1 **Local conditions effects on seed germination of *Hypericum balearicum***

2 **L. in response to temperature**

3 Running head title: Local conditions and temperature requirements

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Abstract

Temperature, which is one of the most relevant abiotic factors affecting seed germination, is strongly influenced by local conditions. Here, we collected seeds of *Hypericum balearicum*, an endemic shrub of the Balearic Islands, from 11 sites of Mallorca. We investigated variations in final germination percentage (FGP), germination rate (t_{50}) and germination timing (t_0) of seeds from different provenances according to the local conditions of each place (here classified in mountain, coast and valley environment) under a range of temperature treatments (12–24 ℃). An environmental data logger was installed in three of the sites to determine the temperature variability for each environment. FGP was neither affected by temperature treatments nor by the environment. The coast environment showed a higher t_{50} and t_0 values in all temperatures tested compared to mountain and valley, especially at low temperatures with mountain environment and at low and high temperatures with valley environment. These differences among environments were consistent with the temperature parameters recorded in each site. *Hypericum balearicum* germinates in the optimal temperature range of Mediterranean plants. Overall, this study demonstrates the importance of taking into account temperature local conditions effects in germination in order to know how plants will respond to environmental changes.

Keywords: Environmental conditions, germination requirements, temperature treatments, Mediterranean plants.

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1. Introduction

Seed germination is a crucial process in plant cycle life as it determines the further development of the plant's life cycle (Fenner and Thompson, 2005; Donohue et al., 2010). Indeed, seed germination affects seedling establishment, survival and plant fitness, which in turn determine species persistence and distribution (Cochrane et al., 2015). Germination has the potential to influence the evolution of plant traits because it is one of the earliest first-stage transition, which provide the background for later development and natural selection. As such, the environmental conditions under which seeds germinate will influence the phenotypic expression of post-germination traits and the natural selection of those traits (Donohue et al., 2010). Seed germination, seedling establishment and recruitment are early stages of the plant life cycle that are heavily influenced by their habitat and climatic parameters such as optimal temperature, soil moisture, oxygen availability, light and physical and chemical soil characteristics (Fenner and Thompson, 2005).

Germination timing, germination rate and germination percentage are considered to be key traits of seed germination. Germination timing is closely related to species fitness because it determines when a seedling begins to be exposed to the environment (Donohue, 2005; Donohue et al., 2010). Germination rate (t_{50}) is related to competitive ability of plants as early seed germination and seedling emergence enhances plant fitness (Verdú and Traveset, 2005; Mummey et al., 2016). Finally, the germination percentage reflects seed viability and the plant colonization capacity (Jiménez-Alfaro et al., 2016).

Several studies have shown that patterns of germination requirements of a species may differ along sites and microhabitats both within and between species, which have been interpreted as an adaptation to environmental variations (McWilliams et al., 1968;

Meyer et al., 1997; Shimono and Kudo, 2003; Liu et al., 2017). This variation in germination response has been considered to be an integration of environmental, especially temperature (Keller and Kollmann, 1999) and genetic factors (McWilliams et al., 1968). Within the phenotypic factors affecting germination, the maternal effect is considered to play a relevant role. These effects are determined by a combination of the environmental conditions experienced by the seed due to its position in the mother plant and the environmental factors to which the plant is exposed (Gutterman, 2000). Besides, seed mass is often affected by environmental factors. Several studies demonstrate how seed mass may vary between sites, both within and between species, in order to increase their fitness according to the local environmental conditions (Wang et al., 2014; Liu et al., 2017), which in turn can have a direct effect on seed germination and dormancy (Vera, 1997; Atia et al., 2009) and on seedling emergence and establishment (Pearson et al., 2002; Baraloto et al., 2005). In seasonal climates, temperature is one of the most relevant abiotic factors in germination, since it regulates seed germination timing, rate and percentage (Fenner and Thompson, 2005; Baskin and Baskin, 2014; Chamorro et al., 2018). In addition, as many studies have shown, the optimal germination temperature may vary between seeds from the same species located in different provenances or localities (Liu et al., 2017; Wu et al., 2019).

Some climatic factors, such as temperature, show large spatial variations in mountainous areas (Erpicum, 1984). These spatial variations have been largely attributed to elevational gradients, with temperature decreasing as elevation increases (Körner, 2007) but also to the orientation of the site and the influence of the local winds (Whiteman, 2000; Cuxart et al., 2007). Several studies indicate that while elevation is the most important factor in estimating temperature variations on large scales, the particular topographical features of each site can change the relationship between elevation and

temperature on smaller scales (Daly, 2006; Jiménez et al., 2015, 2020). Particularly, in mountain peaks or summit sites weather conditions correspond to those from the free atmosphere resulting in winds and temperatures related to those from large-scale origin with a no clear diurnal cycle; on the other hand, valleys and depressions are more sheltered from the large-scale winds resulting in records of wind and temperature with clear diurnal cycle controlled by the solar radiation (Pepin and Norris, 2005) and strongly influenced by the local features (topography, soil cover, etc). Particularly, during nighttime in the abscense of solar heating or significant winds effects, cool and dense air drains into local valleys and depressions causing temperature inversions (Clements et al., 2003) and an opposite circulation is found during daytime (Whiteman, 2000). Finally, it is also important to highlight that in coastal regions, the diurnal cycle of the temperature is influenced by the sea and land breezes, a local wind generated due to the thermal gradient between the land and sea during daytime or nighttime, respectively. As a result, the amplitude of the diurnal cycle is reduced in comparison to the inland regions because of the cold advection generated under sea-breeze conditions (Cuxart et al., 2014). Hence, at a regional or local level, important variations in temperature traits due to local features may be observed.

Overall, the intra-species variation in germination temperature requirements in different localities or provenances may be attributed to local adaptation to climatic factors. Besides, other plant reproductive characteristics, such as flowering phenology, pollination and breeding systems, have been used to understand plant responses to environmental changes (Jiménez et al., 2014; Segui et al., 2018). In this line, the knowledge of the reproductive biology of a species in natural plant populations is vital for their sustainable use, management and conservation (Gaudeul and Till-Bottraud, 2004; Cursach and Rita, 2012a). Therefore, studies about temperature germination

requirements of seeds from different provenances are important in order to understand the adaptation strategies to environmental changes, which in turn would have also implications for plant conservation.

Germination studies of seeds from different origins are available for some *Hypericum* species (Carta et al., 2016; Pérez-García et al., 2006). However, there are no reported studies of germination requirements as a function of local seed origin in *Hypericum balearicum*.

The main objective of this study was to assess if differences among sites in temperature germination requirements of a Mediterranean shrub derived from the local conditions of each site are present. Specifically, we aimed (i) to determine how seed germination percentage, germination rate and germination timing from different local conditions origins respond to temperature treatments; (ii) to evaluate if the possible changes of germination requirements among sites were consistent with the temperature traits of each environment; and (iii) to determine whether the mass of the seeds was influenced by the local conditions characteristics of each site. The target species was *Hypericum balearicum* L., an endemic shrub of the Balearic Islands (Western Mediterranean Basin), since its distribution in Mallorca (the largest island of the Archipelago) is widely varied covering from the coastal to mountainous areas up to 1400 m asl, where local conditions can modify temperature fluctuations across very close locations. Here, we selected 11 sites of *H. balearicum* located in three areas with particular climatic conditions (mountain, coast and valley environment), and we performed in vitro germination tests under a range of temperatures (from 12 to 24 ℃). We hypothesised that *H. balearicum* would exhibit differences among sites in germination requirements according to temperature associated with the local temperature traits of each environment.

2. Materials and methods

2.1.Study species

Hypericum balearicum L. (Hypericaceae) is a woody evergreen shrub, of at least one meter in height (Ramos, 1993), which has hermaphroditic and homogamous flowers. The fruit is a dehiscent, glandular and viscous capsule, and the five carpels and stigmas of the gynaecium persist in the ripe fruit. Ripe fruits do not detach from the mother plant. Seeds are black and shiny with longitudinal grooves which are released when the plant is moved by the wind, although many of the seeds remain stuck to the wall of fruits due to its viscosity. For this reason, seed dispersal requires the drying of the fruit (Tébar, 1992). The species flowers all year round, although the main flowering peak occurs during late spring and early summer (May to mid-July), which is when maximum floral visitations are reached. Besides, during the rainy season of the Balearic Islands (autumn and winter) (Jansà et al., 2014) a second flowering peak appears but to a lesser degree than the last spring and early summer peak (Tébar et al., 2004; Rodríguez-Pérez and Traveset, 2016).

Hypericum balearicum is the diagnostic species of *Hypericion balearici* O. de Bolòs et Molinier 1958 plant community which is characterized by being shrub communities with cushion-like formations (xeroacanthic thickets) that are typical of mountain peak landscapes, but not exclusively, as they also appear in other mountain or coastal areas (Llorens and Gil, 2017). These environments are characterised by intense wind exposure (with saline deposition in coastal areas) and shallow soils. The species is located in Mallorca, Menorca, Cabrera and Eivissa, although the greatest representation of the species is found in Mallorca. Currently, *H. balearicum* is not subject to significant threats or legal protection (Sáez et al., 2017).

2.2.Study area

The selected study site was the island of Mallorca. This island consists of two

mountain ranges (at the northwest and southeast) and an elevated area in the center,

resulting in three main basins (Fig. 1a). The complexity of the terrain and the surface

features in Mallorca are responsible for the locally-generated climate regimes (Jiménez

et al., 2020), such as the sea or land breezes in the three main basins (Cuxart et al., 2014)

or the slope winds (Cuxart et al., 2007). Besides, cold pools are frequently reported in the

bottom parts of the valleys and basins, due to the accumulation of cold air enhanced by

the slope winds (Jiménez et al., 2015). As a result, the amplitude of the diurnal cycle (here

computed as the difference between the temperature maximum and minimum) is often

larger in the bottom parts of the valleys/basins and reduced in the coast, meanwhile it is

nearly zero at the mountain peaks.

2.3.Seed sampling

We collected mature seeds of *H. balearicum* from 11 sites located in different areas of

Mallorca (Balearic Islands). These sites were divided into three groups taking into

account the local condition features of each site. Thus, the selected groups were: mountain

(M1, M2, M3, M4, M5), coast (C1, C2, C3) and valley (V1, V2, V3) (Table 1). The

criterion taken into account to classify the sites by the three environments was based in

the amplitude of the diurnal cycle of the temperature and the influence of the locally-

generated winds. The sites close to the sea are influenced by the presence of sea and land

breezes and with the diurnal cycle reduced by the advection from the sea were classified

as coast. On the other hand, the sites more influenced by the large-scale winds (prevailing

winds of the region) and with diurnal temperature amplitudes reduced were considered

as mountain. Finally, the valley sites were those influenced by the local winds and with a

184   larger diurnal cycle (during nighttime very often the temperature at lower levels cools

185   down and cold pools are generally formed, Jiménez et al., 2020). An example of this

186   thermal amplitudes for a particular day is shown in Fig. 1b for a mesoscale simulation (at

187   1km x 1km resolution over the island of Mallorca, see more details in Cuxart et al., 2014).

188   Mature fruits were collected in 2018 during the period of maximum natural

189   dispersal (August and September), and were thus coming from the most pronounced

190   flowering peak of the species (spring–summer). In sites with a small population size, we

191   collected fruits from all the mother plants in order to collect the maximum genetic

192   variability, whereas in sites with a large population size, fruits were collected from 20–

193   50 mother plants randomly selected across the entire distribution area (Bacchetta et al.,

194   2008). In the laboratory, fruits were opened to remove the seeds. Finally, seeds of each

195   site were stored separately in paper bags at environmental temperature until the start of

196   germination tests (next November).

197

198   *2.4.Climatic data*

199   A wireless environmental data logger (Kestrel® DROP D2) was installed inside a

200   ventilated protective box, about five centimetres above the soil, in three out of the 11

201   study sites (M3, C2 and V1) to obtain air temperature data during autumn, winter and

202   spring. The wire environmental data loggers were strategically placed in a location in the

203   mountain, coast and valley environment: Puig Major (M3), Cala Figuera (C2) and Cúber

204   (V1), respectively. Temperature data (hourly) for this study were collected from

205   4/11/2019 to 4/05/2020. After the sampling season, the mean daily temperature was

206   calculated, as well as minimum and maximum daily values and the amplitude of the

207   diurnal cycle of temperature. A climatological study carried out in Mallorca showed that

there is a correlation between soil and surface temperature, which depends on the time of the year and whether it is day or night (Simó et al., 2019).

These temperature parameters were only used to compare the thermal features of the three environments. These values might change for the different sites within each category due to the local features. In this way, we were able to compare if seed germination of *H. balearicum* varies according to the temperature traits of each environment.

2.5. Germination experiments

Seed batches from the 11 sites were tested for germination separately. Nevertheless, the results from the sites belonging to the same environment were pooled in the data analysis after the germination test. Therefore, the results were not analysed by each site individually but by the defined environments.

Germination experiments were performed to investigate the effects of temperature (at 2 degree intervals from 12 ºC to 24 ºC) and two types of light requirement (12 h of irradiance per day and total darkness) on germination percentage, germination rate, and germination timing. The temperature germination range performed in this study was selected according to the typical germination temperature range of Mediterranean species (15 ºC – 20 ºC) (Bell et al., 1995; Thanos and Doussi, 1995).

The germination experiments were carried out in a growth chamber (Ing. Climas, GROW/HR 260) from November 2018 to June 2019. In each germination test, temperature was kept constant both in the light (12 h of irradiance per day) and in total darkness. In this study, we selected constant temperature treatments because they have been used in other recent studies in order to assess differences among sites in germination temperature requirements (Liu et al., 2017; Zettlemoyer et al., 2017; Wu et al., 2019).

Seeds of each site were sown on agar plates (1% in distilled water) inside Petri dishes (15 cm diameter × 2.5 cm deep). Seeds were not pre-treated as there is no evidence that *H. balearicum* have seed dormancy (Galmés et al., 2006; Tébar, 1992). Seeds were incubated under seven constant temperatures (12, 14, 16, 18, 20, 22 and 24 ℃). Specifically, seeds from Mortitx were not tested at 18 ℃ and 20 ℃.

In each temperature treatment the two types of light requirement were assessed in the same germination chamber, equipped with four white fluorescent lamps (Philips TL-D 30W/830 with a photosynthetic photon flux density of 30 μmol m-2 s-1). Thus, in dark treatments Petri dishes were wrapped in aluminium foil in order to avoid any contact with light. Petri-dishes were randomly distributed in the germination chamber and their position was changed every 2–3 days. For each temperature treatment and site, 8 Petri dishes replicates of 25 seeds each were set (4 light conditions replicates and 4 total darkness condition replicates). All seeds used in germination tests were previously weighed with an analytical balance measuring to the nearest 0.0001 g. Thus, the 25 seeds of each replicate were weighed separately for each site and temperature.

Over a 36-day incubation period, seed germination was checked every 2–3 days and germinated seeds were counted and removed. Seed germination incubated in darkness was checked under a green light chamber to avoid light stimulating effects on seed germination (Luna et al., 2004). An emerged radicle exceeded 1 mm in length was used as the criterion for germination.

At the end of the germination tests, non-germinated seeds were cut and inspected under a stereomicroscope in order to evaluate seed viability. Seeds evaluated were divided into two groups: (1) fresh, firm and possibly viable seeds and (2) dead seeds (with soft and brown embryo colour) and empty seeds. Those seeds classified in the second group (dead and empty seeds) were not included in the study, and consequently, all the results

258 presented were corrected for fresh and firm seeds (Bonner, 1974; Shimono and Kudo,

259 2003; Kołodziejek et al., 2018).

260 The following parameters were calculated for each germination test and replicate:

261 1. Final germination percentage (FGP) (Bonner, 1974; Shimono and Kudo, 2003;

262 Kołodziejek et al., 2018):

$$263 \quad FGP\ (\%) = \frac{Germinated\ seeds}{Total\ number\ of\ seeds - Dead\ and\ empty\ seeds} \times 100$$

264 2. The number of days required to reach 50% of final germination percentage (t_{50})

265 (Côme, 1970; Thanos and Doussi, 1995):

$$266 \quad T_{50} = \frac{[\left(\frac{N}{2}\right) - N_1](T_2 - T_1)}{N_2 - N_1} + T_1$$

267 Where N is the final percentage of germinated seeds, N1 is the percentage of seeds

268 germinated below N/2, N2 is the percentage of seeds germinated above N/2, T1 is the

269 number of days corresponding to N1, and T2 is the number of days corresponding to

270 N2.

271 3. The germination timing or the first germination day (t_0)

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273 **_2.6.Statistical analyses_**

274 All statistical analyses were performed in R version 3.6.3 (R Development Core Team

275 2020).

276 *2.6.1. Climatic data*

277 In order to evaluate differences in daily mean, maximum and minimum temperatures and

278 the amplitude of the diurnal cycle among the environments, we performed a one-way

279 analysis of variance (ANOVA) or a non-parametric Kruskall-Wallis test (Sall et al.,

280 2012).

281 *2.6.2. Germination data*

Before grouping the sites into the three environments, differences between the two light requirements treatments of the three response variables (FGP, t_{50} and t_0) were analysed for each site. Generalized linear models (GLM) were performed for FGP and t_0, which were fitted to a binomial and Poisson distribution, respectively. To assess differences in light requirements treatments of t_{50} data we performed a t-Student test or the non-parametric test Mann-Whitney-Wilcoxon was applied. In spite of few exceptions, the sites did not show differences in light requirements for the three response variables. For this reason, the two light requirement treatments were not taken into account for the following data analyses.

In order to evaluate differences in seed mass among environments, a two-way nested ANOVA was performed including "Location" as a nested factor in "site".

We evaluated the effect of temperature and the environment on FGP and t_0 using a nested Generalized Linear Model (GLM) with "Sites" as a nested factor in "Environment", fitting FGP to a binomial distribution and t_0 to a Poisson distribution. In order to analyse t_{50}, we performed a two-way nested ANOVA including "Site" as a nested factor in "Environment". Seed mass variable was introduced in the germination variables analysis as a covariate.

Significant differences highlighted by two-way nested Anova and nested GLM were analysed using post-hoc Tukey multiple comparison tests.

3. Results

3.1. Climatic data

The three sites where the temperature data were evaluated (from 4/11/2019 to 4/05/2020) showed significant differences in all climate variables (Table 2). The lowest mean,

maximum and minimum temperatures were found in the mountain site. In contrast, the coast site showed the highest mean and minimum temperatures. Finally, for the valley site the maximum temperature is the largest whereas the minimum is the lowest resulting in the largest daily amplitude.

3.2. Seed mass

Seed mass show significant differences among the three environments ($F_{2,589}$ = 57.93, P = < 0.0001) and among sites nested in environment ($F_{8,589}$ = 60.86, P = < 0.0001). Seed mass from the valley environment was significantly larger than the mountain and coastal environments (Table 3).

3.3. Germination percentage

For each environment, the cumulative percentage of germination tended to increase with the incubation time and was stabilized at the end of the germination test (Fig. S1). This pattern was not observed under the lowest temperature treatment (12ºC).

Neither the temperature nor the seed mass showed a significant effect on the FGP (χ^2 = 3.239, df = 6, P = 0.778 and χ^2 = 3.818, df = 2, P = 0.051, respectively). Environment showed a significant effect on FGP in the nested ANOVA (χ^2 = 6.752, df = 2, P = 0.034), although the post-hoc analysis by Tukey's multiple comparison test did not show significant differences in FGP among environments. Finally, site nested in environment showed a significant effect in FGP (χ^2 = 44.357, df = 8, P < 0.0001).

The mean FGP values of the three environments for each temperature treatment are as follows: 12 ºC (56.85±2.45), 14 ºC (64.66±2.41), 16 ºC (63.39±2.24), 18 ºC (67.10±2.17), 20 ºC (61.73±2.35), 22 ºC (68.55±2.32) and 24 ºC (63.52±2.40).

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3.4.Germination rate (t_{50})

Temperature, environment and site nested in environment showed a significant effect on the number of days to achieve 50% of final germination percentage (t_{50}) ($F_{6,572} = 199.213$, $P < 0.001$; $F_{2,572} = 36.583$, $P < 0.001$; $F_{8,572} = 20.822$, $P < 0.001$, respectively). Seed mass did not showed a different effect on t_{50} in the three environments ($F_{2,572} = 0.467$, $P = 0.627$).

The treatment at 12 ºC showed significant differences with all temperature treatments in the three environments, with the 12 ºC treatment exhibiting the highest t_{50}. At high temperatures, the treatments at 22 ºC and 24 ºC are those that exhibited the lowest t_{50} (Fig. 2). Treatment at 14 ºC showed differences in the three environments with 18, 22 and 24 ºC. The mountain and valley environment showed significant differences with the coast environment on t_{50} in all temperatures tested being the coast the one with the highest t_{50} in all the mentioned temperatures. On the other hand, there were no significant differences in t_{50} between mountain and valley. Differences between the mountain and the coast environment were more pronounced in the lowest temperature treatments (12, 14 and 16 ºC). However, differences between the valley and coast environment were more pronounced in the lowest and in the highest temperature treatments (12, 14, 16 ºC and 22, 24 ºC).

3.5.Germination timing (t_0)

In the same way as t_{50}, temperature, environment and site nested in environment showed a significant effect on the germination timing ($\chi^2 = 509.1$, df = 6, P = <0.0001; $\chi^2 = 79.29$, df = 2, P = <0.0001; $\chi^2 = 34.21$, df = 8, P = <0.0001, respectively). Seed mass did not show different effects on t_0 in the three environments ($\chi^2 = 1.67$, df = 2, P = 0.434).

357   *Hypericum balearicum* begun to germinate between the 5th and the 20th day from

358   the sown date depending on the temperature treatment and the environment, except for

359   the 12 ℃ treatment in the coast environment (Fig. S1; Fig. 3). This treatment exhibited

360   the highest t_0 in all environments (18.3, 18.9, 24.9 days on average for mountain, valley

361   and coast environment, respectively). Concerning the treatments at 22 ℃ and 24 ℃, they

362   showed the lowest value compared to the rest of temperatures tested (valley: 8.2 and 7.8

363   days; mountain: 9.2 and 9.2 days; coast: 10.8 and 11.1 days at 22 ℃ and 24 ℃,

364   respectively). In all environments, treatments at 14, 16, 18 and 20 ℃ exhibited similar t_0

365   values (Fig. 3).

366   Mountain and valley showed significant differences with coast on t_0 in all

367   temperatures tested being the coast environment the one with the highest t_0 in all the

368   mentioned temperatures. On the other hand, there were no significant differences in t_0

369   between mountain and valley. In addition, differences in t_0 between the mountain and

370   valley environment compared to the coast environment were more pronounced in the low

371   temperature treatments (12, 14 and 16 ℃) (Fig. 3).

372

373   **4. Discussion**

374   In this study, we show that *Hypericum balearicum*, an endemic shrub of the Balearic

375   Islands (Western Mediterranean Basin), does not exhibit differences in the final

376   germination percentage (FGP) related to temperature among environments (mountain,

377   coast and valley). However, the species exhibits a different germination rate (t_{50}) and

378   germination timing (t_0) behaviour among environments: both germination variables

379   values were lower in mountain and valley compared to coast in all the temperatures

380   treatments.

381

4.1.The general germination requirements of H. balearicum

Both the optimal germination temperature (niche position) and temperature range in which most germination occurs (niche breadth) are important in order to determine the distribution and persistence of species (Luna and Moreno, 2010). *Hypericum balearicum* does not show significant differences in the FGP among temperature treatments in any environment, suggesting that *H. balearicum* in this temperature range has a great plasticity in terms of the germination percentage. Although there were not statistically significant differences between temperatures, the treatment at 12 ºC is the one that exhibit the lowest FGP value and a different germination cumulative percentage pattern compared to the rest of temperatures tested, suggesting that 12 ºC may be the limit of the optimal germination range of *H. balearicum*..

There are few studies about germination temperature requirements of *Hypericum* species. The plasticity in terms of germination percentage was also reported by Galmés et al., (2006) who incubated seeds of *H. balearicum* at three alternating temperatures (12/12h): 5/15 ºC, 10/20 ºC and 15/25 ºC to simulate the diurnal temperature fluctuations of winter, autumn / spring and summer, respectively. In that case, *H. balearicum* did not show significant differences in the FGP between the alternating temperatures tested. In the same way, Pérez-García et al., (2006) and Porceddu et al., (2020), found that temperature had no significant effect on final germination percentages in *H. perforatum* and *H. scruglii*, respectively. In addition, in the same way of our results, Porceddu et al., (2020) also reported that temperature had a significant effect on the germination rate (t_{50}). Contrary to our findings, seeds of other *Hypericum* species have light requirements for germination (Pérez-García et al., 2006; Sánchez-Coronado et al., 2015; Carta et al., 2016)

405 and they need specific treatments to promote germination (e.g. alternating temperature

406 regime, gibberellins) (Sánchez-Coronado et al., 2015; Carta et al., 2016).

407   The final germination percentages results suggest that *H. balearicum* germinates

408 well within the typical temperature range of Mediterranean species, at relatively low

409 temperatures, between 15 ℃ and 20 ℃ (Bell et al., 1995; Thanos and Doussi, 1995; Podda

410 et al., 2017; Martínez-Díaz et al., 2018). The Mediterranean climate of the Balearic

411 Islands is characterized by the alternation of two main seasons: a hot and dry summer and

412 a wet and mild season (November to April), during which unpredictable and heavy

413 precipitations are frequent during autumn (Homar et al., 2010). For this reason, in order

414 to increase the probability of establishment and survival of seedlings (Bell et al., 1995),

415 the temperature germination period of most of the Mediterranean species is consistent

416 with the rainy period of the Mediterranean climate (Céspedes et al., 2012).

417   On the other hand, the species showed the highest values of t_{50} and t_0 (i.e., lower

418 speed and more days to germinate) at 12 ℃ and the lowest values in the treatments at 22

419 ℃ and 24 ℃ in all the environments. In the same way, some studies indicate that higher

420 temperatures accelerate seed germination by increasing both the germination rate and the

421 germination timing (Milbau et al., 2009; Chamorro et al., 2013; Zettlemoyer et al., 2017).

422 Specifically, Chamorro et al., (2013) reported that other three Mediterranean shrubs

423 (*Cistus ladanifer* L., *Lavandula pedunculata* Mill. (Cav.) and *Thymus mastichina* L.)

424 show a higher germination rate as temperature increases. In the same way, both *Naufraga*

425 *balearica* Constance & Canon, an endemic plant of Mallorca (Balearic Islands) and

426 *Apium bermejoi* L. Llorens, an endemic plant of Menorca (Balearic Islands) germinated

427 faster (lower t_{50}) under a higher temperature regime (25 ℃/15 ℃ light/dark) compared to

428 a lower temperature regime (20 ℃/16 ℃ light/dark) (Cursach and Rita, 2012a, 2012b).

429   It has been reported that seed dispersal of *H. balearicum* occurs mainly during

430   summer (Tébar, 1992; Rodríguez-Pérez and Traveset, 2016). Thus, the ability to

431   germinate at high temperatures (22–24 ºC), the increase in the germination rate and

432   germination timing at such high temperatures, as well as the lack of a dormant period

433   could be harmful for the seedling recruitment of the species as the seeds would begin to

434   germinate in case there was an input of moisture (i.e., summer rains). Consequently, *H.*

435   *balearicum* could germinate in a period which is not suitable for the typical species of the

436   Mediterranean climate (Bell et al., 1995; Podda et al., 2017; Martínez-Díaz et al., 2018).

437   However, during the Balearic Islands summer, higher temperatures occur than those

438   tested in this study (autumn and spring), and thus studies focusing on the germination

439   requirements of *H. balearicum* at higher temperatures than 24 ºC are necessary in order

440   to determine whether there is a germination blockage at higher temperatures (Galmés et

441   al., 2006).

442   Under a context of climate change, it is predicted that the average temperature of

443   the earth's surface will increase up to 4 °C by 2100 (Pachauri et al., 2014), and the

444   Mediterranean Basin is one of the regions where the greatest changes are expected

445   worldwide (Giorgi and Lionello, 2008). Besides, it is important to mention that winter

446   2019/20 and spring 2020 in the Balearic Islands was classified as extremely warm in

447   comparison to the period 1965–2020, according to the AEMET seasonal bulletins

448   (AEMET ESPAÑA, 2020). Plant species will only be able to survive these new climatic

449   conditions by adapting through natural selection to the new environmental situation or by

450   migrating to conditions where they are adapted (Nicotra et al., 2010). Therefore, due to

451   the plasticity in the germination percentage according to temperature of *H. balearicum*

452   and the higher germination rate and germination timing at high temperatures (22 ºC and

453   24 ºC), this species is expected to be less affected by climate change than Mediterranean

454 species adapted to low temperatures, as has been observed in other cases (Chamorro et

455 al., 2013; Krichen et al., 2014).

456

457 **4.2.Differences among** *sites* **in temperature germination requirements of H.**

458 **balearicum due to the local conditions features**

459 The seed mass of *H. balearicum* show significant differences among the three

460 environments. Several studies have shown that seed mass may vary within species

461 depending on the environmental characteristics of each site (Winn and Gross, 1993; Liu

462 et al., 2017), and this variation in seed mass of the same species can directly affect seed

463 germination (Vera, 1997; Liu et al., 2017). However, our statistical analyses determine

464 that changes in germination temperature requirements experienced among the

465 environments were not determined by differences in seed mass.

466 The three germination variables (FGP, t_{50} and t_0) showed significant differences

467 between sites. In spite of this, the variables t_{50} and t_0 also showed significant differences

468 between the environments. The final germination percentage among sites showed a large

469 variability (6.25 % to 100 %) compared to t_{50} (8 to 33 days) and t_0 (6 to 30 days). This

470 high variability in the FGP is also observed in *Hypericum perforatum* (Pérez-García et

471 al., 2006). Probably, this great diversity in FGP between sites determines the lack of

472 differences among the environments for this variable. Thus, the observed differences in

473 FGP among sites and not among environments could reflect local adaptation to particular

474 environments that have not been taken into account in the classification of the local

475 environments of this study (mountain, coast and valley).

476 The three environments studied here showed differences in minimum and

477 maximum daily temperatures and the amplitude of the diurnal cycle. These temperature

478 differences are mainly related to the local conditions characteristics of each site (Jiménez

479   et al., 2015, 2020) (see Fig. 1b). Mountain sites are placed at higher levels, resulting in

480   lower temperature values as the temperature decreases with height. Besides, the

481   temperature in these sites is not influenced by small-scale topography. Coast sites are

482   influenced by the sea (responsible for the generation of the sea breeze during daytime and

483   for a less descending temperatures compared with the inland regions during nighttime).

484   Finally, during daytime, the valley sites are in the shade of the sea-breeze generated in

485   the coast regions and the temperature increases due to the solar radiation whereas the

486   temperature cools down more than in coast regions where other mixing events can occur

487   during nighttime (Daly, 2006).

488   The germination rate of *H. balearicum* is affected by temperature and the

489   environment. Differences in germination rate according to temperature between the coast

490   environment and the valley and mountain environment, could be related to the smaller

491   temperature amplitude and the higher average daily temperature of coast sites compared

492   to the valley and mountain sites, which would determine that seeds of coast sites would

493   be adapted to a narrower optimal temperature range for t_{50}, especially at low temperatures.

494   Although the valley and the mountain environments present differences between

495   temperature ranges, they did not show differences in the germination rate pattern

496   according to temperature, probably because they showed fewer differences in the mean

497   daily temperature amplitude than with the coast site. However, valley environment

498   showed higher differences in 22 and 24 ºC treatments with the coast environment,

499   compared to the mountain environment. These higher differences could be related to the

500   highest maximum daily temperature experienced in the valley environment. Regarding

501   the germination timing, there were statistically significant differences between

502   temperature treatments and the environment. The mountain and valley environment

503   showed smaller t_0 values than coast for all the temperatures tested, although the

21

504 differences in the t_0 mean value were greater in treatments at 12, 14 and 16 ℃. This

505 temperature pattern could be due to the fact that the coast environment has a higher daily

506 temperature average than the valley and mountain, which would determine that seeds of

507 *H. balearicum* from mountain and valley sites are adapted to germinate earlier in all

508 temperatures tested, but with more difference, at lower temperatures (12, 14 and 16 ℃).

509 In the same way as t_{50} and t_0 results of *H. balearicum*, some studies show that seeds found

510 at higher elevations, and therefore subjected to colder climates, are adapted to germinate

511 earlier and more quickly at lower temperatures (Mariko et al., 1993; Wu et al., 2019).

512   Both the plasticity of FGP over a wide temperature range and the lack of

513 differences in FGP as a function of temperature among the environments suggest that the

514 final percentage of germination is not playing a decisive role in the adaptation of *H.*

515 *balearicum* compared to the germination rate and the germination timing. In fact, some

516 studies have suggested that the final germination percentage, in some cases, does not

517 provide relevant information about species fitness, but being more important the

518 germination rate and germination timing because they will determine in which

519 environmental conditions the seedlings will emerge (Washitani, 1985; Donohue et al.,

520 2010).

521   Overall, variations in the germination rate and the germination timing as a

522 function of temperature of *H. balearicum* in the three environments are consistent with

523 several studies which demonstrate that variations in germination temperature

524 requirements are due to differences in the particular environmental conditions of each site

525 (McWilliams et al., 1968; Meyer et al., 1997; Liu et al., 2017; Wu et al., 2019).

526

527   **5. Conclusions**

Hypericum balearicum exhibits plasticity in the final percentage of germination as a function of temperature. The species germinates in the optimal temperature range of typical Mediterranean species. Seed mass of *H. balearicum* was significantly higher in valley compared to mountain and coast, although this variation did not determine differences in the temperature germination requirements that occur among the environments. The final germination percentage as a function of temperature did not differ among the environments. However, the germination rate and germination timing did show differences among the environments as a function of temperature due to the local origin of seeds. The coast environment showed a higher germination rate, especially at low temperatures with the mountain and at low and high temperatures with the valley. In addition, the coast environment also showed higher germination timing, especially at low temperatures with mountain and valley. These variations are consistent with the temperature traits of each environment. Finally, the results of this study demonstrate the importance of taking into account studies about differences among sites on germination temperature requirements in order to understand how plants will respond to environmental changes.

553

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779 **Table 1.** Location and local features information of each study site of *Hypericum balearicum*.

Site **information**	Site code	Environment	Latitude (°N)	Longitude (°E)	Elevation (m.a.s.l.)	Number of mother plants from which seeds were collected
Na Burguesa	**M1**	Mountain	39.581	2.563	397	**10**
Puig de Randa	**M2**	Mountain	39.529	2.926	508	**4**
Puig Major	**M3**	Mountain	39.800	2.786	1200	**25**
Puig Major	**M4**	Mountain	39.810	2.797	1408	**25**
Massanella	**M5**	Mountain	39.806	2.853	1300	**22**
Cala Bóquer	**C1**	Coast	39.925	3.092	79	**25**
Cala Figuera	**C2**	Coast	39.950	3.175	105	**25**
Ermita de Betlem	**C3**	Coast	39.739	3.314	261	**25**
Cúber	**V1**	Valley	39.777	2.795	722	**25**
Mortitx	**V2**	Valley	39.876	2.913	365	**50**
Galatzó	**V3**	Valley	39.622	2.467	270	**21**

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783　**Table 2.** Daily mean temperature (T), maximum (Tmax) and minimum (Tmin) values and the
784　amplitude of the diurnal cycle (A) for the mountain, coast and valley site (from 4/11/2019 to
785　4/05/2020). Mean ($\pm$ SE) values are shown (mountain: n = 183; coast: n = 168; valley: n = 153).
786　Letters show significant differences among sites according to Tukey's post-hoc test (T, Tmin and
787　A) or the non-parametric comparison test of Wilcoxon (Tmax) (P < 0.05).

Site	Tmean (°C)	Tmax (°C)	Tmin (°C)	A
Mountain	7.7 $\pm$ 0.2　**c**	14.9 $\pm$ 0.4 **c**	3.6 $\pm$ 0.2 **c**	11.3 $\pm$ 0.4 **b**
Coast	13.1 $\pm$ 0.2 **a**	17.1 $\pm$ 0.3 b	10.5 $\pm$ 0.2 **a**	6.7 $\pm$ 0.3 **c**
Valley	10.6 $\pm$ 0.3 **b**	20.1 $\pm$ 0.5 **a**	5.9 $\pm$ 0.2 **b**	14.2 $\pm$ 0.5 **a**

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791 **Table 3.** Seed mass of *Hypericum balearicum* in the three environments (Mountain, Coast and

792 Valley). Mean values ($\pm$ SE) are shown ($n_{Mountain} = 280$; $n_{Coast} = 168$; $n_{Valley} = 152$). Different letters

793 indicate statistically significant differences by Tukey's multiple comparison test ($P < 0.05$).

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Environment	Seed mass (mg)
Mountain	4.61±0.039 **b**
Coast	4.57±0.047 **b**
Valley	5.18±0.065 **a**

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802 **Fig. 1 (a)** Elevation (m a.s.l.) of Mallorca (western Mediterranean Sea) together with the 10 m-
803 wind vectors during sea-breeze conditions (on 5th June 2010 at 1200 UTC obtained from a
804 mesoscale numerical simulation; more details in Cuxart et al., 2014). **(b)** Daily thermal
805 amplitude computed from the same mesoscale simulation. Symbols show the sites where mature
806 seeds were collected (see Table 1 for further details) and ~~black~~ red dots indicate those with
807 observations of temperature

808 **Fig. S1** Cumulative germination percentage with standard errors (mountain: n = 40; coast: n =
809 24 and valley: $n_{12,14,16,22,24\,°C} = 24$ and $n_{18,20\,°C} = 16$) of *Hypericum balearicum* in the three
810 environments under different temperature regimes: (a) mountain, (b) coast and (c) valley

811 **Fig. 2** The number of days required to reach 50% of final germination percentage (t_{50}) of
812 *Hypericum balearicum* from mountain, coast and valley environment under different temperature
813 treatments. Mean values ($\pm$ SE) are shown per each temperature (mountain: n = 40; coast: n = 24
814 and valley: $n_{12,14,16,22,24\,°C} = 24$ and $n_{18,20\,°C} = 16$). Different letters indicate statistically significant
815 differences by Tukey's multiple comparison test (P < 0.05) between temperatures within each
816 environment (small letters) or within the same temperature among the environments (capital
817 letters)

818 **Fig. 3** The number of days to the first germination (t_0) of *Hypericum balearicum* from mountain,
819 coast and valley environment under different temperature treatments. Mean values ($\pm$ SE) are
820 shown per each temperature (mountain: n = 40; coast: n = 24: n = 16; valley: $n_{12,14,16,22,24\,°C} = 24$
821 and $n_{18,20\,°C} = 16$). Different letters indicate statistically significant differences by Tukey's
822 multiple comparison test (P < 0.05) between temperatures within environment (small letters) or
823 within the same temperature between the environments (capital letters)

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