From seed to seedling: A critical transitional stage for the Mediterranean psammophilous species Dianthus morisianus (Caryophyllaceae)

D. Cogoni a, E. Mattana a, G. Fenu a & G. Bacchetta a

a Centro Conservazione Biodiversità (CCB), Dipartimento di Scienze della Vita e dell'Ambiente, Università degli Studi di Cagliari, Viale S. Ignazio da Laconi 13, Cagliari, 09123, Italia


To cite this article: D. Cogoni, E. Mattana, G. Fenu & G. Bacchetta (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: Official Journal of the Societa Botanica Italiana, 146:4, 910-917

To link to this article: http://dx.doi.org/10.1080/11263504.2011.647106

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.
From seed to seedling: A critical transitional stage for the Mediterranean psammophilous species *Dianthus morisianus* (Caryophyllaceae)

D. COGONI, E. MATTANA, G. FENU, & G. BACCHETTA

Centro Conservazione Biodiversità (CCB), Dipartimento di Scienze della Vita e dell’Ambiente, Università degli Studi di Cagliari, Viale S. Ignazio da Laconi 13, Cagliari 09123, Italia

**Abstract**
Seed germination, seedling emergence and seed persistence in the soil were investigated for *Dianthus morisianus* (Caryophyllaceae), a psammophilous endemic species of Sardinia. Stored and freshly collected seeds were incubated in a range of constant temperatures (5–25°C) and an alternating temperature regime (25/10°C). The effect of seed burial depth on seedling emergence was investigated under controlled environmental conditions. Seed persistence in the soil was verified by *in situ* experimental seed burials. Seeds of this species were non-dormant, and all seed lots germinated both in the light and darkness, mainly at low temperatures (<20°C), with a maximum at 15°C (>95%). Optimal seedling emergence was obtained when seeds were buried at a depth of 1–2 cm, and a declining emergence with increasing depth was observed. *D. morisianus* was also unable to form a persistent soil seed bank. The fate of the seeds that, after dispersal, do not emerge from the soil in the spring is, therefore, presumably to die before the next favourable growing season.

**Keywords:** endemic, sand dune, Sardinia, seed burial, seed germination, soil seed bank

**Introduction**
Sandy coastal dunes are highly variable ecosystems because of shifting substrate, burial by sand, bare areas among plants, the porous nature of sands and little or no organic matter, especially, during the early stages of dune development (Maun 2009). In the coastal strands and foredunes, there are many risks that limit the transformation of a seed to a seedling owing to the spatial and temporal variation in the substrate (Maun 1994). The micro-environmental variability mediated by wind and wave action creates rather harsh and uncertain conditions for seed germination, emergence of seedlings and their establishment (Maun 1994).

The transition from seed to seedling is a high-risk period in the life cycle of most plants (Harper 1977). Consequently, mechanisms that minimise the risk to this transition will be under strong selection pressure (Meyer et al. 1997), and natural selection should favour seed germination patterns that increase the probability of successful seedling establishment.

When a species is restricted in distribution to a peculiar part of an environmental gradient, its seed germination characteristics are likely to be adapted to that particular set of conditions (Daws et al. 2002).

Under a Mediterranean climate, characterised by a highly seasonal alternation of favourable and unfavourable conditions, plant growth and reproduction must occur in a window of favourable conditions that may vary in length and in which environmental cues and constraints play a central role (Thanos et al. 1995; Doussi & Thanos 2002; Gresta et al. 2010). The optimal germination temperature for Mediterranean species is typically within the range of 5–15°C; these species are also characterised by having a low germination rate (GR) and being negatively affected by prolonged chilling (Thanos et al. 1989; Skordilis & Thanos 1995; Doussi & Thanos 2002). In particular, a surface-avoiding mechanism of seedling establishment based on photo-inhibition has been described in the seeds of many Mediterranean species such as *Glaucium flavum* Crantz (Thanos et al. 1989), *Allium staticiforme* Sibth. &
Matthiola tricuspidata (L.) Hoffmanns. & Link subsp. maritimus (Thanos et al. 1991). However, seeds of *Otanthus maritimus* (L.) R. Br., another photo-inhibited widespread annual plant of Mediterranean sand dunes, did not conform with the Mediterranean germination physiology, showing a wide range of permissive temperatures and a high GR (Thanos et al. 1994). Rapid germination has been suggested to be an adaptation for rapid establishment before soil drying in unpredictable environments (Daws et al. 2002). However, low GR and a narrow range of cool temperatures are considered an advantageous ecological adaptation to the unpredictable rainfall pattern of species living under ‘typical’ Mediterranean climate conditions, by limiting germination to winter and, thereby, maximising the length of the growing season before the onset of summer drought (Thanos et al. 1995).

Seedlings are the most vulnerable stage of the life cycle of plants, being subjected to several abiotic and biotic constraints that may affect their emergence, survival and establishment (Harper 1977; Moles & Westoby 2004; Leck et al. 2008). Seedling establishment is an especially critical phase in the life cycle of plants inhabiting dry environments (Yang et al. 2010). Studies on the survival and establishment of seedlings in coastal sand dunes suggest that nutrient deficiency, lack of moisture, sand accretion, salt spray and predation are probably the most important limiting factors. Seedling recruitment of dune species coincides with periods of high moisture availability and occurs in years with high, well-distributed rainfall (Maun 1994). Seed burial depth is an important factor regulating seed germination and seedling emergence as seed germination is directly related to seed size and the depth at which seeds were buried (Bond et al. 1999; Ren et al. 2002).

The ability to form a persistent soil seed bank is crucial to the survival of many rare or declining species (Keddry & Reznicek 1982; Rowell et al. 1982; Quilichini & Debussche 2000; Eckstein et al. 2006) by conferring a degree of resilience in the face of modern and intensive land use (Thompson et al. 1993) and protecting populations from local extinction when above-ground vegetation is removed (Arroyo et al. 2006). Moreover, this ability is correlated to seed size and shape, with persistent seeds being generally smaller and more rounded than transient ones (Thompson & Grime 1979; Thompson 1987; Thompson et al. 1993; Funes et al. 1999; Cerabolini et al. 2003). However, Yu et al. (2007) found that, in a Mediterranean coastal dune ecosystem, species with bigger seeds tend to form a more persistent soil seed bank than those with smaller seeds. Therefore, these authors concluded that the relationships between seed mass, shape and persistence may be habitat specific and affected by the spatial scale of flora investigation. They also suggested that differences in climate may determine diverse seed persistence patterns.

*Dianthus morisianus* Vals. (Caryophyllaceae) is a psammophilous chamaephyte that grows on stabilised dunes in contact with micro-forests of *Juniperus* spp. (*J. oxycedrus* L. subsp. *macrocarpa* (Sibth. & Sm.) Neir. and *J. phoenicea* L. subsp. *turbinata* (Guss.) Nyman) and *Quercus calliprinos* Webb, only in a small area near Buggerru (southwest Sardinia) (Bacchetta et al. 2010). *D. morisianus* is listed in the National Red List as endangered (Conti et al. 1992) and in the Regional Red List as vulnerable (Conti et al. 1997); more recently, it has been inserted in the European threatened plant list (Sharrock & Jones 2009), and the Critically Endangered International Union for Conservation of Nature (IUCN) category has been proposed for it (Bacchetta & Pontecorvo 2005; Fenu et al. 2010). However, the biology and ecology of this species have been scarcely investigated.

In this study, reproductive traits, namely (i) seed germination requirements, (ii) seedling emergence and (iii) seed persistence in the soil were investigated, with the main aim to better understand the transitional phases from seed to seedling in a Mediterranean narrow endemic species, growing in a highly specialised habitat such as the Mediterranean coastal dune ecosystem.

**Materials and methods**

**Study species** *Dianthus morisianus* Vals. is a perennial suffrutex, characterised by numerous woody stocks and erect stems, 20–45 cm long, and by a basal rosette with thin and linear leaves, 1–15 cm long. The stems bear terminal multi-flowered heads (normally 2–18 flowers/head); the calyx is characterised by lanceolate teeth, membranaceous on the margin; the colour of the corolla is normally pink (Bacchetta et al. 2010). The flowering season lasts from early May to late June, whereas ripe fruits can be found during June and July (Fenu et al. 2010).

**Seed lot details**

Ripe seeds were collected in 2006, 2008 and 2009, from *ca.* 100 individuals of the natural population in Portixeddu (Buggerru, southwestern Sardinia, N 40° 14’ E 09° 25’) at an altitude of 10–55 m a.s.l. in the fixed dune system. Available climatic data for the population site are reported in Table I. Seeds collected in 2006 and 2008 were stored at the
Sardinian Germplasm Bank (BG-SAR), where they were placed in a dry room at 15 °C and 15% relative humidity (r.h.) and then stored at +5 °C and −25 °C, respectively. Seeds collected in 2009 where kept under laboratory conditions (ca. 20 °C and 45–50% r.h.) till the start of germination experiments in November 2009. The mean seed masses (±1 standard deviation, SD) of the three seed lots, calculated by weighing 10 replicates of 20 seeds each, were 1.28 ± 0.07, 1.29 ± 0.03 and 1.19 ± 0.08 mg for 2006, 2008 and 2009 collections, respectively.

Germination tests

Three replicates of 25 seeds per treatment were sown on the surface of 1% water agar, to provide solid, non-sterile medium for germination, in 60-mm plastic Petri dishes and incubated at both constant (5, 10, 15, 20 and 25 °C) and alternating temperatures (25/10 °C). In all treatments, seeds were exposed to irradiance for 12 h per day. In an alternating temperature regime, the 12-h light period coincided with the elevated temperature period. Germination was considered to have occurred at the time of visible radicle emergence. Germination was scored daily for 45 days and the germinated seeds were removed. To verify the effect of light on germination, a preliminary test was carried out by sowing three extra replicates at 15 °C in the dark, achieved by wrapping dishes in an aluminium foil. Seeds in this experiment were only scored once, at the end of the test, to avoid any exposure to irradiance. At the end of the germination tests, a cut test was carried out to determine the viability of the remaining seeds, and the final germination percentage was calculated on the basis of the total number of filled seeds as the mean of the three replicates ± 1 SD.

Seedling emergence

Three replicates of 25 seeds each were sown at different burial depths (0, 1, 2, 5 and 10 cm) in 15 square pots filled with sand and kept in a greenhouse, where the air temperature throughout the experiment was 16.5 ± 0.7 °C, with a photoperiod of 12 h of irradiance per day. Pots were watered daily. During these 45 days, the emerged seedlings (i.e. the emergence of cotyledons on the sand surface) were scored daily and removed. At the end of the experiment, the sand from the pots was examined, and non-germinated intact seeds and split empty seed coats were recorded for each replicate. Non-emerged seedlings were determined by subtracting the number of emerged seedlings from the number of empty seed coats for each replicate. Any remaining, intact non-germinated seeds were sown immediately at 15 °C in the light on the surface of 1% water agar in 60-mm plastic Petri dishes to check their viability and germination capacity.

Experimental seed burial

Experimental seed burials were carried out in July 2009 at the time of natural seed dispersal at the site of the original population, following the protocol of Arroyo et al. (2004), modified as reported below. Sets of three replicates containing 10 seeds each were introduced into fine grain nylon mesh envelopes that were placed in plastic nets. The envelopes were filled with sieved local soil and buried so that the seed envelopes were at a depth of 5, 10 and 15 cm. After 1 year, the replicates were exhumed. Any remaining, intact, non-germinated seeds were sown immediately at 15 °C in the light on the surface of 1% water agar in 60-mm plastic Petri dishes to check their viability and germination capacity.

Data analysis

Theoretical cardinal temperatures were evaluated by determining the seed GR, defined as the reciprocal of $T_{50}$ (time to reach 50% of the maximum germination in one replicate) for the germination tests carried out at constant temperatures (5–25 °C). The data for each seed lot were regressed using a linear model to estimate the base temperature ($t_b$) and ceiling temperature ($t_c$), by the average of $x$-intercept for both sub-optimal and supra-optimal temperature
ranges (Ellis et al. 1986). The optimum temperature ($t_o$) was calculated as the intercept of sub- and supra-optimal temperature response functions (Hardegree 2006). The mean time to seedling emergence (MTE) was estimated according to the formula: $MTE = \frac{\sum(n_i \times d_i)}{N}$, where $n_i$ is the number of emerged seedlings at day $i$, $d$ the incubation period in days and $N$ the total number of emerged seedlings in the treatment. Analysis of variance (one-way and two-ways ANOVA) was applied for final germination and seedling emergence percentages and MTE values, after verifying that the ANOVA assumptions were met (i.e. homogeneity of variance and normal distribution, verified by Bartlett’s test and the Shapiro-Wilk test, respectively). Subsequently Fisher’s post hoc least significant difference test (LSD) was carried out. All the statistical analyses were carried out by using R v. 2.11.1 (R Development Core Team 2009).

Results

Seed germination

At the end of the preliminary germination test carried out at 15°C, D. morisianus seeds reached high germination percentages both in the light (100%, 97.33 ± 4.62% and 94.67 ± 6.11% for 2006, 2008 and 2009 seed lots, respectively) and in the dark (97.33 ± 2.31%, 94.67 ± 6.11% and 94.67 ± 6.11% for 2006, 2008 and 2009 seed lots, respectively). The two-way ANOVA highlighted no statistical differences ($P > 0.05$) for photoperiod ($P = 0.3107$) and seed lot ($P = 0.4160$), and for their interaction ($P = 0.7375$).

When seeds were sown at different temperature regimes in the light, the three seed lots showed the same germination behaviour, achieving their maximum germination at 15°C (100%, 97.33 ± 4.62% and 94.67 ± 6.11% for 2006, 2008 and 2009 seed lots, respectively; $P > 0.05$ by the LSD test) and lower values at either colder or warmer temperatures (Figure 1). While the two stored seed lots (2006 and 2008) did not show any statistical differences in their final germination percentages, the 2009 seed lot reached a lower germination relative to the other two lots at 5°C and 25/10°C, and at 10 and 20°C compared with the 2006 seed lot ($P < 0.05$ by the LSD test) (Figure 1). At 5°C, final germination percentages were 78.33 ± 4.93%, 78.84 ± 19.43% and 46.67 ± 11.65% for 2006, 2008 and 2009 seed lots, respectively; while at 25°C, they were less than 50% (49.33 ± 12.22%, 41.33 ± 12.22% and 26.53 ± 10.76% for 2006, 2008 and 2009 seed lots, respectively; Figure 1). An alternating temperature regime (25/10°C) did not improve final germination for any of the three seed lots ($P < 0.05$ by the LSD test; Figure 1). The two-way ANOVA highlighted a significant effect ($P < 0.001$) for both the seed lot and temperature factors, while no effect was detected for their interaction ($P > 0.05$).

The three seed lots showed their maximum GR at 15°C (with values ranging from 0.19 day$^{-1}$ for the 2009 seed lot to 0.25 day$^{-1}$ for the 2006 seed lot) and lower values at both colder and warmer temperatures (Figure 2), as highlighted by their final germination percentages (Figure 1). The 2009 seed lot had a slower germination than the stored seed lots at all the tested temperatures, and the low final germination percentage reached by this seed lot at 25°C did not allow calculation of the $T_{50}$ for this temperature. The regression equations and the cardinal temperatures are reported in Table II. The $t_b$ ranged from ca. 0°C for the 2009 seed lot to...
2.29°C for the 2008 seed lot, with a mean of 1.37°C, the $t_o$ from ca. 28.5°C for the 2006 seed lot to 35.16°C for the 2008 seed lot, with a mean of approximately 30°C. The optimal temperature was close to 15.5°C for the 2006 and 2008 seed lots as well as the overall value (Table II).

### Seedling emergence

No seedlings emerged when sown at a burial depth of 10 cm. A few of them (15.28 ± 13.39%) emerged from 5 cm, and their percentage increased significantly ($P < 0.05$ by post hoc Fisher’s LSD test; Figure 3) at depths ranging from 2 cm to soil surface (>65%), achieving their maximum at a depth of 1 cm (100%). Non-emerged seedlings, detected by examining the sand on the pots at the end of the experiment, were 90.28 ± 13.39% and 73.55 ± 20.88% at 10 and 5 cm of burial, respectively, while all germinated seeds emerged at lower depths (Figure 3). The relationship between soil depth and emerged and non-emerged seedlings was assessed by sigmoidal regression curves (Figure 3).

The MTE ranged from 11.70 ± 1.84 days for 5 cm to 18.54 ± 2.67 days for 0 cm, with these differences being statistically significant ($P < 0.05$ by one-way ANOVA; Figure 3). Emergence was faster as the depth of burial decreased, even if this trend was not statistically significant ($P > 0.05$ for linear regression analysis).

Germination and viability for each burial depth was assessed at 15°C in the light on the retrieved intact seeds (Figure 3). At the depth of 10 cm, 5.56 ± 9.62% of the seeds had died, and 4.29 ± 4.35% were still viable dormant seeds. At 5 cm depth, 4.17 ± 7.22% of the seeds germinated after their exhumation and 7.19 ± 5.56% were viable dormant seeds. At 2 cm, the majority of the seeds (5.79 ± 27.35%) germinated in the lab, 1.39 ± 2.61% were still dormant, and only 6.85 ± 8.10% were dead. After sowing on the surface of the sand, none of the retrieved seeds germinated in the lab, with 20.81 ± 16.15% being dead, and 10.64 ± 7.63% were viable dormant seeds.

### Seed persistence in the soil

After one year of burial, very few seeds were retrieved intact at all the depths tested (5, 10 and 15 cm). The great majority of the seeds (73.33 ± 5.77% at 5 cm and 90.00 ± 10.00% at 10 and 15 cm) germinated during the year, and empty seed coats were retrieved, showing no statistical differences between depths of burial ($P > 0.05$ by one-way ANOVA). All the exhumed intact seeds germinated after sowing immediately in the incubator at 15°C in the light.

### Discussion

According to the results obtained in this study, D. morisianus seeds are non-dormant (*sensu* Baskin & Baskin 2004), as all seed lots germinated under a wide range of conditions, without any pre-treatment. Maximum germination was achieved at 10–15°C (>80%), while germination percentages mainly decreased at temperatures >15°C, as detected for other Mediterranean species (Thanos et al. 1989, 1995). However, seeds collected in 2006 and 2008 and then stored at 5°C for 3 years, and at –25°C, for 1 year showed a wider germination range and higher (although not always statistically significant) germination percentages, both at 5°C and >15°C, when compared with the fresh seeds collected in 2009. Moreover, stored seed lots germinated faster at all the tested temperatures than the fresh seed lot, with the differences being higher in the supra-optimal range (>15°C). A similar effect was detected by Thanos et al. (1995) in seeds of Satureja thymbra L., where germination range, final germination percentage and GR were positively related to the age of the seed lot.

<table>
<thead>
<tr>
<th>Seed lot</th>
<th>Function type</th>
<th>Model</th>
<th>$r^2$</th>
<th>$P$</th>
<th>$t_o$ (°C)</th>
<th>$t_c$ (°C)</th>
<th>$t_o$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>Sub-optimal</td>
<td>$y_1 = -0.0269 + 0.0173x$</td>
<td>0.98</td>
<td>&lt;0.0001</td>
<td>1.55</td>
<td>28.57</td>
<td>15.55</td>
</tr>
<tr>
<td></td>
<td>Supra-optimal</td>
<td>$y_2 = 0.5315 - 0.0186x$</td>
<td>0.67</td>
<td>&lt;0.0137</td>
<td>28.57</td>
<td>28.57</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>Sub-optimal</td>
<td>$y_1 = -0.0448 + 0.0196x$</td>
<td>0.99</td>
<td>&lt;0.0001</td>
<td>2.29</td>
<td>35.16</td>
<td>15.33</td>
</tr>
<tr>
<td></td>
<td>Supra-optimal</td>
<td>$y_2 = 0.4536 - 0.0129x$</td>
<td>0.74</td>
<td>=0.0124</td>
<td>35.16</td>
<td>28.57</td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Sub-optimal</td>
<td>$y_1 = 0.0006 + 0.0132x$</td>
<td>0.89</td>
<td>=0.0014</td>
<td>-0.04</td>
<td>-0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Supra-optimal</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Overall</td>
<td>Sub-optimal</td>
<td>$y_1 = -0.022 + 0.016x$</td>
<td>0.90</td>
<td>&lt;0.0001</td>
<td>1.37</td>
<td>29.85</td>
<td>15.87</td>
</tr>
<tr>
<td></td>
<td>Supra-optimal</td>
<td>$y_2 = 0.504 - 0.017x$</td>
<td>0.60</td>
<td>=0.0003</td>
<td>29.85</td>
<td>29.85</td>
<td></td>
</tr>
</tbody>
</table>

$t_o$: base temperature, $t_c$: ceiling temperature, and $t_o$: optimal temperature.
A widening of the temperature range for germination is one of the effects of ‘dry after-ripening’, i.e., a period of usually several months of dry storage of freshly harvested, mature seeds; the rate of dormancy release is usually faster at warmer temperatures (Finch-Savage & Leubner-Metzger 2006). After dispersal in summer, *D. morisianus* seeds experience a dry period (from June to September), characterised by high temperatures associated to soil aridity caused by the lack of rainfall and a low capillarity and water-holding capacity of the sandy soil. This period, during which conditions are unsuitable for germination may, however, enhance germination in the subsequent autumn–early spring. From November, mean air temperatures drop to less than 20°C and water availability achieves its maximum as highlighted by the climatic data (Table I), thus allowing seeds to germinate.

The maximum GR detected for this species (T50 ranging from 4 to 5 days at 15°C) was higher than in other Mediterranean maritime plants such as *Glau-cium flavum* (T50 of ca. 30 days for untreated seeds at 10°C; Thanos et al. 1989) and *Muscari* spp. (T50 ranging from 12 to 16 days at 10°C; Doussi & Thanos 2002). Rapid germination has been suggested to be an adaptation for rapid establishment in advance of soil drying in unpredictable environments (Daws et al. 2002). The fast germination of *D. morisianus* seeds suggests, therefore, that this species is well adapted to a rapid establishment in harsh conditions, as described by Thanos et al. (1994) for *Matthiola tricuspidata* (maximum germination reached within 3 days at 15 and 20°C), a psammophilous Mediterranean species with a ‘weedy’ opportunistic strategy, and by Mattana et al. (2010) for *Centranthus ruber* L. (T50 of 4.5 days at 15°C) a widespread species with a mainly Mediterranean distribution, living in stressed habitats.

The photo-inhibition for germination detected by Thanos et al. (1989, 1991) in Mediterranean maritime plants enables seeds (and eventually seedlings) to avoid germinating (and becoming established) under the harsh conditions of the surface (e.g. in the water-stressed sand or shingle of Mediterranean beaches). However, *D. morisianus* seeds, which achieved high germination percentages both in the light and in the dark, did not show this kind of surface-avoiding mechanism. This indifference to irradiance, and the lack of an alternating temperature regime requirement for germination, suggest that *D. morisianus* seeds are more likely to germinate under the canopy of *Juniperus* spp. and *Quercus calliprinos* micro-forests. In fact, under tree canopy, diurnal temperature alternations are known to be much lower than in the open gaps (Daws et al. 2002). On the other hand, light acts as a depth-sensing mechanism, thereby avoiding possible fatal germination of small...
seeds buried too deeply in the soil (Grime et al. 1981; Milberg et al. 2000). Small seeds are more likely to require light to germinate, as found in several studies both in tropical (seed mass < 0.7 ± 0.4 mg for neotropical pioneer species; Pearson et al. 2002) and temperate climates (seed mass < ca. 1.5 mg for temperate forest herbs; Jankowska-Blaszczuk & Daw 2007). The light indifference for germination observed for *D. morisianus* seeds is consistent with the mean seed mass of ca. 1.2 mg, allowing the buried seeds to germinate far from the arid sand surface. Bond et al. (1999) found that there is an allometric correlation between the maximum depth at which seedling emergence occurs and seed mass [maximum depth (mm): $27.3 \times \text{seed weight}^{0.334}$ (mg)]. According to this model, *D. morisianus* seedlings should not be able to emerge from depths > 2.9 cm. Laboratory experiments showed that seedlings belonging to the seeds placed on the soil surface established poorly, while the optimal seedling emergence for this species was between 1 and 2 cm of burial depth. However, few seedlings (ca. 15%) emerged from a depth of 5 cm and, therefore, deeper than the theoretical maximum depth of about 3 cm. This emergence pattern may be an adaptation to the spatial and temporal variation of the incoherent sand substrate, which can expose seeds buried too close to the surface.

Thompson et al. (1993) found a relationship between seed shape and mass and seed longevity in the soil, and concluded that small and nearly spherical seeds, with a seed mass < 3 mg, tend to form persistent soil seed banks. *D. morisianus* seeds, with a mean size of ca. 1.2 mg, should be persistent, but the results of the seed burial experiments showed that *D. morisianus* is unable to form a persistent soil seed bank. Even though *D. morisianus* seeds are below the threshold indicated by Thompson et al. (1993), they have a flattened shape, so they may have a higher variance of seed dimensions to form a persistent soil seed bank, as confirmed by the findings of Cerabolini et al. (2003) who reported a transient soil seed bank for three other species of *Dianthus* group (Caryophyllaceae) in central-southern Italy, Sicily and Sardinia. In a study on seed persistence in a sand soil of northern China, Zhao et al. (2011) found that seed mass and shape were significantly and negatively correlated to persistence in the soil, but no obvious threshold of seed mass and seed shape was detected as established by Thompson et al. (1993) to distinguish persistent from transient species. Moreover, Yu et al. (2007) found that, in a Mediterranean coastal sand dune ecosystem, species with bigger seeds tend to have a more persistent soil seed bank than those with smaller seeds. Many plant species of dune systems, and of the foredunes, in particular, create a transient soil seed bank as they grow in habitats with recurrent sand movement, high wind velocities and full sunlight conditions that are, therefore, too variable to create a permanent soil seed bank (Planisek & Pippen 1984; Ehrenfeld 1990; Maun 2009). The lack of a permanent soil seed bank for *D. morisianus* also seems to confirm this pattern for species of coastal backdunes.

Among the phases of the transitional stage from seed dispersal to seedling establishment analysed in this study for *D. morisianus*, seedling emergence seems to be the most critical for the long-term persistence of this species. While seeds could germinate with high percentages under a broad range of conditions, they germinated best after being sown at the narrow optimal depth of 1–2 cm, a situation that avoids both the arid soil surface and deep burials. In a dynamic environment such as the coastal backdunes where this plant grows, very few seeds per year can match these conditions allowing seedling establishment and their subsequent recruitment. Moreover, the lack of a persistent soil seed bank indicates that the fate of the seeds that do not emerge from the soil in the spring after dispersal is to die before the next favourable growing season.

**Acknowledgements**

The authors thank M. Porceddu (CCB) for help with using R software. This research was supported by the ‘Assessorato Difesa Ambiente–Regione Autonoma della Sardegna’.

**References**


Meyer SE, Allen PS, Beckstead J. 1997. Seed germination in
Mattana E, Daws MI, Bacchetta G. 2010. Comparative germina-
Maun MA. 1994. Adaptations enhancing survival and establish-
Keddy PA, Reznicek AA. 1982. The role of seed banks in the
Grime JP, Mason G, Curtis AV, Rodman J, Band MA, Mowforth
Fenu G, Cogoni D, Mattana E, Bacchetta G. 2010. Ecophysiology of seed germina-
Doussi MA, Thanos CA. 2002. Ecophysiology of seed germina-
Eckstein RL, Höckel N, Danhelika J. 2006. Biological flora of
Hardegree SP. 2006. Predicting germination responses to tempera-
Leck MA, Parker VT, Simpson RL, editors. 2008. Seedling ecolo-
y and evolution. Cambridge: Cambridge University Press.
Mattana E, Daws MI, Bacchetta G. 2010. Comparative germina-