



## Short communication

# Rapid adaptation of seed germination requirements of the threatened Mediterranean species *Malcolmia littorea* (Brassicaceae) and implications for its reintroduction



Marcello De Vitis<sup>a,\*</sup>, Charlotte E. Seal<sup>b</sup>, Tiziana Ulian<sup>b</sup>, Hugh W. Pritchard<sup>b</sup>, Sara Magrini<sup>a</sup>, Giuseppe Fabrini<sup>c</sup>, Efsio Mattana<sup>b</sup>

<sup>a</sup> Banca del Germoplasma della Tuscia, Università degli Studi della Tuscia, 01100 Viterbo, Italy

<sup>b</sup> Seed Conservation Department, Royal Botanic Gardens, Kew, Wellcome Trust Millennium Building, Wakehurst Place, Ardingly, West Sussex RH17 6TN, UK

<sup>c</sup> Banca del Germoplasma dell'Orto Botanico di Roma, Dipartimento di Biologia Ambientale, Università Sapienza di Roma, 00185 Roma, Italy

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## ABSTRACT

*Malcolmia littorea* (Brassicaceae) is a threatened species growing in the coastal sandy dunes of the west-Mediterranean basin. In this study, the seed germination and seedling emergence requirements of this species were investigated in the only remaining native population in Italy. The highest germination percentage was achieved in darkness with scoring under safe green light at 5–10 °C. Seedling emergence was highest when seeds were buried between 1 and 10 mm in depth. The results suggest that germination and seedling emergence are adapted to Mediterranean coastal habitats by employing a common mechanism of light-inhibited germination and by germinating at cooler temperatures before the onset of the summer drought. Seeds were also collected from plants cultivated at a botanical garden and from plants reintroduced by sowing or by transplanting. For those populations, germination was maximal between 10–25 °C, suggesting that the thermal germination behaviour may be affected by the maternal environment of seed production within one generation. It is suggested to use seeds produced in the same environment to which they will be used for the reintroduction of this species.

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## 1. Introduction

Mediterranean coastal habitats are particularly vulnerable to disturbance, being subjected to a dynamic equilibrium due to several natural factors and human activities (Carboni et al., 2009; Curr et al., 2000; Lomba et al., 2008; Ma and Liu, 2008). In places where disturbance has been particularly intense or prolonged in time, plant populations have been fragmented with a risk of local extinction of species, and coenoses have often completely disappeared (Acosta et al., 2006; Carranza et al., 2010).

It is important to understand which factors restrict the establishment and growth of rare species to support their in situ conservation (Schemske et al., 1994) such as through reintroduction. While species reintroduction from seed is usual for many commonly occurring plants, little is known about the germination characteristics of many rare, native plant species (Cochrane et al., 2002). Several key factors influence

seed germination, including temperature and light (Probert, 2000; Thanos et al., 1994). The optimal temperatures for total germination of coastal Mediterranean species are typically within the range of 5–15 °C, with germination percentages decreasing at higher temperatures (Thanos et al., 1989, 1995; Tlig et al., 2008). These species are also characterised by having a low germination rate and being negatively affected by prolonged chilling (Doussi and Thanos, 2002; Skordilis and Thanos, 1995).

The stimulation of seed germination by alternating temperatures is also common in species from arid zones (Mahmoud et al., 1983; Probert, 2000). The requirement for alternating temperatures and light may represent an adaptation of small-seeded species to ensure that germination occurs close to the soil surface in vegetation gaps (Probert, 2000). These responses can be interpreted in terms of the ecology of the species, in that larger seeds can emerge from greater depths, and thus there is less need for depth-sensing mechanisms (Murdoch, 1983). In coastal species of Mediterranean climates, light-inhibited seed germination has been reported (e.g. Thanos et al., 1989, 1991, 1994). This mechanism is of adaptational value in the sea-shore or sandy habitats, since germination on the surface of the sand might present a great hazard for newly germinated seedlings, due to rapid evaporation and large temperature fluctuations (Thanos et al., 1989).

\* Corresponding author at: Dipartimento di Scienze Ecologiche e Biologiche, Università degli Studi della Tuscia, Largo dell'Università s.n.c.-Blocco C, 01100 Viterbo, Italy. Tel.: +39 0 333 8252929.

E-mail addresses: [devitis@unitus.it](mailto:devitis@unitus.it), [marcello.devitis@yahoo.it](mailto:marcello.devitis@yahoo.it) (M. De Vitis).

In this study, the seed germination behaviour of *Malcolmia littorea*, a suffruticous chamaephyte belonging to the Brassicaceae family, was investigated. This species grows in sandy coastal habitats along transition and fixed dunes (Del Vecchio et al., 2012), and has a west-Mediterranean distribution (Pignatti, 1982), occurring in Italy, France, Spain, Portugal, Morocco and Algeria (Greuter et al., 1986). In Italy the species is represented by just one native population in the Latium region. Severe urbanisation of the area and tourist pressure are the most intensive threats for this isolated population (Del Vecchio et al., 2012) and as a result, it is listed as critically endangered in the Italian Red List (Rossi et al., 2013). During 2009–2011, using seeds produced by the Italian native population, an experimental reintroduction was performed by in situ seed sowing and plant transplanting (ex situ seed sowing and transfer of the grown plants), and resulted in an experimental population (sensu Caplow, 2004) of the species, established inside its historical range (De Vitis, 2011).

The main aim of this study was to characterise the seed germination behaviour of the native population of *M. littorea* by (1) determining light, storage and temperature effects on seed germination, and (2) assessing the optimal burial depth for seed germination and seedling emergence. As the environment during seed production and cultivation is likely to affect the germination performance (Evans, 1993; Rojas-Arèchiga et al., 2001), and therefore reintroduction success, the germination of seeds from the native population was also compared with seeds from three experimental populations.

## 2. Material and methods

### 2.1. Seed lot details

Ripe siliques of *M. littorea* were collected directly from plants in the native population (San Felice Circeo, Latina, Italy: NA1), in one population cultivated at the Botanical Garden of Rome (BG) and in two reintroduced populations (formed by in situ sowing (RE1), and ex situ sowing followed by transplanting of the grown plantlets (RE2)), at the time of natural dispersal in summer 2011 (Table 1). All the fruits were cleaned in the laboratory and the seeds were stored in glass vials at room conditions (+20 °C) for three months until the experiments started. During Summer 2013 seeds from plants in the native population were collected again and sown two days after collection (NA2). In each population, siliques were collected from 10 to 50 individuals, according to their population size (Table 1), as specified below. Average seed mass was calculated for each seed lot by weighing five replicates of 50 seeds each.

### 2.2. Germination tests

In all the experiments, five replicates of 20 seeds were sown on the surface of 1% water-agar, which provided a solid, non-sterile medium for germination, in 90 mm plastic Petri dishes and incubated at different temperatures and irradiances as specified below. Experiments lasted for a maximum of 26 days, at which time no further germination was observed. Germination was defined as visible radicle emergence. At the end of the germination tests, a cut-test was carried out to determine

the viability of the remaining seeds (soft or firm) and the final germination percentage was calculated on the basis of the total number of filled seeds as the mean of the five replicates  $\pm$  standard error (SE).

### 2.3. Effect of light irradiance, temperature and storage

Stored (NA1) and fresh (NA2) seeds belonging to the native population were incubated at 15 °C under different light conditions. In particular, seeds were exposed to a 12 h photoperiod (12 h) with a ratio of red:far red of ca. 2.0, a light quantity of ca. 7  $\mu\text{mol}/\text{m}^2/\text{s}$  and density of 50–100  $\text{W}/\text{m}^2$ , and in the dark, achieved by wrapping the dishes in two layers of aluminium foil. Seeds were scored for germination every two days. In the darkness seeds were either scored under safe green light (GL; light density of 14.0  $\text{W}/\text{m}^2$ ) or only at the end of the experiment (D; 26 days).

Seeds belonging to NA1, RE1, RE2 and BG were incubated in the dark and scored every two days under safe green light at both constant (5, 10, 15, 20 and 25 °C) and alternating (15/5, 20/10 and 25/15 °C) temperatures. As more seeds of NA1 were available for experimental work, they were also germinated at constant temperatures of 30 and 35 °C.

### 2.4. Experimental seed burials

Five replicates of 20 seeds of NA1 were placed on top of the 55 mm diameter Whatman no. 1 filter paper and buried at different depths in six black plastic boxes (174  $\times$  115  $\times$  60 mm) with transparent lids. Each box was filled with 1060 g of sand, with grain sizes between 0.8 and 0.005 mm, pH 6.0–7.5 (ISTA, 2006), and moistened with 105 ml of distilled water, calculated on the basis of previous experiments with seeds of comparable size (unpublished internal report of the Seed Conservation Department, Royal Botanic Gardens, Kew, UK). Replicates were buried at 1, 5, 10, 15, 20 and 25 mm. The boxes were incubated at the constant temperature of 15 °C and 12 h photoperiod (ratio of red:far red of ca. 2.0, a light quantity of ca. 7  $\mu\text{mol}/\text{m}^2/\text{s}$  and density of 50–100  $\text{W}/\text{m}^2$ ) and checked every two days for seedlings to emerge beyond the sand surface. Emerged seedlings were removed. After 26 days, the replicates were exhumed and the numbers of germinated non-emerged seedlings were scored and the viability of the non-germinated seeds was checked by a cut-test. The percentage of emerged seedlings was then recalculated on the basis of the filled seeds, as the mean of the five replicates  $\pm$  standard error (SE).

### 2.5. Data analysis

Differences amongst final germination and  $t_{50}$  (time to reach 50% of the maximum germination in one replicate) were analysed with a t-test (paired and unpaired; for normally distributed data) or with the Wilcoxon (paired) and Mann–Whitney (unpaired) tests (for data not normally distributed) to compare two groups of data. When more than two groups were compared, a one-way ANOVA (for normally distributed data) or Kruskal–Wallis (for data not normally distributed), was followed by a post-hoc test (Tukey's or Mann–Whitney pairwise comparison). Two-way ANOVA was also performed to identify a

**Table 1**

Provenance, habitat, population size, number of sampled individuals and the average seed mass ( $\pm$ SD) for each seed lot of *M. littorea* investigated in this study. One-way ANOVA was performed to compare average seed masses; values with different letters are significant at  $P < 0.05$ .

Seed lot	Provenance	Plant habitat	Altitude (m a.s.l.)	Population size	Sampled individuals	Seed mass (mg)
NA1	Native population	Coastal sandy dune	3	30	20	0.14 $\pm$ 0.01b
NA2	Native population	Coastal sandy dune	3	30	20	0.15 $\pm$ 0.01b
RE1	Reintroduced population (by sowing)	Coastal sandy dune	2	120	50	0.15 $\pm$ 0.01b
RE2	Reintroduced population (by transplanting)	Coastal sandy dune	2	13	10	0.17 $\pm$ 0.00a
BG	Botanical Garden of Rome	Living collection	17	10	10	0.16 $\pm$ 0.01b

significant effect of independent variables, and their interaction, on a dependent variable. Software Past 2.13 (Hammer et al., 2001) was used.

### 3. Results

#### 3.1. Effect of light irradiance and storage

At 15 °C, exposure to 12 h photoperiod resulted in a very low germination percentage (1%) both for stored (NA1) and fresh (NA2) seeds, with no significant differences between them ( $U = 12.50, P > 0.05$ ). Under dark condition with scoring every 2 days under green light (GL), seeds of NA2 reached their maximum germination ( $37 \pm 8.9\%$ ) in 14 days, while those of NA1 in 6 days with a significantly higher germination percentage ( $68 \pm 3.4\%$ ;  $t = 3.30, P < 0.05$ ). In contrast, under continuous darkness (D) germination of NA2 ( $13 \pm 3.4\%$ ) was significantly higher than that of NA1 (0%;  $U = 0.50, P < 0.05$ ; Fig. 1).

#### 3.2. Effect of temperature

At constant temperatures, seeds from NA1 showed the highest final germination at 5 °C ( $75.0 \pm 2.7\%$ ) and 10 °C ( $64.0 \pm 7.6\%$ ) and a statistically significant decreasing trend ( $P < 0.001$ ) with increasing temperature, reaching less than 2.0% germination at 30 and 35 °C (Fig. 2). In contrast, the lowest germination percentage was detected at 5 °C in the other seed lots (RE1, RE2 and BG), with maximal germination between 10 and 25 °C ( $P < 0.05$ ; Fig. 2). A two-way ANOVA, applied on final germination percentages of all seed lots at 5–25 °C, highlighted that the effect of provenance (Pr), temperature (T) and their interaction ( $Pr \times T$ ) were highly significant ( $P < 0.0001$ ).

Under alternating temperatures, RE1, RE2 and BG showed high germination percentages in all the tested conditions, ranging from ca. 70 to ca. 90%, but significantly lower germination percentages of NA1 at 15/5 and 25/15 °C (Fig. 3). A paired comparison of the germination percentages between alternating temperatures and their corresponding average constant temperatures showed that alternating temperatures did not affect the germination of NA1 and BG. For RE1, germination was higher at 15 °C than at 20/10 °C ( $W = 15, P < 0.05$ ) and for RE2, germination was higher at 20 °C than at 25/15 °C ( $t = 2.8, P < 0.05$ ).

Under constant temperature regimes,  $t_{50}$  of all seed lots were significantly longer at 5 °C after which  $t_{50}$  shortened with increasing temperature (Fig. 2). A two-way ANOVA applied on  $t_{50}$  values of all seed lots at 5–25 °C, showed that the effect of provenance (Pr) was not significant, while the effects of temperature (T) and of the interaction of the two variables ( $Pr \times T$ ) were highly significant ( $P < 0.0001$ ). Alternating

temperatures did not decrease the  $t_{50}$  in comparison with their corresponding average constant temperatures in any seed lot (data not shown).

#### 3.3. Effect of burial depth

The highest percentage of seedling emergence was detected between 1 and 10 mm in depth (Fig. 4). From below 10 mm very few seedlings emerged (1% of all the buried seeds at depths  $\geq 15$  mm). At 1 mm,  $20 \pm 4\%$  of the seeds were dead and  $9 \pm 8\%$  of seedlings died after germination and all the emerged seedlings ( $51 \pm 10\%$ ) were found to have an elongate hypocotyl under the very fine sand layer. At 5 mm  $69 \pm 3\%$  of the seeds germinated and emerged and  $25 \pm 5.0\%$  seeds germinated but did not emerge. At 10 mm the percentage of emerged seedlings was  $41 \pm 11\%$  and the percentage of germinated non-emerged seedlings was  $21 \pm 2\%$ . At the depth 15 mm,  $45 \pm 7\%$  of the seeds died and  $48 \pm 6\%$  germinated without reaching the surface; at 20 mm,  $31 \pm 7.3\%$  of the seeds germinated without emerging, a small percentage of seeds died ( $9 \pm 4\%$ ) and a greater percentage remained viable under the sand ( $57 \pm 12\%$ ); while at 25 mm almost all seeds died ( $90 \pm 3\%$ ) and a few seeds germinated but did not emerge ( $7 \pm 3\%$ ).

### 4. Discussion

#### 4.1. Germination behaviour and soil depth-sensing of the native population

Information on the seed biology and ecology of threatened species must be considered in order to ensure their long term conservation and reintroduction. For seeds of the threatened *M. littorea*, the lower germination of fresh seeds in comparison to stored seeds suggest that physiological dormancy is likely, broken by after-ripening in dry storage (sensu Baskin and Baskin, 1998, 2004). Short exposure (e.g. a few minutes every two days) of seeds in the dark to the green safelight was found to positively affect seed germination rather than a condition of complete darkness, without receiving any light impulse, or the exposure to 12 h of white light. Purportedly, short bursts of light may stimulate germination in the late stages of dormancy breakage (Baskin et al., 2006). Mediterranean species have previously been reported to be sensitive to green light. Luna et al. (2004) evaluated the effect of green safelight with respect to absolute darkness on the germination response of a set of 12 Mediterranean species; starting the germination tests several months after seed collection, the germination of six species were sensitive (five positively and one negatively) to short-duration exposures to green safelight.

Coastal Mediterranean species typically germinate within the range of 5–15 °C, therefore limiting germination to the winter so plants can establish before the onset of the summer drought (Thanos et al., 1989, 1995). At 5–10 °C, the germination of the native population (NA1) of *M. littorea* was the highest amongst the tested temperatures, conferring to this species ecological adaptation to the unpredictable rainfall pattern of species living under “typical” Mediterranean climate conditions, reducing the risk of losing a cohort of plants. Rapid germination has also been suggested to be an adaptation in advance of soil drying in unpredictable environments (Daws et al., 2002) as prevalent in the Mediterranean. Compared to other Mediterranean species such as *Glaucium flavum* ( $t_{50}$  of ca. 30 days for untreated seeds at 10 °C; Thanos et al., 1989), *Muscari* spp. ( $t_{50}$  ranging from 12 to 16 days at 10 °C; Doussi and Thanos, 2002), *Centranthus ruber* ( $t_{50}$  of 4.5 days at 15 °C; Mattana et al., 2010) and *Dianthus morisianus* ( $t_{50}$  ranging from 4 to 5 days at 15 °C; Cogoni et al., 2012), NA1 showed faster germination, with  $t_{50}$  values of 3.4 days at 15 °C and 5.6 days at 10 °C.

In Mediterranean climates, germination on the soil surface is unfavourable due to high irradiance. Many species employ surface-avoiding mechanisms (Thanos et al., 1989), based on diurnally fluctuating temperatures and light quality, to detect depth of seed burial (Koutsovoulou et al., 2014), as germination from too deep within the

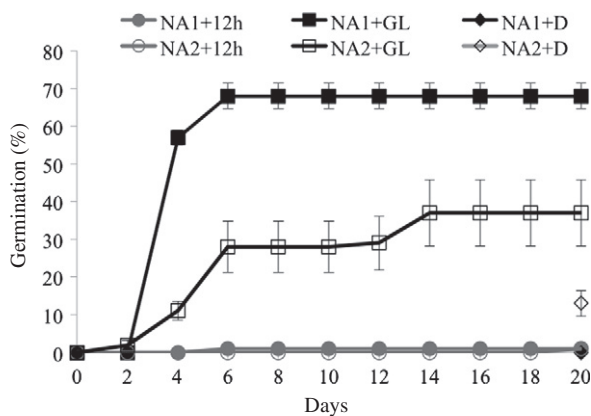
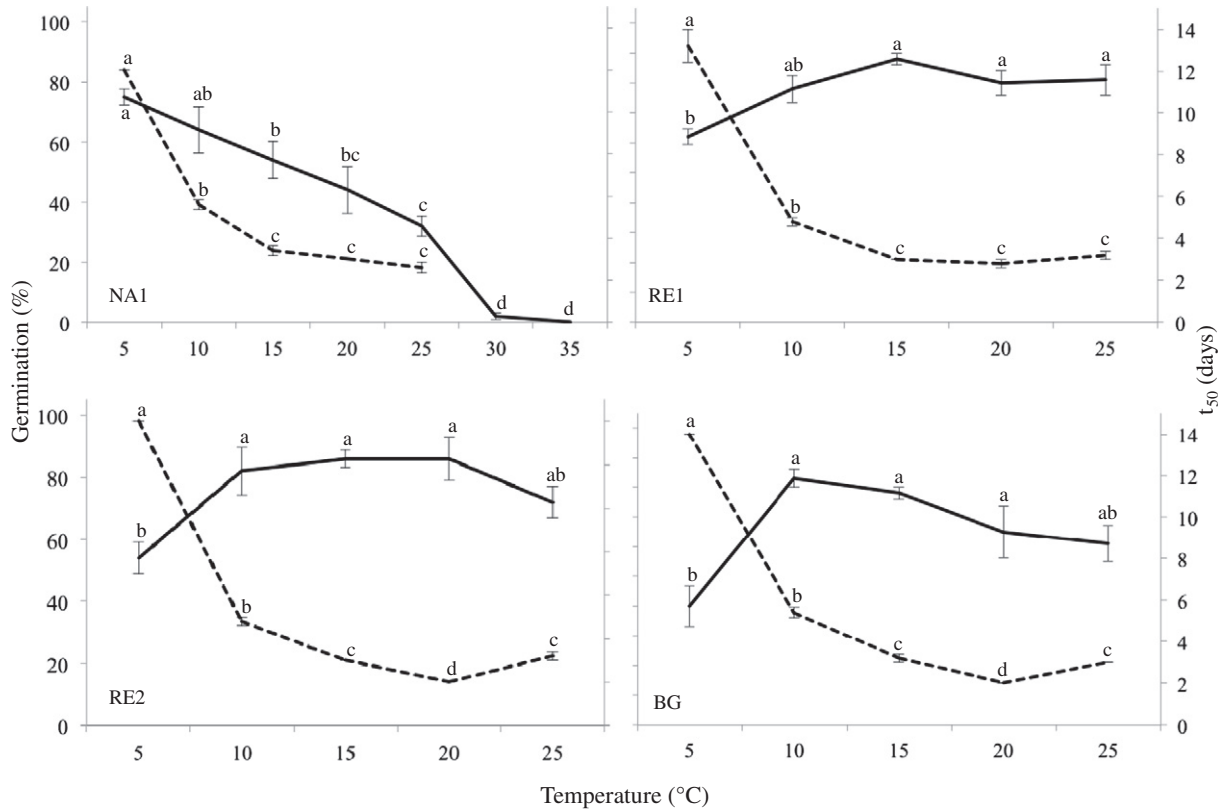


Fig. 1. Germination percentages (mean  $\pm$  SE) of the natural population under 15 °C and different light (exposure to 12 h of white light, 12 h; dark with short exposure to green safelight for scoring, GL; and continuous darkness with scoring at the end of the experiment, D) and storage conditions (stored, NA1; and fresh, NA2).



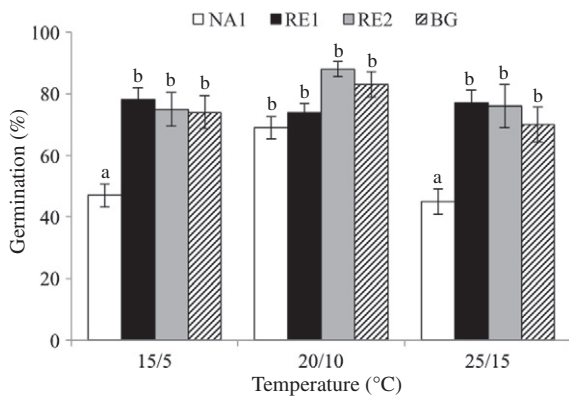
**Fig. 2.** Germination percentage (G%; solid line; mean ± SE) and  $t_{50}$  (dotted line; mean ± SE) under constant temperatures in the dark with scoring under green safelight for NA1, RE1, RE2 and BG. Within each seed lot, a significant difference amongst G% and  $t_{50}$  was detected by one-way ANOVA (for normally distributed data) and Kruskal–Wallis (for data not normally distributed) at  $P < 0.01$ . Bars with different letters are significant at  $P < 0.05$  (post-hoc tests: Tukey's for normally distributed data and Mann–Whitney for data not normally distributed).

soil will lead to seedling exhaustion before surface emergence (Benvenuti et al., 2001). The application of alternating temperature regimes did not improve the final germination percentage nor the  $t_{50}$  of NA1 in comparison with constant temperatures, and this is consistent with previous studies where seeds smaller than 2 mg did not respond to alternating temperatures (Cogoni et al., 2012; Mattana et al., 2010; Rojas-Arèchiga et al., 2001). Bond et al. (1999) found that there is an allometric correlation between seed mass and the maximum depth at which seedling emergence occurs [maximum depth (mm):  $27.3 \times \text{seed weight}^{0.334}$  (mg)]. According to this model, *M. littorea* seeds should not be able to emerge from depths > 15 mm. Laboratory experiments confirmed the model validity for *M. littorea*, as very few seedlings emerged from burial depths > 15 mm (1%), while a considerable

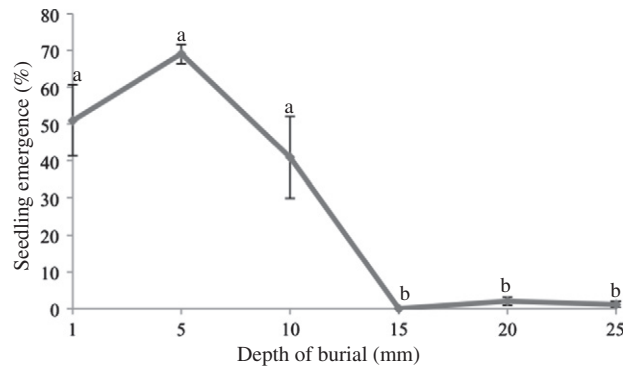
emergence of seedlings was evident between 1 and 10 mm. Further interpretation of the seedling emergence experiment is precluded by the fact that the irradiance of the incubator lighting was lower than expected in nature. Nonetheless, *M. littorea* appears to germinate as a typical Mediterranean species avoiding high irradiance and by germinating at cooler temperatures.

4.2. Impact of maternal environment and implications for conservation

Seeds produced and matured on the mother plants in the wild (NA1) had a different germination response to those produced from the experimental populations, suggesting that seeds may show a rapid alteration of fitness within one generation. This has previously been shown in seeds of the cactus *Stenocereus stellatus* (Rojas-Arèchiga et al., 2001). If the maternal environment during seed development is a determinant



**Fig. 3.** Final germination percentages (mean ± SE) of the different seed lots under alternating temperature treatments. One-way ANOVA detected a significant difference amongst them ( $P < 0.001$ ); bars with different letters are significant at  $P < 0.05$  (post-hoc Tukey's test).



**Fig. 4.** Seedling emergence of five replicates (mean ± SE) at 15 °C for the tested burial depths. Significant differences were tested by Kruskal–Wallis test ( $P < 0.001$ ); bars with different letters are significant at  $P < 0.05$  (by Mann–Whitney pairwise comparison).

for the germination behaviour of *M. littorea*, then the source of seeds could have a major impact on the success of conservation programmes. Therefore, seeds for reintroduction should be harvested from plants produced in the same environment into which they will be sown. If seeds from the native population must be collected, it is suggested that only small quantities are removed so as to reduce the risk of depauperation, and then sown *ex situ* to produce plants for reintroduction. Finally, we suggest seeds should be dried upon collection, after-ripened to break any physiological dormancy, and sown *in situ* within 1 to 10 mm of the soil surface at 10–15 °C.

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### References

- Acosta, A., Ercole, S., Stanisci, A., Blasi, C., 2006. Sandy coastal ecosystems and effects of disturbance in Central Italy. *Journal of Coastal Research* 2, 985–989.
- Baskin, C.C., Baskin, J.M., 1998. Seeds: ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego, CA.
- Baskin, J.M., Baskin, C.C., 2004. A classification system for seed dormancy. *Seed Science Research* 14, 1–16.
- Baskin, C.C., Thompson, K., Baskin, J.M., 2006. Mistakes in germination ecology and how to avoid them. *Seed Science Research* 16, 165–168.
- Benvenuti, S., Macchia, M., Miele, S., 2001. Light, temperature and burial depth effects on *Rumex obtusifolius* seed germination and emergence. *Weed Research* 41, 177–186.
- Bond, W.J., Honig, M., Maze, K.E., 1999. Seed size and seedling emergence: an allometric relationship and some ecological implications. *Oecologia* 120, 132–136.
- Caplow, F., 2004. Reintroduction plan for golden paintbrush (*Castilleja levisecta*). Washington Natural Heritage Program, Washington Department of Natural Resources, Olympia.
- Carboni, M., Carranza, M.L., Acosta, A., 2009. Assessing conservation status on coastal dunes: a multiscale approach. *Landscape and Urban Planning* 91, 17–25.
- Carranza, M.L., Carboni, M., Feola, S., Acosta, A., 2010. Landscape-scale patterns of alien plant species on coastal dunes: the case of iceplant in central Italy. *Applied Vegetation Science* 13, 135–145.
- Cochrane, A., Kelly, A., Brown, K., Cunneen, S., 2002. Relationships between seed germination requirements and ecophysiological characteristics aid the recovery of threatened native plant species in Western Australia. *Ecological Management and Restoration* 3, 47–60.
- Cogoni, D., Mattana, E., Fenu, G., Bacchetta, G., 2012. From seed to seedling: A critical transitional stage for the Mediterranean psammophilous species *Dianthus morisianus* (Caryophyllaceae). *Plant Biosystems—An International Journal Dealing with all Aspects of Plant Biology* 146, 910–917.
- Curr, R.H.F., Koh, A., Edwards, E., Williams, A.T., Davies, P., 2000. Assessing anthropogenic impact on Mediterranean sand dunes from aerial digital photography. *Journal of Coastal Conservation* 6, 15–22.
- Daws, M.I., Burslem, D.F.R.P., Crabtree, L.M., Kirkman, P., Mullins, C.E., Dalling, J.W., 2002. Differences in seed germination responses may promote coexistence of four sympatric *Piper* species. *Functional Ecology* 16, 258–267.
- De Vitis, M., 2011. Development of an experimental protocol for the reintroduction of *Malcolmia littorea* (L.) R. Br., species included in the regional Red Lists of the Italian Flora. Master's thesis Department of Environmental Biology, University La Sapienza of Rome.
- Del Vecchio, S., Giovi, E., Izzi, C.F., Abbate, G., Acosta, A., 2012. *Malcolmia littorea*: the isolated Italian population in the European context. *Journal for Nature Conservation* 20, 357–363.
- Doussi, M.A., Thanos, C.A., 2002. Ecophysiology of seed germination in Mediterranean geophytes. 1. *Muscari* spp. *Seed Science Research* 12, 193–201.
- Evans, L.T., 1993. *Crop Evolution, Adaptation and Yield*. Cambridge University Press, Cambridge.
- Greuter, W., Burdet, H.M., Long, G., 1986. *Med-Checklist*, vol. III. Conservatoire Botanique de Genève, Genève.
- Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. Past: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4, 9 ([http://palaeo-electronica.org/2001\\_1/past/issue1\\_01.htm](http://palaeo-electronica.org/2001_1/past/issue1_01.htm)).
- ISTA, 2006. *International Rules for Seed Testing*. Basseersdorf, Switzerland.
- Koutsouvolou, K., Daws, M.I., Thanos, C.A., 2014. Campanulaceae: a family with small seeds that require light for germination. *Annals of Botany* 113, 135–143.
- Lomba, A., Alves, P., Honrado, J., 2008. Endemic sand dune vegetation of the Northwest Iberian peninsula: diversity, dynamics, and significance for bioindication and monitoring of coastal landscapes. *Journal of Coastal Research* 24, 113–121.
- Luna, B., Pérez, B., Fernández-González, F., Moreno, J.M., 2004. Sensitivity to green safe-light of 12 Mediterranean species. *Seed Science and Technology* 32, 113–117.
- Ma, J., Liu, Z., 2008. Spatiotemporal pattern of seed bank in the annual psammophyte *Agriophyllum squarrosum* Moq. (Chenopodiaceae) on the active sand dunes of north-eastern Inner Mongolia, China. *Plant and Soil* 311, 97–107.
- Mahmoud, A., El Sheikh, A.M., Abdul Baset, S., 1983. Germination of two halophytes: *Halopeplis perfoliata* and *Limonium axillare* from Saudi Arabia. *Journal of Arid Environments* 6, 87–98.
- Mattana, E., Daws, M.I., Bacchetta, G., 2010. Comparative germination ecology of the endemic *Centranthus amazonum* (Valerianaceae) and its widespread congener *Centranthus ruber*. *Plant Species Biology* 25, 165–172.
- Murdoch, A.J., 1983. Environmental control of germination and emergence in *Avena fatua*. *Aspects of Applied Biology* 4, 63–69.
- Pignatti, S., 1982. *Flora d'Italia*. Edagricole, Bologna, Italy.
- Probert, R.J., 2000. The role of temperature in the regulation of seed dormancy and germination. In: Fenner, M. (Ed.), *Seeds: The Ecology of Regeneration in Plant Communities*. CAB International, Wallingford, UK, pp. 261–292.
- Rojas-Arèchiga, M., Casas, A., Vázquez-Yanes, C., 2001. Seed germination of wild and cultivated *Stenocereus stellatus* (Cactaceae) from the Tehuacán-Cuicatlán Valley, Central Mexico. *Journal of Arid Environments* 49, 279–287.
- Rossi, G., Montagnani, C., Gargano, D., Peruzzi, L., Abeli, T., Ravera, S., Cogoni, A., Fenu, G., Magrini, S., Gennai, M., Foggi, B., Wagensommer, R.P., Venturella, G., Blasi, C., Raimondo, F.M., Orsenigo, S., 2013. Lista Rossa della Flora Italiana. 1. Policy species e altre specie minacciate. Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare.
- Schemske, D.W., Husband, B.C., Ruckelshaus, M.H., Goodwillie, C., Parker, I.M., Bishop, J.G., 1994. Evaluating approaches to the conservation of rare and endangered plants. *Ecology* 75, 584–606.
- Skordilis, A., Thanos, C.A., 1995. Seed stratification and germination strategy in the Mediterranean pines *Pinus brutia* and *P. halepensis*. *Seed Science Research* 5, 151–160.
- Thanos, C.A., Georghiou, K., Skarou, F., 1989. *Glaucium flavum* seed germination: an ecophysiological approach. *Annals of Botany* 63, 121–130.
- Thanos, C.A., Georghiou, K., Douma, D.J., Marangaki, C.J., 1991. Photoinhibition of seed germination in Mediterranean maritime plants. *Annals of Botany* 68, 469–475.
- Thanos, C.A., Georghiou, K., Delipetrou, P., 1994. Photoinhibition of seed germination in the maritime plant *Matthiola tricuspidata*. *Annals of Botany* 73, 639–644.
- Thanos, C.A., Kadis, C.C., Skarou, F., 1995. Ecophysiology of germination in the aromatic plants thyme, savory and oregano (Labiatae). *Seed Science Research* 5, 161–170.
- Tlig, T., Gorai, M., Neffati, M., 2008. Germination responses of *Diplotaxis harra* to temperature and salinity. *Flora-Morphology, Distribution, Functional Ecology of Plants* 203, 421–428.