

## **Seed Production and in Situ Germination of *Lamyropsis microcephala* (Asteraceae), a Threatened Mediterranean Mountain Species**

Authors: Mattana, Efisio, Fenu, Giuseppe, and Bacchetta, Gianluigi

Source: Arctic, Antarctic, and Alpine Research, 44(3) : 343-349

Published By: Institute of Arctic and Alpine Research (INSTAAR),  
University of Colorado

URL: <https://doi.org/10.1657/1938-4246-44.3.343>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

# Seed Production and *in situ* Germination of *Lamyropsis microcephala* (Asteraceae), a Threatened Mediterranean Mountain Species

Efisio Mattana\*†  
Giuseppe Fenu\* and  
Gianluigi Bacchetta\*

\*Centro Conservazione Biodiversità (CCB), Dipartimento di Scienze della Vita e dell'Ambiente, Università degli Studi di Cagliari, Viale Sant'Ignazio da Laconi 13, 09123 – Cagliari, Italy

†Corresponding author:  
mattana.efisio@gmail.com.

## Abstract

Seed reproduction is considered a critical bottleneck of the plant life cycle, constraining population growth, especially in the Mediterranean area. In this study, we investigated seed reproduction of *Lamyropsis microcephala* (Asteraceae), a threatened species occurring only in the Gennargentu massif (CE Sardinia, Italy). Seed output was quantified in two of the four localities where the species occurs, which differed in population size. Germination of seeds from all the four localities was assessed, both in the field and under controlled conditions, and the annual trend of soil temperature recorded by data-loggers. Plants had ca. 60 cypselas (i.e. the fruits of Asteraceae) per capitulum in the larger Rio Aratu and ca. 30 in the smaller Pisargiu locality, with only ca. 1.7 and 0.3 germinating cypselas per capitulum, respectively. Under controlled conditions, seeds of the two large localities (Bau 'e Laccos and Rio Aratu) germinated above 80%, while those of the two small ones (Brunco Spina and Pisargiu) did not reach 55%. All seeds sown in the field germinated in April–June, when diurnal fluctuations of temperatures were almost 10 times higher than in winter, limiting the length of the growing season before the onset of summer drought, and highlighting an increasing threat from global warming.

DOI: <http://dx.doi.org/10.1657/1938-4246-44.3.343>

## Introduction

High seed yield represents a successful trait for plant establishment and population growth. However, in the Mediterranean area, seed reproduction represents a lottery in colonizing open, even distant, suitable patches, and multiple demographic strategies, such as plant longevity and vegetative reproduction, enable persistence of threatened long-lived plants (García and Zamora, 2003), especially in high Mediterranean mountains whose vegetation usually consists of patchy communities (Escudero et al., 2004). A negative relationship has been reported between a narrow population size (or a low population density) and seed production, with the latter being reduced to small, isolated populations because of both increased inbreeding and reduced number of compatible mates (Vergeer et al., 2003; Campbell and Husband, 2007).

In seasonal climates, temperature is usually the main environmental factor governing seed germination in moist soil (Fenner and Thompson, 2005). In particular, diurnally fluctuating temperatures vary according to season, topography, and global climate, being lower in winter and under cloud cover (Dai et al., 1999). Since the amplitude of the diurnal temperature fluctuations vary among seasons, they may serve to detect different signals at different times of the year, such as moist conditions in winter or drought in spring (Saatkamp et al., 2011). Plants in environments that experience frost have been found to be more likely to possess some form of seed dormancy than species of milder environments (Allen and Meyer, 1998; Jurado and Flores, 2005), and populations from habitats with severe winters tend to have a higher fraction of dormant seeds and require a longer period of moist chilling to germinate (Allen and Meyer, 1998; Pendleton and Meyer, 2004). In alpine regions, spring emergence prevails due to temperatures being too low to stimulate germination following autumn dispersal or due to

a requirement of cold stratification over winter (Baskin and Baskin, 1998). Conversely, under Mediterranean climate, germination is limited to winter, thereby maximizing the length of the growing season before the onset of summer drought (Thanos et al., 1991, 1995; Doussi and Thanos, 2002). The Mediterranean-type alpine (or cryoromediterranean) zone, characterized by the presence of thorny cushion communities, is present in the most notable mountain areas that extend beyond the potential treeline, such as Sierra Nevada, High Atlas, Mount Etna, some peaks of the Hellenids, including the mountain in Crete, the Thaurus, and the Lebanese Mountains (Nagy and Grabherr, 2009). In these regions, even the high mountains may experience drought in the summer months, as a trend perceptibly increasing from north to south and from west to east (Nagy and Grabherr, 2009). However, high mountain Mediterranean plants do not seem to differ in germination characteristics from “typical” alpine, except for the high number of species ready to germinate without any pre-treatment (Giménez-Benavides et al., 2005). In addition, increases in temperature have been predicted and reported for the Mediterranean mountain ranges due to climate change (Peñuelas and Boada, 2003; Giménez-Benavides et al., 2007). As a consequence, seed germination of some species may be anticipated from summer to autumn, thereby determining seedling demise during winter, as predicted for *Nepeta sphaciotica* (Thanos and Fournaraki, 2010).

The Mediterranean basin has been recognized as one of the 34 most important global biodiversity hotspots (Myers et al., 2000; Conservation International, 2007). Médail and Quézel (1997) defined 10 regional hotspots of plant biodiversity within this area and, more recently, Médail and Diadema (2009) identified 52 putative glacial refugia, with 26 of them (i.e. 50%) occurring within the regional hotspots. Sardinia, situated in the West Mediterranean

basin, has been included in the ‘‘Tyrrhenian Islands’’ regional hot-spot (Médail and Quézel, 1999) and NE Sardinia, including the Gennargentu massif, is considered a glacial refugium *sensu* Médail and Diadema (2009).

*Lamyropsis microcephala* (Moris) Dittrich et Greuter (Asteraceae) is a Sardinian endemic, growing exclusively in the Gennargentu massif, and has been reported to be mainly vegetatively propagated, by virtue of both low seed set and low seed germination (Diana Corrias, 1977). More recently, Mattana et al. (2009) reported that germination success for *L. microcephala* was higher than 60% at temperatures  $\geq 20$  °C, after a cold stratification of 3 months at 5 °C. These authors concluded that seeds of this species showed a type 2 of non-deep physiological dormancy (i.e. seeds germinate also at low temperatures after dormancy is released), following the dormancy classification system (Baskin and Baskin, 2004). Seed dispersal takes place in late summer (Diana Corrias, 1977), and *L. microcephala* seeds, having a transient soil seed bank, presumably germinate in the following late spring, after experiencing the low winter temperatures that accelerate germination (Mattana et al., 2009). However, germination data reported by Mattana et al. (2009) were calculated for viable filled seeds and no quantitative data are available on the production of viable seeds for this species and on the environmental factors that promote germination in the wild.

The aim of this study was to assess the effectiveness of seed reproduction of *L. microcephala* by (1) quantifying its seed output, (2) assessing its seed germination both in the field and under controlled conditions, and (3) characterizing the annual trend of soil temperature in its localities.

## Materials and Methods

### STUDY SPECIES

*Lamyropsis* (Charadze) Dittrich (Asteraceae) belongs to the tribe Cardueae Cass., subtribe Carduinae Dumort, and comprises

eight perennial species distributed from S Europe eastwards to SW Asia (Mabberley, 2008) and, in particular, in the Caucasus region, where temperate continental bioclimate prevails (Rivas-Martínez et al., 2004). *Lamyropsis microcephala* is the westernmost and the most threatened species of the genus, being the only one included under the Critically Endangered IUCN category (Fenu et al., 2011), listed as a priority species in the Annex II of the European Habitats Directive (DIR. 92/43/EEC), and in the European threatened plant list (Bilz et al., 2011).

### STUDY AREA AND SEED COLLECTION

The Gennargentu massif (CE Sardinia) is characterized by a maximum elevation of 1834 m a.s.l. and by several ridges higher than 1500 m. The *L. microcephala* population is situated in small catchment areas on the northern and western slopes of this massif (Fenu et al., 2011). Vegetation is characterized by open grassland, where hemicryptophytes and cushion chamaephytes prevail (*Cariaci-Genistetea lobelioidis* vegetation class; Fenu et al., 2011), included in the ‘‘Endemic Oro-Mediterranean heaths with gorse (code 4090) and the subtype Cyrno-Sardinian hedgehog-heaths (code 31.75)’’ habitat (Biondi et al., 2009). Tourism and other outdoor activities represent the main threats to the population of this species, followed by expansion of pastoral activities and nomadic grazing of sheep, cattle, horses, and pigs (Fenu et al., 2011). The population is constituted by four localities which differ in their population size and area (Fenu et al., 2011; Table 1). For this study, eight experimental sites were identified: three in each of the two large localities (Rio Aratu and Bau ‘e Laccos), in order to cover the elevation range (L: low elevation, M: medium, and H: high; see Table 1), while for the two small ones (Bruncu Spina and Pisargiu), the experimental sites coincided with their central points (Table 1).

TABLE 1  
Data of the four localities and experimental sites (BA, BS, PG, and RA).

Locality	Code	Elevation range (m a.s.l.) <sup>a</sup>	Mean aspect (°) <sup>a</sup>	Mean slope (°) <sup>a</sup>	Area (m <sup>2</sup> ) <sup>a</sup>	Estimated N of reproductive ramets <sup>a</sup>	Mean N of capitula per reproductive ramet <sup>a</sup>	Experimental site code	Elevation (m a.s.l.)	Aspect (°)	Temperature Logger
								(L: low, M: medium, and H: high altitude)			
Bau ‘e Laccos (Fonni, NU)	BA	1450–1590	N (10)	15	12,500	66,268	2.69 ± 1.8 (N = 188)	BA L	1455	NW (305)	
								BA M	1520	NW (335)	X
								BA H	1555	NW (335)	
Bruncu Spina (Fonni, NU)	BS	1625–1637	N (355)	45	600	3076	2.46 ± 1.8 (N = 100)	BS	1630	N (355)	
Pisargiu (Fonni, NU)	PG	1580–1590	N (5)	35	200	1080	1.98 ± 1.5 (N = 100)	PG	1585	N (5)	
Rio Aratu (Desulo, NU)	RA	1450–1820	W (280)	20	240,000	1,170,120	2.59 ± 1.7 (N = 144)	RA L	1565	W (270)	
								RA M	1665	SW (235)	X
								RA H	1705	W (280)	

<sup>a</sup> Data from Fenu et al. (2011).

Cypselas (the achene fruits of the Asteraceae) were collected after obtaining the permits required by European and national laws for the species listed in the Habitats Directive (DIR. 92/43/EEC). Harvesting was conducted when seed ripening was complete in August 2009 for two localities (Rio Aratu and Pisargiu) and in August 2010 for all of them (see Table 1), as specified below.

#### SEED OUTPUT ASSESSMENT

In 2009, a capitulum was collected from 30 reproductive ramets in the Rio Aratu and Pisargiu localities, chosen as representative of two different population sizes (see Table 1). Each capitulum was analyzed and cypselas were counted and evaluated according to the following categories: “filled”: cypselas containing well-developed seeds; “aborted”: cypselas containing aborted seeds; and “empty”: cypselas with no seeds. Filled cypselas differed in size from the empty ones or those containing aborted seeds, allowing evaluation by external visual analysis. Filled cypselas of each capitulum were sown, after removing the pappus, on the surface of 1% solid water agar in plastic Petri dishes and incubated at 25 °C in the light (12 h of irradiance per day) after a pre-chilling period of three months at 5 °C. These conditions were chosen on the basis of the results achieved for this species in a previous work (Mattana et al., 2009). At the end of the experiment a cut test (ISTA, 2006) was carried out to check the viability of any remaining non-germinated seeds.

#### GERMINATION EXPERIMENTS

Cypselas (hereafter seeds) were collected from all localities in 2010. A subsample of filled seeds was taken in the laboratory and four plastic Petri dishes of 25 seeds each were sown at the above-reported conditions. At the end of the experiment, a cut test was carried out to check the viability of any remaining non-germinated seeds.

At the time of the harvesting, three sets of 20 filled seeds each were also placed in envelopes made by plastic mesh and sown in their field, after removing the pappus, at a depth of ca. 2 cm in the eight experimental sites (Table 1). This depth was chosen considering a maximum depth of seed burial for seedling emergence of 3.6 cm, as calculated following the formula: maximum depth (mm) =  $27.3 \times \text{seed weight (mg)}^{0.334}$  (Bond et al., 1999) for seeds with a fresh seed mass of  $2.32 \pm 0.69$  mg. Envelopes were checked for emerged seedlings in April 2011, when emergence was checked by gently removing the soil over the envelopes to avoid damaging seedlings, while they were exhumed and analyzed in the laboratory in June 2011.

In the medium elevation sites of the two large localities, a temperature logger (TidbiT® v2 Temp logger, Onset Computer Corporation, Cape Cod, Massachusetts, U.S.A.) was also sown in order to record soil temperature at 90-min intervals during a year (Table 1).

#### STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA), followed by a *post hoc* Fisher least significant difference test (LSD) was carried out, when the ANOVA assumptions were met, otherwise a non-para-

metric Wilcoxon test was applied. ANOVA was used, for seed collected in 2010, to test whether localities differed in final germination percentages under controlled conditions and if seasons differed in mean values of diurnally fluctuating temperatures (square-root transformed data; Saatkamp et al., 2011), calculated as differences between daily maximum and minimum temperatures (Thompson et al., 1977). The Wilcoxon test was carried out to verify differences between localities, for seeds collected in 2009, in the mean percentage of filled seeds and the number of germinated seeds per capitulum. All the statistical analyses were carried out by using R v. 2.13.0 (R Development Core Team, 2010).

## Results

#### SEED OUTPUT

*Lamyropsis microcephala* plants at the two localities investigated in 2009 showed significant differences in their seed production. While, in the Rio Aratu, capitula had an average of  $57.42 \pm 17.64$  (mean  $\pm$  SD) cypselas, in Pisargiu the mean number of cypselas per capitulum was  $29.90 \pm 9.79$  ( $N = 30$ ;  $P < 0.001$  by Wilcoxon test). However, only  $7.74 \pm 13.40\%$  (mean  $\pm$  SD) and  $2.82 \pm 3.99\%$  of the cypselas per capitulum were filled for Rio Aratu and Pisargiu, respectively ( $N = 30$ ;  $P < 0.05$  by Wilcoxon test; Fig. 1). The great majority of the cypselas collected in both localities were not filled, with  $61.74 \pm 17.00\%$  and  $80.39 \pm 14.33\%$  having aborted seed and  $30.37 \pm 15.20\%$  and  $16.79 \pm 13.30\%$  being empty for Rio Aratu and Pisargiu, respectively (Fig. 1). After incubation of the filled cypselas for germination under controlled conditions, a mean number of  $1.77 \pm 1.80$  and  $0.27 \pm 0.47$  seeds per capitulum germinated for Rio Aratu and Pisargiu, respectively ( $N = 30$ ;  $P < 0.001$  by Wilcoxon test). All non-germinated seeds died during the experiment.

#### SEED GERMINATION AND PHENOLOGY OF SEEDLING EMERGENCE

Localities sampled in 2010 differed for final germination percentages under controlled conditions. While seeds belonging to the two main localities reached germination percentages above 80% ( $86.45 \pm 10.42\%$  and  $80.74 \pm 3.29\%$ , for Bau ‘e Laccos and Rio Aratu, respectively), those of the two small localities did not germinate more than 55% ( $52.38 \pm 18.75\%$  and  $47.48 \pm 11.46\%$ , for Bruncu Spina and Pisargiu, respectively). The difference on final germination among populations was statistically significant ( $P < 0.01$  by one-way ANOVA; Table 2), and all non-germinated seeds died during the experiment.

During the first measurement period of the envelopes buried in the field in April 2011, few emerged seedlings were found in the medium and high elevation experimental sites of the Bau ‘e Laccos locality (ca. 12% and 7%, for BA M and BA H, respectively), while no emerged seedlings were found in the lower elevation site (BA L) as well as in all the other localities (Table 2). At the end of the experiment in June 2011, emerged seedlings were found only for Rio Aratu locality (95% and ca. 42% for RA L and RA H, respectively; see Table 2). After opening the envelopes, only empty split seed coats were found, indicating that all the sown seeds germinated before exhumation. The presence of emerged seedlings in the lowest locality (Bau ‘e Laccos) in April and in the highest (Rio Aratu) in June, as well as the fact that all sown seed

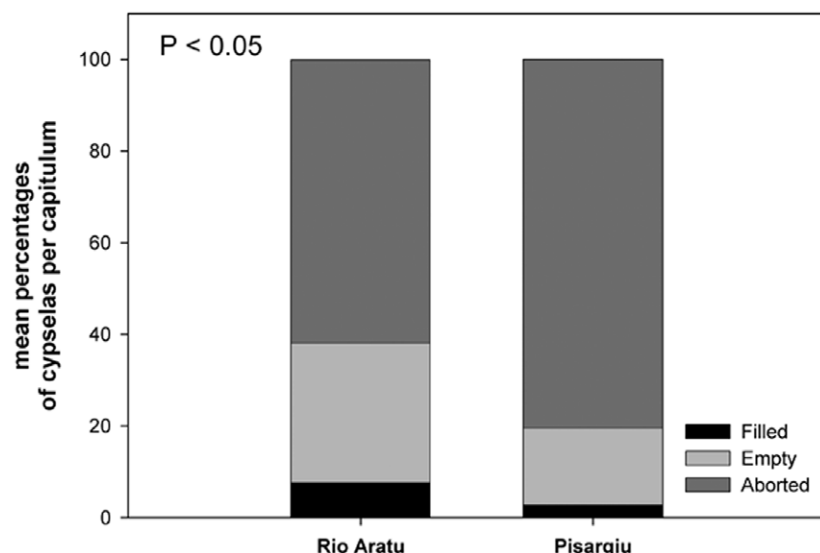


FIGURE 1. Evaluation categories expressed as percentages of the total number of cypselas per capitulum for the two localities investigated in 2009 (Rio Aratu and Pisargiu). Difference between localities in the percentage of filled cypselas was tested by non-parametric Wilcoxon test ( $N = 30$ ).

germinated before exhumation, indicate that *L. microcephala* seed germination occurs from April to June (Fig. 2).

The soil temperatures recorded by the two data loggers were very similar for the two localities with an annual mean temperature of ca. 10 °C, with mean values of ca. 1.5 °C in winter and ca. 20 °C in summer. In Bau 'e Laccos, temperatures ranged from a minimum of -1.7 °C (14 December 2010) to a maximum of 43.5 °C (29 August 2010), while in Rio Aratu, they ranged from 0.1 °C (13 March 2011) to 35.2 °C (21 August 2011). The snow continuously covered the soil from the end of January to the middle of March 2011 in both localities for a total of ca. 50 days, with mean temperatures close to 0°C, while mean daily temperatures were lower than 5 °C (cold stratification period) from November 2010 to March 2011 for a total of ca. 135 days. In April/June, when seed germination occurred, mean soil temperature was ca. 11 °C,

ranging from minimum temperatures of ca. 8 °C to maximum temperatures of ca. 17 °C (70 days). The analysis of the mean diurnally fluctuating temperatures recorded during a year period highlighted an average  $\Delta T$  value of  $4.6 \pm 3.4$  °C in autumn (from equinox to solstice), a minimum  $\Delta T$  in winter (from solstice to equinox) with  $1.1 \pm 1.4$  °C and then it increased in spring ( $\Delta T = 8.2 \pm 4.2$  °C) and in summer when reached its maximum of  $12.7 \pm 3.6$  °C ( $P < 0.001$  by one-way ANOVA, followed by *post hoc* Fisher's LSD test; Fig. 3).

## Discussion

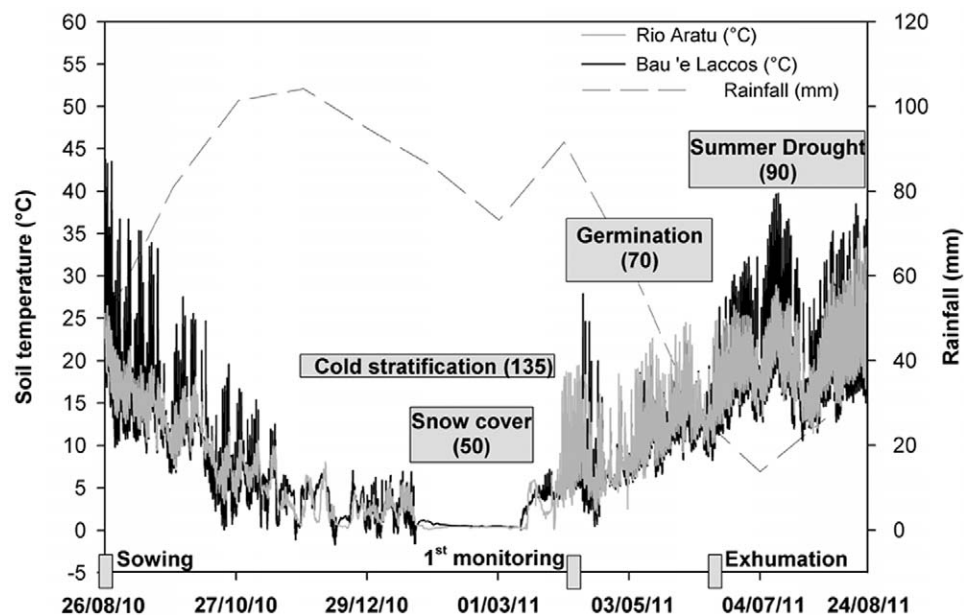
### SEED PRODUCTION

The number of germinated seeds per capitulum of *L. microcephala* reported in this study confirmed the observations of Diana

TABLE 2

Final germination percentages under controlled conditions of seeds collected in 2010 from each locality and values of seedling emergence in the field. Seeds were sown in the laboratory at 25°C, with a photoperiod of 12 h of irradiance per day, after a pre-chilling of 3 months at 5°C. Data are the mean of 4 replicates of 25 seeds each; values with the same letters are not different at  $P > 0.05$  by *post hoc* Fisher least significant difference test. Values of seedling emergence in the field are expressed as percentages of three replicates of 20 seeds each. Seeds were sown on 26 August 2010. At the first monitoring (18 April 2011) envelopes were not exhumed, but emergence was checked by gently removing the upper soil to avoid damaging seedlings. The experiment was ended on 12 June 2011, when envelopes were exhumed and remaining seeds analyzed in the laboratory.

Locality	Germination under controlled conditions (%; mean $\pm$ SD) ( $P < 0.01$ )	Site code	18 April 2011 Emerged (%; mean $\pm$ SD)	12 June 2011 Seedlings (%; mean $\pm$ SD)	Empty split seed coats (%; mean $\pm$ SD)
BA	86.45 $\pm$ 10.42 <sup>a</sup>	BA L	0	0	100.0 $\pm$ 0
		BA M	11.7 $\pm$ 20.2	0	100.0 $\pm$ 0
		BA H	6.7 $\pm$ 7.6	0	100.0 $\pm$ 0
BS	52.38 $\pm$ 18.75 <sup>b</sup>	BS	0	0	100.0 $\pm$ 0
PG	47.48 $\pm$ 11.46 <sup>b</sup>	PG	0	0	100.0 $\pm$ 0
RA	80.74 $\pm$ 3.29 <sup>a</sup>	RA L	0	95.0 $\pm$ 5.0	5.0 $\pm$ 5.0
		RA M	0	0	100.0 $\pm$ 0
		RA H	0	41.7 $\pm$ 30.5	58.3 $\pm$ 30.5

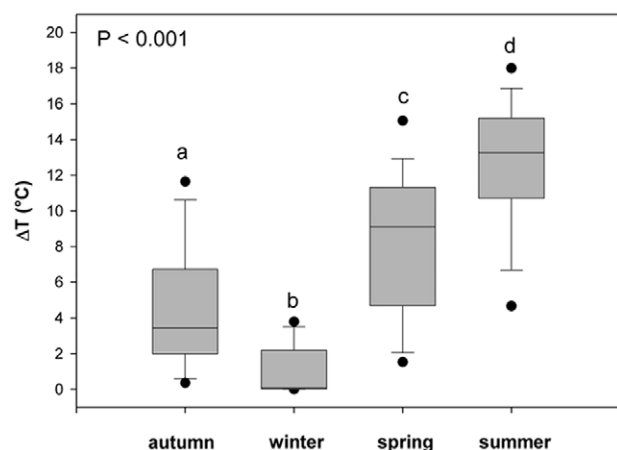


**FIGURE 2.** Soil temperature (°C) recorded by the dataloggers with a 90-min interval in the two investigated localities (Bau 'e Laccos, 1520 m and Rio Aratu, 1665 m a.s.l.). Dates are expressed as DD/MM/YY. The times of the sowing of the seeds in the field, their first monitoring, and their exhumation are indicated, as well as the period of snow cover and, according to results achieved in the field experiments, seed germination time. Length of the gray bars correspond to the duration of the phases, and they are expressed in days (numbers in parentheses). Historical annual trend of rainfalls (mm) in the study area is also reported (data from the nearby weather station of Forni at ca. 1000 m a.s.l.).

Corrias (1977), who suggested a low seed set occurred in this species. However, it should be taken into account that multiplying the number of germinated seeds per capitulum calculated in this study per the estimated number of reproductive ramets per locality by the mean number of capitula per ramet reported in Fenu et al. (2011), gives ca. 5,000,000 and 600 germinable seeds for Rio Aratu and Pisargiu, respectively.

Localities of *L. microcephala* with different population size differed significantly also in their seed viability, with a higher percentage of filled cypselas per capitulum in the larger locality (Rio Aratu, ca. 1,000,000 reproductive ramets) than in the smaller one (Pisargiu, ca. 1000 reproductive ramets). These results are consistent with several previous studies investigating the significance of population size for the reproductive success of rare (e.g. Fischer and

Matthies, 1998; Leimu et al., 2006) and endangered plant species (Mattana et al., 2010). Small populations may fail to attract sufficient pollinators, leading to pollen limitation and reduced seed set (Ågren, 1996; Colling et al., 2004). This process may be the cause of reduced fecundity in small populations of several insect-pollinated species such as *Senecio integrifolius* (Widén, 1993), *Gentiana germanica* (Fischer and Matthies, 1998), and *Primula elatior* (Jacquemyn et al., 2002). Although self-incompatibility is found within Asteraceae (Matton et al., 1994), mating system and pollination strategy of *L. microcephala* are still not investigated. Thus, the low seed output reported for the species in general, and particularly for the small locality of Pisargiu, may also be due to inbreeding depression (Ågren, 1996; Fischer and Matthies, 1998; Campbell and Husband, 2007).



**FIGURE 3.** Diurnally fluctuating soil temperatures ( $\Delta T$ ) in the study area, recorded by the dataloggers with a 90-min interval. Data are the mean of the values ( $N = 90$ ) recorded in each of the two investigated localities (Bau 'e Laccos, 1520 m and Rio Aratu, 1665 m a.s.l.).  $P < 0.001$  by one-way ANOVA; boxes with different letters are different at  $P < 0.05$  by *post hoc* Fisher's LSD test.

#### SEED GERMINATION AND SEEDLING FATE

The high germination percentages (>80%) reached under controlled conditions by seeds from the two main localities (Rio Aratu and Bau 'e Laccos) and the significantly lower germination (ca. 50%) detected for seeds of the two small localities (Brunco Spina and Pisargiu), suggests that the size of the localities may have an effect also on seed germination. Menges (1991) found that germination of *Silene regia* was relatively low in small populations and uniformly high in populations with more than 150 plants, and suggested that this trend could have been determined by the presence of inbreeding depression and a reduced pollinator visitation in small populations. However, all seeds sown in the field germinated before exhumation, without differences among localities (see Table 2). In laboratory experiments, seeds were exposed to constant temperatures, which do not represent natural conditions (Baskin and Baskin, 1998), while seed germination is often increased by both the number and amplitude of temperature alternations (Thompson et al., 1977; Probert, 1992; Saatkamp et al., 2011). Therefore, the higher germination percentages observed in the seeds from the two larger localities under these controlled (un-natural) conditions are likely due to a difference in their seed vigor (Menges, 1991).

The results of the field experiments highlighted a spring germination for this species. However, while in a previous study under controlled conditions, seeds germinated better at constant temperatures  $>20^{\circ}\text{C}$  or at the alternating temperature regime of  $30/15^{\circ}\text{C}$ , suggesting a germination in late spring/early summer (Mattana et al., 2009), in the natural localities seeds germinated between April and June, with mean temperatures ranging around  $10^{\circ}\text{C}$ . All the seeds germinated as soon as snow melted, when diurnal fluctuations of temperatures achieved  $\Delta T$  values almost 10 times higher than in winter, confirming the role of high temperature fluctuations on the germination enhancement (Thompson et al., 1977; Probert, 1992; Saatkamp et al., 2011). Alternating diurnal temperatures are reported to promote germination in many alpine species, leading to early spring germination in order to ensure enough time for plant establishment (Bliss, 1971). This adaptation is essential for Mediterranean high mountain species, where topsoil remains moistened for only few weeks after snowmelt and an intense water deficit characterizes the summer when temperatures are high enough to enable growth (Giménez-Benavides et al., 2005). The difference in times of germination recorded between the lowest (Bau 'e Laccos) and the highest locality (Rio Aratu) is therefore likely to be due to a different time of snowmelt determined by their altitudes.

The soil temperatures recorded in the two main *L. microcephala* localities highlighted that although seeds germinate in early spring when soil is still moistened by the snowmelt and rainfall, in the subsequent summer seedlings face very harsh environmental conditions, with soil temperatures rising to values of ca.  $20^{\circ}\text{C}$  with  $\Delta T$  of ca.  $15^{\circ}\text{C}$ . Considering that from the end of June to the end of August rainfall is drastically reduced, it is not surprising that no seedling recruitment was observed (Diana Corrias, 1977), as newly established seedlings might survive only in rare moist microsites, where water is still available during the main part of the summer (Mattana et al., 2009).

## CONCLUSIONS

The results of this study confirmed that low seed set occurs in the endangered plant *Lamyropsis microcephala* (Diana Corrias, 1977) and identified spring as the season of seed germination in the field for this species, when soil is still moistened by snowmelt and rainfall. These results suggest that reproduction by seeds is limited by both a low seed production and the current Mediterranean climate, which reduces the number and quality of available sites for seedling establishment. These results are in agreement with the findings of García-Camacho et al. (2010), who suggested that species of high altitude Mediterranean communities may be both seed and microsite limited, and of García and Zamora (2003) who considered plant longevity and vegetative reproduction as major traits enabling persistence of threatened long-lived plants in the Mediterranean area. However, more studies are needed to verify if vegetative propagation alone may guarantee long term *in situ* persistence of this species. In addition, the high increases in temperature predicted for the Mediterranean mountain ranges (Peñuelas and Boada, 2003) are likely to exacerbate the environmental conditions, making still more difficult population recruitment by seeds of this endangered species.

## Acknowledgments

The authors thank D. Cogoni and M. Porceddu (CCB) for helping with fieldwork and J. Vila Traver for helping with seed counting and germination experiments.

## References Cited

- Ågren, J., 1996: Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology*, 77: 1779–1790.
- Allen, P. S., and Meyer, S. E., 1998: Ecological aspects of seed dormancy loss. *Seed Science Research*, 8: 183–191.
- Baskin, C. C., and Baskin, J. M., 1998: *Seeds—Ecology, Biogeography and Evolution of Dormancy and Germination*. San Diego: Academic Press, 666 pp.
- Baskin, J. M., and Baskin, C. C., 2004: A classification system for seed dormancy. *Seed Science Research*, 14: 1–16.
- Bilz, M., Kell, S. P., Maxted, N., and Lansdown, R. V., 2011: *European Red List of Vascular Plants*. Luxembourg: Publications Office of the European Union, 130 pp.
- Biondi, E., Blasi, C., Burrascano, S., Casavecchia, S., Copiz, R., Del Vico, E., Galdenzi, D., Gigante, D., Lasen, C., Spampinato, G., Venanzoni, R., and Zivkovic, L., 2009: Manuale Italiano di Interpretazione degli Habitat della Direttiva 92/43/CEE. Società Botanica Italiana – Ministero dell’Ambiente e della Tutela del Territorio e del Mare. Direzione Protezione della Natura, <<http://vnr.unipg.it/habitat>>, accessed 26 March 2010.
- Bliss, L. C., 1971: Arctic and alpine plant life cycles. *Annual Review of Ecology and Systematics*, 2: 405–438.
- Bond, W. J., Honig, M., and Maze, K. E., 1999: Seed size and seedling emergence: an allometric relationship and some ecological implications. *Oecologia*, 120: 132–136.
- Campbell, L. G., and Husband, B. C., 2007: Small populations are mate-poor but pollinator-rich in a rare, self-incompatible plant, *Hymenoxys herbacea* (Asteraceae). *New Phytologist*, 174: 915–925.
- Colling, G., Reckinger, C., and Matthies, D., 2004: Effects of pollen quantity and quality on reproduction and offspring vigor in the rare plant *Scorzonera humilis* (Asteraceae). *American Journal of Botany*, 91(11): 1774–1782.
- Conservation International, 2007: Biodiversity hotspots, <<http://www.biodiversityhotspots.org>>, accessed 22 March 2012.
- Dai, A., Trenberth, K. E., and Karl, T. R., 1999: Effects of clouds, soil moisture, precipitation, and water vapor on diurnal temperature range. *Journal of Climate*, 12(8): 2451–2473.
- Diana Corrias, S., 1977: Le piante endemiche della Sardegna: 6–7. *Bollettino della Società Sarda di Scienze Naturali*, 16: 287–294.
- Doussi, M. A., and Thanos, C. A., 2002: Ecophysiology of seed germination in Mediterranean geophytes. 1. *Muscari* spp. *Seed Science Research*, 12: 193–201.
- Escudero, A., Giménez-Benavides, L., Iriondo, J. M., and Rubio, A., 2004: Patch dynamics and islands of fertility in a high mountain Mediterranean community. *Arctic, Antarctic, and Alpine Research*, 36(4): 518–527.
- Fenner, M., and Thompson, K., 2005: *The Ecology of Seeds*. Cambridge: Cambridge University Press, 250 pp.
- Fenu, G., Mattana, E., and Bacchetta, G., 2011: Distribution, status and conservation of a Critically Endangered, extremely narrow endemic: *Lamyropsis microcephala* (Asteraceae) in Sardinia. *Oryx*, 45: 180–186.
- Fischer, M., and Matthies, D., 1998: Effects of population size on performance in the rare plant *Gentianella germanica*. *Journal of Ecology*, 86: 195–203.
- García, D., and Zamora, R., 2003: Persistence, multiple demographic strategies and conservation in long lived Mediterranean plants. *Journal of Vegetation Science*, 14: 921–926.
- García-Camacho, R., Iriondo, J. M., and Escudero, A., 2010: Seedling dynamics at elevation limits: complex interactions beyond seed

- and microsite limitations. *American Journal of Botany*, 97(11): 1791–1797.
- Giménez-Benavides, L., Escudero, A., and Pérez-García, F., 2005: Seed germination of high mountain Mediterranean species: altitudinal, interpopulational and interannual variability. *Ecological Research*, 20: 433–444.
- Giménez-Benavides, L., Escudero, A., and Iriondo, J. M., 2007: Reproductive limits of a late-flowering high-mountain Mediterranean plant along an elevational climate gradient. *New Phytologist*, 173: 367–382.
- ISTA, 2006: *International Rules for Seed Testing*. Edition 2006. Basersdorf: The International Seed Testing Association (ISTA).
- Jacquemyn, H., Brys, R., and Hermy, M., 2002: Patch occupancy, population size and reproductive success of a forest herb (*Primula elatior*) in a fragmented landscape. *Oecologia*, 130: 617–625.
- Jurado, E., and Flores, J., 2005: Is seed dormancy under environmental control or bound to plant traits? *Journal of Vegetation Science*, 16: 559–564.
- Leimu, R., Mutikainen, P., Koricheva, J., and Fischer, M., 2006: How general are positive associations between population size, fitness, and genetic variation? *Journal of Ecology*, 94: 942–952.
- Mabberley, D. J., 2008: *Mabberley's Plant-Book*. Third edition. Seattle: University of Washington Botanic Gardens, 1021 pp.
- Mattana, E., Daws, M. I., and Bacchetta, G., 2009: Seed dormancy and germination ecology of *Lamyropsis microcephala*: a mountain endemic species of Sardinia (Italy). *Seed Science and Technology*, 37: 491–497.
- Mattana, E., Daws, M. I., Fenu, G., and Bacchetta, G., 2010: Ecological and morphological seed traits of *Polygala sardoa* and *P. sinisica*: a comparative study on two endemic species of Sardinia. *Flora*, 205: 825–831.
- Matton, D. P., Nass, N., Clarke, A., and Newbiggin, E., 1994: Self-incompatibility: how plants avoid illegitimate offspring. *Proceedings of the National Academy of Sciences*, 91: 1992–1997.
- Médail, F., and Diadema, K., 2009: Glacial refugia influence plant diversity patterns in the Mediterranean Basin. *Journal of Biogeography*, 36(7): 1333–1345.
- Médail, F., and Quézel, P., 1997: Hot-spots analysis for conservation of plant biodiversity in the Mediterranean Basin. *Annals of the Missouri Botanical Garden*, 84: 112–127.
- Médail, F., and Quézel, P., 1999: Biodiversity hotspots in the Mediterranean Basin: setting global conservation priorities. *Conservation Biology*, 13: 1510–1513.
- Menges, E. S., 1991: Seed germination percentage increases with population size in a fragmented prairie species. *Conservation Biology*, 5(2): 158–164.
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. B., and Kent, J., 2000: Biodiversity hotspots for conservation priorities. *Nature*, 403: 853–858.
- Nagy, L., and Grabherr, G., 2009: *The Biology of Alpine Habitats*. New York: Oxford University Press, 376 pp.
- Pendleton, B. K., and Meyer, S. E., 2004: Habitat-correlated variation in blackbrush (*Coleogyne ramosissima*: Rosaceae) seed germination response. *Journal of Arid Environments*, 59: 229–243.
- Peñuelas, J., and Boada, M., 2003: A global change-induced biome shift in the Montseny Mountains (NE Spain). *Global Change Biology*, 9: 131–140.
- Probert, R. J., 1992: The role of temperature in germination ecophysiology. In Fenner, M. (ed.), *Seeds: the Ecology of Regeneration in Plant Communities*. Wallingford: CAB International, 261–292.
- R Development Core Team, 2010: R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
- Rivas-Martínez, S., Peñas, A., and Díaz, T. E., 2004: Bioclimatic map of Europe, bioclimates. Cartographic Service, University of León, Spain. <<http://www.globalbioclimatics.org/form/maps.htm>>, accessed 2 November 2011.
- Saatkamp, A., Affre, L., Baumberger, T., Dumas, P. J., Gasmi, A., Gachet, S., and Arène, F., 2011: Soil depth detection by seeds and diurnally fluctuating temperatures: different dynamics in 10 annual plants. *Plant and Soil*, 349(1–2): 331–340.
- Thanos, C. A., and Fournaraki, C., 2010: Global warming and seed germination—The case of *Nepeta sphaciotica*, an Alpine Cretan endemic. In Pendleton, R., Meyer, S., and Schultz, B. (eds.), *Proceedings of the Seed Ecology III Conference: Salt Lake City: The Third International Society for Seed Science Meeting on Seeds and the Environment: Seeds and Change; 2010 Jun 20–24.*, 176–177.
- Thanos, C. A., Georghiou, K., Douma, D. J., and Marangaki, C. J., 1991: Photoinhibition of seed germination in Mediterranean maritime plants. *Annals of Botany*, 68: 469–475.
- Thanos, C. A., Kadis, C. C., and Skarou, F., 1995: Ecophysiology of germination in the aromatic plants thyme, savory and oregano. *Seed Science Research*, 5: 161–170.
- Thompson, K., Grime, J. P., and Mason, G., 1977: Seed germination in response to diurnal fluctuations of temperature. *Nature*, 267: 147–149.
- Vergeer, P., Rengelink, R., Copal, A., and Ouborg, N. J., 2003: The interacting effects of genetic variation, habitat quality and population size on performance of *Succisa pratensis*. *Journal of Ecology*, 91: 18–26.
- Widén, B., 1993: Demographic and genetic effects on reproduction as related to population size in a rare, perennial herb, *Senecio integrifolius*. *Biological Journal of the Linnean Society*, 50(3): 179–195.

MS accepted April 2012