exposing the fruits to light. To achieve total darkness, we covered the Petri dishes with aluminium foils that were retired at the end of the germination tests.

Between 14 and 18 June 2004, we prepared five Petri dishes for each combination of the factor levels, each with two filter papers and 30 or 50 fruits, depending on their availability. On 19 June, we added to the dishes 5 mL of the corresponding solution, covered them with transparent film to prevent evaporation and placed them into Sanyo MLR-350H growth chambers with a 12 h photoperiod. The solution of all the Petri dishes was renewed weekly, except for those dishes of the darkness treatment. Germination was checked every 3 d, and the germinated fruits were removed from the dishes. We considered a fruit to have germinated when the radicle emerged from the seed. After 48 d, the Petri dishes were left to dry completely. We then opened the fruits that had not germinated, as well as those fruits that had not been used in the germination experiments, and checked for the presence of seeds in all of them using a binocular microscope with 10–40× magnification. We considered that a fruit contained a seed when the seed completely filled the internal space of the fruit, and its tissues were white or creamy-white and turgid. We did not apply the tetrazolium test to determine seed viability since some authors found inconsistent results when applied to *S. rosmarinus* seeds (Madeiras et al., 2009), possibly because of their high oil content (see Wood et al., 2005; Quílez et al., 2020).

For the statistic analyses, we considered that the fruits were replicates and that the Petri dishes were a random factor clustering the fruits (see Sileshi, 2012, for an explanation of this approach). We fitted generalised linear mixed models with binomial error distribution and Nelder–Mead optimiser to the data from each population to predict *S. rosmarinus* germination probability in relation to the factors (temperature regime, water potential and light), first using all fruits and then only those fruits containing seeds. In the models, we use positive values for soil water potential, so that the higher the water potential value, the greater the stress on the seeds in terms of water absorption. Models with fruits that had seeds proved to be singular (the random term had no influence) for the population of Barranc dels Horts for the effect of temperature and water potential, and for the populations of Barranc dels Horts and La Murta for the effect of temperature and light, so we adjusted them with generalised linear models.

Statistic analyses were performed with R version 4.5.2 (R Core Team, 2025): package *lme4* to adjust the generalised linear mixed models, package *stats* to adjust generalised linear models and package *jtools* for the summary output of the models.

3 Results

We collected a total of 17 184 fruits of *Salvia rosmarinus* from the four populations (Table 2), of which only 17.2 % contained seeds (Table 2). Of the 8955 fruits we used in the germination experiments, only 1732 (19.3 %) contained seeds (Table 2), which considerably reduced the number of replicates (fruits with seeds) used per treatment level and population (Table 3). The proportion of fruits that germinated from among all those used in the germination experiments, a germination rate of only 11.2 % was observed, but

Table 1. Location and environmental characteristics of the populations where the fruits of *Salvia rosmarinus* were collected. Date: recollection date (year/month/day); Long: geographical longitude (decimal degrees based on the European Terrestrial Reference System 1989); Lat: geographical latitude (decimal degrees based on the European Terrestrial Reference System 1989); Alt: altitude (metres above sea level); T: temperature (°C); P: precipitation (mm). Temperature and precipitation data are annual averages for the 1971–2000 period and were obtained from "Visor del Atlas Climático de la Península Ibérica" (managed by AEMET, http://agroclimap.aemet.es/, last access: 20 December 2024).

	Date (y/m/d)	Long	Lat	Alt	T	P
LA MURTA	2004/02/10	−0.36	39.13	204	16.3	726
POBLA DE VALLBONA	2004/02/07	−0.56	39.57	99	16.7	423
BARRANC DELS HORTS	2004/05/18	−0.09	40.39	703	11.8	717
PUEBLA DE SAN MIGUEL	2004/05/27	−1.14	40.04	1313	10.6	529

this percentage rose to 57.9 % when only the fruits containing seeds were used (Table 2). All these proportions vary depending on the population from which the fruits came and the treatments used in the germination experiment (Table 2). Overall, the population with the highest level of filled fruits was Barranc dels Horts, followed by La Murta, Puebla de San Miguel and Pobla de Vallbona (Table 2). Likewise, the population with the highest level of germinated seeds was La Murta (16.3 %), followed by Barranc dels Horts (14.3 %), Pobla de Vallbona (9.8 %) and Puebla de San Miguel (8.9 %). If we calculated the proportions based on the number of fruits with seeds in each population, the values and the order of the populations changed, with La Murta again having the highest germination level (78.7 %), followed by Pobla de Vallbona (69.9 %), Barranc dels Horts (53,3 %) and Puebla de San Miguel (45.7 %), the lowest value.

Regarding the effects of temperature and water potential on germination, we observed differences in all populations between the models fitted using all the fruits and those fitted with the fruits containing seeds (Table 4 and Fig. S1 in the Supplement). The models fitted using all fruits yielded three different responses depending on the population. Thus, the germination of the fruits from La Murta was not effected by any factor; the germination of the fruits from Barranc dels Horts and Puebla de San Miguel was negatively affected by the increase in water potential but not by the increase in temperature or by their interaction (Table 4 and Fig. S1 in the Supplement; and in the case of fruits from Pobla de Vallbona, the germination was negatively affected by water potential but only at the highest temperature treatment level. However, when we built the models with only those fruits containing seeds, we found two groups of models: one for the fruits from La Murta, Barranc dels Horts and Puebla de San Miguel, in which the increase in water potential decreased germination and the increase in temperature increased germination; and another group for the fruits from Pobla de Vallbona, in which only water potential had a negative effect on germination (Table 4 and Fig. S1 in the Supplement). The standardised size of this positive effect of germination temperatures (Table 4) was indistinguishable between populations with the highest and lowest average temperatures (Table 1), as was the size of the effect of water potential, which was similar in all populations regardless of their average annual precipitation and temperatures.

Regarding the effect of temperature and light on germination, of all the populations, only that of Pobla de Vallbona showed differences in the effect of the factors on germination between the models fitted with all the fruits and the models fitted with the fruits with seeds (Table 5 and Fig. S2 in the Supplement). When we used all the fruits to adjust the models, and neither temperature nor continuous darkness affected the germination of the fruits from La Murta and Barranc dels Horts, while the germination of the fruits from Puebla de San Miguel was higher in alternating light and dark conditions than in continuous dark conditions, and that of the fruits from Pobla de Vallbona was higher under alternating light and dark conditions than under continuous dark conditions, although this only occurred at the highest temperature level used in the experiment (Table 5 and Fig. S2 in the Supplement). When we used only fruits with seeds to fit the models, we obtained a more consistent result. Thus, neither the temperature regime and light nor their interaction affected germination in any of the populations, except the Puebla de San Miguel fruits, which was negatively affected by the exposure to continuous darkness (Table 5 and Fig. S2 in the Supplement).

4 Discussion

The percentage of fruits of the different populations that germinated in the experiments was low, with values comparable to those found by us in other populations in eastern Spain (Garcia-Fayos et al., 2018) and to those reported in the studies on the germination of *S. rosmarinus* that we had reviewed. However, if we calculated the germination rate from the number of fruits containing seeds, the germination level increases 4 to 7 times, depending on the population, exceeding half of the fruits germinating when considering all the fruits from all the populations together.

For a given population, the effect of the factors on fruit germination differed depending on whether data from all fruits were used or data only from those fruits that contained

Table 2. Characteristics of the fruits of *Salvia rosmarinus* collected in the different populations: total number of fruits collected (Fruits) and number of fruits containing seeds (Filled) in the whole sample, number of fruits (Fruits), number of fruits containing seeds (Filled), and number of fruits that germinated (Germinated) in the experiment of Temperature and Water Potential and in the experiment of Temperature and Light [indicates percentages].

	Whole sample		Temperature × Water Potential			Temperature × Light		
	Fruits	Filled	Fruits	Filled	Germinated	Fruits	Filled	Germinated
LA MURTA	5623	1016 [18.1]	1942	401 [20.7]	296 [15.2]	902	187 [20.7]	167 [18.5]
P. VALLBONA	3298	421 [12.8]	1349	165 [12.2]	107 [7.9]	900	151 [16.8]	114 [12.7]
B. DELS HORTS	4117	875 [21.3]	1927	448 [23.3]	205 [10.6]	886	303 [34.2]	197 [22.2]
P. SAN MIGUEL	4146	651 [15.7]	1943	295 [15.2]	126 [6.5]	900	259 [28.8]	127 [14.1]
TOTAL	17 184	2963 [17.2]	7161	1309 [18.3]	734 [10.2]	3588	900 [25.1]	605 [16.9]

seeds. Thus, in the Temperature × Water Potential experiment, in all four populations the effect of the factors on germination changed from models using data from all fruits to those models using data from fruits containing seeds (Table 4 and Fig. S1 in the Supplement). In the case of the Temperature × Light experiment, the effect of the factors changed in only two of the populations (Table 5 and Fig. S2 in the Supplement).

If we focus on how the germination of *S. rosmarinus* is affected by the temperature regime, water potential, and light, we would conclude that each population responds idiosyncratically to these factors if we consider the data from all the fruits used in the experiments, whereas if we use the data only from those fruits that contain seeds, we would conclude that two of the populations respond in the same way, while the other two respond idiosyncratically to the factors.

Despite the improvement of the models when we used data from seed-bearing fruits rather than data from all fruits to fit the models, we found some differences between populations in relation to the factors affecting the germination of *S. rosmarinus* (Tables 4 and 5). In the Temperature × Water Potential experiment, all populations, with the exception of that of Pobla de Vallbona, showed a positive effect of temperature and a negative effect of water potential on germination; and in the Temperature × Light experiment, all populations but that of Puebla de San Miguel showed no effect of temperature and light on germination. Perhaps the populations of Pobla de Vallbona and Puebla de San Miguel really do have different germination requirements from those of the other populations, or perhaps these differences are the unintended consequence of the very small number of replicates.

Several studies have indicated that different populations of the same plant species may exhibit differences in their response to environmental factors affecting germination and that, when these differences correlate with environmental differences between populations, they can be regarded as an adaptation of the populations to their environment or as a local adaptation (Donohue et al., 2010; Walck et al., 2011). However, in our study, the fruits from populations that experienced higher mean annual temperatures did not germinate better at higher temperatures than those from other populations, nor was the negative effect of water potential on germination related to the mean precipitation or temperatures of the population from which the fruits were collected. Furthermore, a population genetics study of *S. rosmarinus*, in which we sampled several populations within the same environmental gradient used in the present study, revealed high levels of genetic diversity, along with a weak population structure caused by high levels of gene flow between populations due to the species' prolonged flowering phenology and its pollination by generalist insects (Segarra-Moragues et al., 2016), which would hinder adaptation to local conditions.

A limited number of replicates could also explain the differences between populations in the effect of factors on germination observed in our study when using fruits with seeds. In many cases, discounting seedless fruits resulted in a small number of replicates (fruits with seeds) per treatment level, with the implications that it has for model fitting (Table 3). Thus, in the Temperature × Water Potential experiment, only the populations of La Murta and Barranc dels Horts had 25 or more fruits with seeds in all treatment levels (Table 3). In contrast, the Pobla de Vallbona population had fewer than 25 fruits with seeds in eight of the nine treatment levels, and the Puebla de San Miguel population had fewer than 25 fruits in two out of the nine, implying that a difference in one germinated fruit between treatments represents more than 4 % of the total germination value. In the Temperature × Light experiment, the populations of Pobla de Vallbona and Puebla de San Miguel had four treatment levels and one treatment level with fewer than 25 replicates, respectively (Table 3). Furthermore, if we consider that 50 fruits is the minimum acceptable number of replicates per treatment level to obtain statistically consistent results, almost all treatment levels across all populations in both germination experiments would have a deficit of replicates. The implications of the low proportion of seeded fruits used in the experiments would be even more serious if we had proceeded as was done in all the studies we reviewed, in which Petri dishes instead of fruits were used as replicates, as we were unable to detect any germination in 30 of the 180 Petri dishes of the Temperature × Water Po-

Table 3. Number of cases – number of fruits of *Salvia rosmarinus* and number of fruits containing seeds – included in each treatment combination of Temperature × Water Potential and Temperature × Light. Temperature levels are expressed in °C, water potential levels in MPa and light treatment levels in hours of light exposure.

	ALL FRUITS			FRUITS WITH SEEDS		
LA MURTA						
	Temperature regime			Temperature regime		
Water Potential	5–15	10–20	15–25	5–15	10–20	15–25
0	152	148	151	30	33	35
−0.1	251	252	244	25	62	49
−0.5	249	247	248	65	58	44
Light						
12	152	148	151	27	33	29
24	150	151	150	30	33	35
POBLA DE VALLBONA						
	Temperature regime			Temperature regime		
Water Potential	5–15	10–20	15–25	5–15	10–20	15–25
0	152	149	149	16	23	45
−0.1	150	150	150	10	10	22
−0.5	150	150	149	15	16	8
Light						
12	152	149	149	29	24	14
24	150	150	150	16	23	45
BARRANC DELS HORTS						
	Temperature regime			Temperature regime		
Water Potential	5–15	10–20	15–25	5–15	10–20	15–25
0	150	149	145	37	67	48
−0.1	250	250	247	64	53	32
−0.5	247	243	246	62	51	34
Light						
12	150	149	145	64	52	35
24	148	150	144	37	67	48
PUEBLA DE SAN MIGUEL						
	Temperature regime			Temperature regime		
Water Potential	5–15	10–20	15–25	5–15	10–20	15–25
0	150	149	150	61	41	41
−0.1	249	250	248	29	26	19
−0.5	249	249	249	29	21	28
Light						
12	150	149	150	43	48	25
24	148	151	152	61	41	41

Table 4. Standardised regression coefficients, coefficient intervals at 95 % and z values of models for the effect of temperature regime and water potential on germination of the four populations of *Salvia rosmarinus*, fitting the models with all the fruits and with the fruits containing seeds. In bold are the significative terms in the models. See the corresponding figures in the Supplement.

	ALL FRUITS				FRUITS WITH SEEDS			
LA MURTA								
	Estimate	2.5 %	97.5 %	z value	Estimate	2.5 %	97.5 %	z value
Intercept	**−1.0966**	−1.2281	−0.9650	−16.3365	**0.7553**	0.5711	0.9395	8.0362
Temperature	0.1004	−0.0304	0.2313	1.5042	**0.2211**	0.0451	0.3971	2.4622
Water Potential	−0.0710	−0.2000	0.0580	−1.0785	**−0.3414**	−0.5181	−0.1646	−3.7851
$T \times$ WP	−0.0695	−0.1989	0.0600	−1.0517	0.0590	−0.1139	0.2320	0.6691
POBLA DE VALLBONA								
	Estimate	2.5 %	97.5 %	z value	Estimate	2.5 %	97.5 %	z value
Intercept	**−1.6069**	−1.7838	−1.4301	−17.8083	**0.4551**	0.1083	0.8019	2.5721
Temperature	0.1360	−0.0273	0.2994	1.6325	0.1137	−0.2133	0.4408	0.6816
Water Potential	**−0.2560**	−0.4252	−0.0867	−2.9647	**−0.3870**	−0.7330	−0.0409	−2.1915
$T \times$ WP	**−0.2384**	−0.4086	−0.0682	−2.7454	−0.1455	−0.4646	0.1737	−0.8935
BARRANC DELS HORTS								
	Estimate	2.5 %	97.5 %	z value	Estimate	2.5 %	97.5 %	z value
Intercept	**−1.3387**	−1.4580	−1.2193	−21.9758	**−0.1213**	−0.2420	−0.0007	−1.9709
Temperature	−0.0062	−0.1220	0.1096	−0.1048	**0.1495**	0.0285	0.2704	2.4215
Water Potential	**−0.3296**	−0.4489	−0.2103	−5.4147	**−0.3902**	−0.5144	−0.2660	−6.1599
$T \times$ WP	−0.0446	−0.1617	0.0725	−0.7461	0.0055	−0.1181	0.1291	0.0874
PUEBLA DE SAN MIGUEL								
	Estimate	2.5 %	97.5 %	z value	Estimate	2.5 %	97.5 %	z value
Intercept	**−1.7322**	−1.9012	−1.5632	−20.0870	**−0.2429**	−0.4334	−0.0524	−2.4994
Temperature	0.0592	−0.0994	0.2178	0.7314	**0.2382**	0.0482	0.4282	2.4575
Water Potential	**−0.4547**	−0.6211	−0.2882	−5.3529	**−0.4527**	−0.6500	−0.2553	−4.4962
$T \times$ WP	0.0646	−0.0969	0.2262	0.7842	0.0062	−0.1835	0.1959	0.0638

tential experiment, nor in 5 of the 120 Petri dishes used in the Temperature × Light experiment. Furthermore, of these Petri dishes, 14 and 4 respectively did not contain fruits with seeds, so there was no possibility of detecting germination in them.

Therefore, to determine which factors influence the germination of species with indehiscent fruits containing a single seed, as well as in reproductive biology experiments and observations of these same species, it is necessary to approximate the number of fruits used per treatment level in the experiments to the proportion of them containing seeds, in order to increase statistical power (Garcia de Santana et al., 2018). However, this does not eliminate the need to verify at the end of the experiments and observations whether the fruits contain seeds, so as to correct the calculations. This problem is not unique to the species used here as a model, as there are many other species in the same family and in many other plant families whose fruits are indehiscent and contain a single seed (see Bobrov and Romanov, 2019). This problem is also not exclusive to germination experiments, since it is also possible to find studies in the scientific literature on the seed biology of species from these same plant families that considered the presence of seeds in the fruits but also other studies that did not.

5 Conclusions

The high proportion of seedless fruits in our experimental samples, and their variation between populations, led to inconsistencies in the statistical models of the factors affecting the germination of *S. rosmarinus*. These inconsistencies were reduced, but they did not disappear when we fitted the models with data from fruits containing seeds. We attribute these inconsistencies to the consequences of the reduction in the number of replicates per treatment level that occurred when considering only fruits containing seeds, since other processes, such as local adaptation, do not appear likely to be involved. Therefore, to determine which factors influence

Table 5. Standardised regression coefficients, coefficient intervals at 95 % and z values of models for the effect of temperature regime and light on germination of the four populations of *Salvia rosmarinus*, fitting the models with all the fruits and with the fruits containing seeds. In bold are the significative terms in the models. See the corresponding figures in the Supplement.

	ALL FRUITS				FRUITS WITH SEEDS			
LA MURTA								
	Estimate	2.5 %	97.5 %	z value	Estimate	2.5 %	97.5 %	z value
Intercept	**−1.0155**	−1.2622	−0.7688	−8.0682	**1.9720**	1.3334	2.6105	6.0530
Temperature	0.0590	−0.1881	0.3062	0.4682	0.1662	−0.4785	0.8110	0.5053
Light	0.0902	−0.2539	0.4343	0.5136	0.3202	−0.6171	1.2576	0.6696
$T \times L$	0.0237	−0.3224	0.3699	0.1345	−0.0978	−1.0340	0.8383	−0.2048
POBLA DE VALLBONA								
	Estimate	2.5 %	97.5 %	z value	Estimate	2.5 %	97.5 %	z value
Intercept	**−1.2940**	−1.4716	−1.1164	−14.2833	**0.5923**	0.1504	1.0342	2.6271
Temperature	−0.1407	−0.3139	0.0326	−1.5913	0.0893	−0.3204	0.4990	0.4273
Light	0.1852	−0.0553	0.4257	1.5093	0.2954	−0.2756	0.8663	1.0138
$T \times L$	**0.5089**	0.2674	0.7503	4.1313	0.2226	−0.3385	0.7838	0.7777
BARRANC DELS HORTS								
	Estimate	2.5 %	97.5 %	z value	Estimate	2.5 %	97.5 %	z value
Intercept	**−0.7738**	−0.9325	−0.6152	−9.5600	**0.7112**	0.3593	1.0631	3.9609
Temperature	−0.0931	−0.2515	0.0653	−1.1521	0.3049	−0.0401	0.6499	1.7322
Light	−0.0114	−0.2346	0.2119	−0.0997	−0.1212	−0.6083	0.3659	−0.4877
$T \times L$	0.1816	−0.0431	0.4063	1.5836	−0.2683	−0.7578	0.2212	−1.0742
PUEBLA DE SAN MIGUEL								
	Estimate	2.5 %	97.5 %	z value	Estimate	2.5 %	97.5 %	z value
Intercept	**−1.2945**	−1.4831	−1.1058	−13.4509	−0.2591	−0.5210	0.0027	−1.9395
Temperature	−0.0385	−0.2284	0.1515	−0.3969	0.1832	−0.0957	0.4621	1.2873
Light	**0.3578**	0.1080	0.6076	2.8070	**0.4196**	0.0666	0.7725	2.3300
$T \times L$	−0.0315	−0.2843	0.2212	−0.2446	−0.0913	−0.4498	0.2672	−0.4992

germination, as well as in reproductive biology experiments and observations involving species in which the indehiscent fruit containing a single seed is the unit of analysis, it is necessary to adjust the number of fruits used per treatment level in the experiments according to the proportion of them containing seeds (as done by Moreira et al., 2010). This optimises the number of fruits with seeds and increases statistical power. However, this does not eliminate the need to check at the end of experiments and observations whether the fruits that did not germinate contain seeds, in order to correct the calculations. This further reinforces the recommendation – which is not always followed – to also determine how many seeds are viable at the end of experiments or observations in which the seed is the unit of measurement.

Code and data availability. The R code and the data used are available at https://doi.org/10.6084/m9.figshare.30609617 (Garcia-Fayos, 2026).

Supplement. The supplement related to this article is available online at https://doi.org/10.5194/we-26-61-2026-supplement.

Competing interests. The author has declared that there are no competing interests.

Acknowledgements. I would like to thank Jorge Sellés for his extraordinary contribution throughout the experimental work on which this article is based. He helped me to collect the fruits, cleaned them all, carried out the germination experiments and checked for the presence of seeds in all the fruits. I would also like to thank the two anonymous reviewers whose suggestions greatly helped me to improve the original paper. Thanks also to the Fundació Caixa Castelló for allowing us to collect the fruits on their property in Mas Vell.

Financial support. This research was made possible by financial support from grants (REN2000-0163-P4-05 ECOFIARB and CGL2009-07262, ROMERO) from the Spanish Ministry of Science and Innovation (MICINN).

Review statement. This paper was edited by Adrian Brennan and reviewed by two anonymous referees.

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