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Conservation aspects for chasmophytic species: Phenological behavior and seed strategies of the Central Apennine threatened endemism *Moehringia papulosa* Bertol.

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Abstract

Chasmophytic vegetation growing on the cracks of cliffs in the Mediterranean and in the Euro-Siberian phytogeographic regions shows a great regional diversity, with a large number of endemic plant species, many of them endangered and at risk of extinction. *Moehringia papulosa* is an example of a threatened plant living in this kind of habitat. It is an endemism of the Marche region in central Italy, whose natural populations are considered as critically endangered (CR) under the IUCN criteria and the habitat is protected by the E.U. (Directive 92/43/EEC) with the habitat 8210 “calcareous rocky slopes with chasmophytic vegetation”. The phenology of natural populations was analyzed, seed morphology is described and type and level of seed dormancy were determined. The effects of different collecting dates, localities and the influence of elaiosome on germination responses were also considered in order to establish germination requirements to provide optimal protocols for conservation and restoration programmes. Interpopulation differences on seed morphological features were not found and our results also confirm the fact that removal of the elaiosome stimulates germination. The seeds of this species show a non-deep physiological dormancy. The pretreatments proposed as optimal for germination are as follows: a combination of scarification and gibberellins, and 12 weeks of cold stratification.

Keywords: Conservation, endangered, endemism, germination, myrmecochory, rocky habitat, seed dormancy, phenology

Introduction

Habitat alteration due to human activities (e.g. resource extraction, agricultural expansion, urban development, extension of transportation infrastructure) is one of the causes for the decrease in the number of species and the fragmentation of populations with a reduction of ecosystem complexity (Stedman-Edwards 1997; Barber et al. 2004). Among the alterations mentioned, the most critical for the landscape is mining, which destroys entire mountains, thereby generating many problems in ecosystem conservation.

The chasmophytic vegetation, with species that grow on the cracks of the cliffs, shows a great regional diversity, with many endemic plant species, both in the Mediterranean and in the Euro-Siberian regions, and is especially affected by these alterations. Many of these plants are endangered and at risk of extinction also because the amount of soil on

the rocks is minimal; this is also why it is difficult to find these species on altered habitats. For all these reasons, the Habitat Directive (92/43/EEC) admits the high importance of habitat group 82 “Rocky slopes with chasmophytic vegetation” particularly the habitat 8210 “Calcareous rocky slopes with chasmophytic vegetation” (European Commission 2007). In phytosociological terms, the vegetation of fissures of limestone cliffs, in the Mediterranean region and in the euro-Siberian from the plain to alpine levels, belonging essentially to the *Potentilletalia caulescentis* Br.-Bl. in Br.-Bl. & Jenny 1926 and *Asplenietalia glandulosi* Br.-Bl. in Meier et Br.-Bl. 1934 orders, corresponds to this habitat (Biondi 2011; Blasi et al. 2011; Pott 2011). On the basis of the Italian interpretation manual of the Habitat Directive (Biondi et al. 2009), the vegetation of *P. caulescentis* in Apennines belongs to the subtypes 62.13 and 62.15-62.1B.

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These subtypes correspond to: 62.13, plant communities of Northern Tyrrhenian Apennine (*Saxifragion ligulatae* Rioux & Quézel 1949) and 62.15-62.1B to plant communities of Central and Southern Apennine and Sicily (*Saxifragion australis* Biondi & Ballelli ex Brullo 1983). The association *Moehringia papulosae*-*Potentilletum caulescentis* Biondi & Ballelli 1982, whose *M. papulosa* is the most important species, belongs to this latter alliance (Biondi & Ballelli 1982; Biondi et al. 2000; Brullo 1983) (Figure 1).

M. papulosa Bertol. is the only Marche region endemism living on the Adriatic side of central Apennine. *M. papulosa* is a suffrutescent, chasmo-phytic perennial species; it is a small bush growing on overhanging rocks on limestone cliffs. Stems are erect, ascending; flowering occurs between April and June (Pignatti 1982), but secondary flowering has been observed up to November (Brilli-Cattarini 1958). *M. papulosa* (Figure 2) is known only in three Apennine locations: Gola del Furlo (*locus classicus*),



Figure 1. The plant in his natural habitat; location between the quarries of Serra San Quirico.



Figure 2. *Moehringia papulosa* flowering.

Gola della Rossa and in the nearby Gola di Frasassi. The first is in the State Nature Reserve of Gola del Furlo (province of Pesaro-Urbino); the other two are in the Regional Nature Reserve of Gola della Rossa and Frasassi (province of Ancona).

At present, this plant can be considered extinct in Gola di Frasassi, because it disappeared from the stations reported by Brilli-Cattarini (1969), although its presence is not unlikely in other areas of the same locality as censusing population as at this site is quite difficult, because of its rocky calcareous cliffs which often exceed 150 m in height.

The conservation status of *M. papulosa* has been recently revised under the IUCN criteria (Biondi & Bianchelli 2008). According to the present knowledge, this species has been considered as critically endangered (CR) at a regional scale [B1 + B2ab (iv-v)]; previously, it fell under the Endangered (EN) category both at a national and global scale (Conti et al. 1992, 1997).

The study of this species, particularly regarding seed germination is the main object of the present research. The germination characteristics of this plant will allows us to establish an optimal experimental protocol for its propagation. From here on, the *ex situ* cultivation to reintroduce the species where it disappeared, or to strengthen the natural populations, will be possible. This study is a part of a broader project aimed at acquiring more information about the characteristics of this plant, also in ecological and phenological terms, which is considered essential to achieve the final objective.

The main aims of this study were to investigate: (1) flowering and fruiting phenology of natural populations, (2) seed morphology, (3) influence of elaiosome removal on germination, (4) seed dormancy level and type, (5) optimal germination protocol, and (6) effect of collecting date and locality on germination responses.

Material and methods

Studied populations

We carried out field work on the five known subpopulations. Demographic censuses were conducted in natural populations (Albert et al. 2003; Keith 2000), in order to establish the number of mature individuals in the different populations in order to programme seed collection, in accord with standardized protocols (Bacchetta et al. 2006; Enssonet 2009).

As this species grows on generally inaccessible rocky cliffs, we counted “visual units” (García et al. 2002) for individual censuses, from ground level to a maximum height of 7 m; thus, an underestimate of population size is possible.

Plants were classified into three categories: seedling, non-reproductive plants (juveniles + non-flowering), and reproductive plants (flowering). We selected a different number of plants for the phenological study depending on population and accessibility of individuals. Phenology was monitored from February to July 2008, approximately once every two weeks, trying to correlate the observation with the climatic traits of this territory (Rivas-Martínez & Rivas-Sáenz 1996–2009). The total number of flowers and fruit was determined at the end of each stage, in order to avoid counting twice. Reproductive success was expressed as the fruit per flower ratio.

Collecting was restricted to the populations from the nucleus of Gola del Furlo depending on the number of accessible individuals. Seeds were collected from natural populations (Table I) mainly from June to July, when they were completely mature, taking into account that this species has the advantage of producing persistent seeds that remain within the capsules long after dehiscence. The number of seeds per fruit was determined, by directly counting them in collected subsamples.

Seed dormancy analyses and the establishment of seed germination protocols for endangered species depends of the amount of seeds collected, and it is necessary to make sure that the natural capacity of regeneration of the populations is always preserved (Bacchetta et al. 2006; Ensconet 2009).

Seed collecting for our investigation without risk for species preservation was possible only in the populations with a higher number of individuals. Due to the small amount of seeds obtained each year from natural populations, various experiments were carried out with several accessions from the same or different populations. Three different collecting dates were required to complete the trials: 7 December 2006, 28 June 2007, and 7 September 2007.

Seed morphological features

To determine seed weight, batches of 50 seeds were weighed using an Orion Cahn C-33 microbalance. Seed surface was analyzed with a SEM Hitachi

S-4100, file emission, from the SCSIE department, electronic microscopy section of the University of Valencia. Samples were mounted on aluminium stubs with a carbon double-sided tape, and were sputter-coated with a 100–200 Å thick layer of gold and palladium by an Ion Sputter (Bio-Rad SC-500), and were examined at an accelerating voltage of 5 KV.

Seed descriptions were according to Werker (1997). The terminology of Stearn (1980) and Barthlott (1981) was adopted for the SEM aspects of the seed-coat. The elaiosome was described using Hind's (1988) classification. The type of embryo was defined according to Martin (1946) and Martin and Barkley (1961). Width and length were measured by light microscopy with micrometer at the longest and widest axis of the seed. In order to determine the seed dimensions, 50 seeds from each species were measured with a binocular microscope (Nikon SMZ-U) at a magnification factor of $4\times$. Means and standard deviations were calculated for each measured character.

Germination experiments

Seed dormancy and establishment of an optimal germination protocol. Seed viability was determined, previously to the germination study, on 10 seeds by the tetrazolium test (ISTA 1999). The elaiosome was first detached and the seed coat chipped with a scalpel, then seeds were maintained in 1% TZ solution at 30°C during 24 h in absolute darkness.

A small production of seeds greatly limits the number, kind and size of experiments. So according to Baskin and Baskin (2004a) recommendations, we use results in taxonomic relatives to develop the experimental design. To investigate the dormancy status of seeds, we previously compiled information about requirements of close species in the available bibliography and database of Royal Botanic Gardens Kew (Seed Information Database 2008). Finally the pretreatments essayed were (1) scarification with sand paper, (2) gibberellic acid (0.722 mM) added to the substrate, (3) a combination of both, and (4) cold stratification at 5°C during 6 and (5) 12 weeks. All pretreatments were compared to a control (0).

Table I. Populations, localities, accessions and collection dates of *Moehringia papulosa* seeds in the Gola del Furlo site used for the following investigations: (a) seed features determination; (b) elaiosome effects on germination; (c) dormancy analysis and optimal germination protocol; (d) effect of collecting date; and (e) subpopulation variation.

Population	Coordinates	Acc.	Date	Experiments
Grotta del Grano	X317092 Y4835980	A	7 December 2006	a, b
		B	28 June 2007	a, b, c, e
Diga ENEL	X316336 Y4835095	C	28 June 2007	a, d, e
		D	14 September 2007	a, b, d

To establish other basic germination requirements, related to light and temperature regime, a factorial experiment was designed with seeds pre-treated with gibberellic acid. We compared germination responses under two different light levels, total darkness and 12 h photoperiod using daylight fluorescent illumination ($125 \mu\text{mol m}^{-2} \text{s}^{-1}$). We checked as well the response with constant (20°C) and fluctuating day/night ($25/15^\circ\text{C}$) temperatures. Seed germination in darkness was monitored under green safe light provided with two green luminescent lamps (Philips TL-D 18W/17) supplemented with a green filter (Roscolux ref. no. 90).

Germination tests were started 3–6 months after the seed collecting dates. All the germination tests were carried out in 6 cm diameter Petri dishes with 0.6% bacteriological agar (Pronadisa, indicate city/state). Four replications of 20 seeds each were used. The germination tests were completed after 30 days.

At the end of the germination period, the final germination percentage, T_{50} (Thanos & Doussi 1995) and the Mean Germination Time (MTG) (Black et al. 2006) were calculated and expressed as mean values \pm typical error (specify the meaning). These indexes were not calculated when the final germination percentage was less than 5%.

Influence of elaiosome removal on germination. Seeds from three different accessions (Table I) were sown after the elaiosome had been carefully removed with a razor blade and results compared with intact seeds without any kind of pretreatment. Germination was tested at 20°C and 12/12 h photoperiod.

Effect of collecting date and locality in germination response. We compared seed responses in two different accessions collected from the same locality, Diga ENEL, in June and September 2007. The tests were carried out at $25/15^\circ\text{C}$ in absolute darkness. Two different pretreatments (gibberellic acid and scarification combined with gibberellic acid) and no pretreatment (control) were used for this comparison.

In order to detect subpopulation differences, we also compared germination of accessions collected

the same day (28 June 2007) from two different localities, i.e. Grotta del Grano and Diga ENEL. The same pretreatments previously described above were applied.

Statistical analysis

For all the tests, the final germination percentages were calculated and arcsine-transformed, and the levels of significance ($P < 0.05$) were obtained by analysis of variance (ANOVA) using SPSS 15.0. One-way ANOVA was used to analyze the influence of the elaiosome, the effect of collecting date and locality, and the pretreatments for rupturing seed dormancy. In this last case, homogeneous groups were determined using Tukey's test. Two-way ANOVA was applied to analyze the effect of temperature and light regimes on final germination percentages. The statistical analysis of T_{50} and MTG was also carried out using the same methods.

Results

Population study

Natural populations are distributed in two main nuclei in the Marche region: Gola del Furlo and Gola della Rossa. The observed data regarding number of monitored individuals and the average of flowering and fructification for each population on the date of study are shown in Table II.

No phenological differences were found between the populations of *M. papulosa*. The opening of the first flowers occurred in April (average temperature min. 11°C max. 16°C ; average sunlight 6.8 h/day), and the maximum of flowering was in May (average temperature min. 14°C , max. 20°C ; average sunlight 8.4 h/day). The fruiting stage started in June (average temperature: min. 18°C , max. 24°C ; average sunlight 8.9 h/day).

From the monitored populations, a mean of 68% (49–80%) of the plants flowered: however, the average fructification per plant was particularly low in some of the subpopulations considered.

Table II. Estimated number of seedlings of non-reproductive plants, and of reproductive and dead plants, mean number of flowers per plant, and percentage fructification per plant in natural populations of *Moehringia papulosa* from different areas within the two main locations in the Marche region: Gola del Furlo and Gola della Rossa.

Population	Seedling	Non-reproductive	Reproductive	Dead	Mean flowers	Fructification (%)
<i>Gola del Furlo</i> (Pesaro-Urbino prov.)						
Passo Furlo Diga ENEL (150 m asl)	101	19	30	9	35	18
Villa Furlo (150 m asl)	7	20	19	4	26	23
Grotta del Grano (150 m asl)	1	3	10	1	53	17
<i>Gola della Rossa</i> (Ancona prov.)						
Ponte di Chiaradovo (157 m asl)		2	8		48	18
c/o Cave Serra S. Quirico (150 m asl)	5	4	10	2	20	53

In all the monitored individuals, a constant number of four seeds per fruit was observed. In most of them, one of the seeds weighed less than the others (Figure 3).

Seed morphological features

No interpopulation differences in *M. papulosa* seed morphology were detected. The seeds (Figure 4) are reniform to elliptic, slightly compressed, small and black. Seed size ranges between 1.1–1.8 mm in length (1.3 ± 0.1) and 0.8–1.2 mm in width



Figure 3. Fruit after dehiscence.

(1.0 ± 0.1). The strophiole/elaiosome is 0.5–1.6 mm long (1.2 ± 0.3) and 0.5–1.3 mm wide (0.9 ± 0.2). Mean seed weight is 0.427 ± 0.057 mg.

The elaiosome is white and clearly visible as a mass of non-lignified cells at the hilar end of the seed (Figure 4a). According to the classification of Hind (1988), it is of the hyphal or coelenterate type.

The seed coat is quite simple in this species; it does not show any kind of cuticular sculptures or secondary wall thickenings (Figure 4b). When analyzing the primary sculpture, we observed that the cells are more or less elongated depending on the area examined, with the anticlinal walls undulated in an S-type, and without significant relief at the cell boundary (Figure 4c).

We found some convex cells near the dorsal zone next to the micropile (Figure 4d). The surface is smooth; the cells do not have any micro-ornamentation on the outer wall nor any kind of papillae.

Regarding the embryo, the seeds are of the peripheral type, with an elongate and curved embryo.

Germination responses

In the three accessions studied, A, B, and D (Table I), we found significant differences in germination between intact seeds and seeds without the elaiosome (Figure 5). In all the tests, intact seed did not germinate in contrast to seeds devoid of the elaiosome which showed the following results: population A: $20.0 \pm 3.3\%$ ($P = 0.000$; $F = 150.0$),

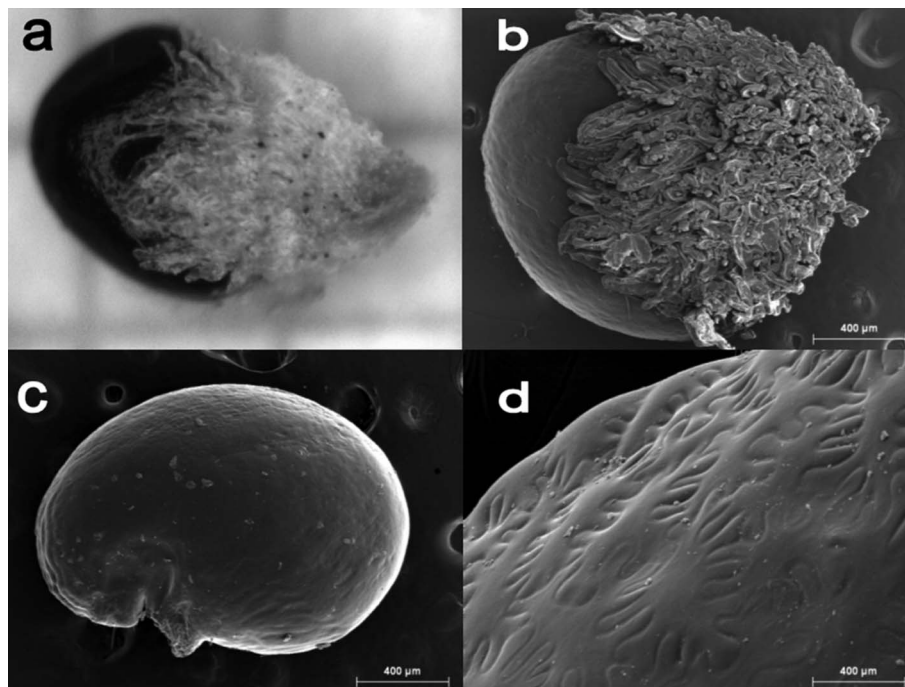


Figure 4. Morphological aspects and seed coat microstructure of *Moehringia papulosa*. (a) seed observed with stereomicroscopy; (b) entire seed with SEM; (c) seed surface after elaiosome detachment and (d) outline of testa cells.

population B: $15.0 \pm 6.0\%$ ($P=0.002$; $F=25.0$), population D: $10.0 \pm 4.0\%$ ($P=0.002$; $F=25.0$).

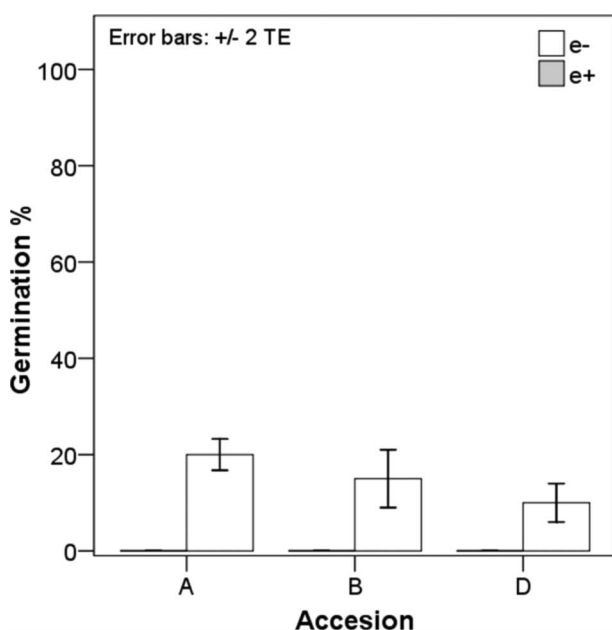


Figure 5. Effect of the elaiosome removal (e+ entire seeds; e- seeds without elaiosome) on seed germination (20°C; 12/12 h photoperiod); accessions A and B and D (see Table I).

Tetrazolium tests revealed different theoretical viability values for the three accessions studied in the germination experiments. The final results for this test were 20%, 56% and 70% for accession B, C, and D, respectively (Table III). Thus, the expected maximum potential germination percentage for the seeds used in this study would be within these values. However, in many cases, real germination values are higher.

Seed germination percentages, MTG and T_{50} values for all the tested conditions are shown in Table III.

The best germination percentages (Figure 6) were obtained with the combination of mechanic scarification and gibberellic acid added to the substrate (pretreatment 3), and with cold stratification during 12 weeks (pretreatment 5). Nevertheless, significant differences in the germination time (MTG, T_{50}) were observed between the two treatments, with cold stratification giving the fastest response.

Different light and temperature regimes, constant or alternating, did not result in improved germination percentages under any of the different conditions tested (Table III). The two way ANOVA indicated that there are no significant ($P > 0.05$) differences due to individual effects of temperature and light regimes, nor to their interactions, on the

Table III. Seed viability from tetrazolium tests (TZ) and seed germination percentages, MTG and T_{50} values for all the tested conditions (Illumination: (0) darkness (1) 12:12 light photoperiod; Pretreatments: (1) scarification with sand paper, (2) gibberellic acid (3) scarification and gibberellic acid (4) stratification at 5°C six weeks (5) stratification at 5°C (12 weeks) for the three accessions studied (B, C, and D, see Table I).

Accession	TZ	Temperature (°C)	Illumination	Pretreatment	%	MTG	T_{50}
B	20%	20	0	0	8.8 ± 5.9	—	—
		20	0	1	20.0 ± 6.1	6.8 ± 0.3	6.4 ± 0.3
		20	0	2	5.0 ± 2.9	—	—
		20	0	3	$41.3 \pm 7.2^*$	8.9 ± 1.3	7.2 ± 0.9
		20	0	4	1.3 ± 1.3	—	—
		20	0	5	$38.9 \pm 6.9^*$	$1.1 \pm 0.1^*$	$0.5 \pm 0.0^*$
		20	1	2	11.3 ± 3.8	7.7 ± 2.7	6.0 ± 2.3
		25/15	0	0	2.5 ± 1.4	—	—
		25/15	0	1	$33.8 \pm 7.7^*$	8.0 ± 1.8	5.5 ± 0.5
		25/15	0	2	8.8 ± 5.2	11.9 ± 2.2	7.8 ± 3.3
		25/15	0	3	$42.5 \pm 4.3^*$	9.1 ± 1.1	8.3 ± 1.6
		25/15	0	4	6.3 ± 2.4	9.3 ± 2.9	8.8 ± 2.9
		25/15	0	5	$33.8 \pm 6.9^*$	$1.2 \pm 0.1^*$	$0.5 \pm 0.0^*$
		25/15	1	2	5.0 ± 2.9	—	—
		25/15	0	0	3.3 ± 1.9	—	—
C	56%	20	0	2	15.0 ± 7.9	9.6 ± 1.9	7.0 ± 1.0
		20	0	3	$75.0 \pm 12^*$	11.9 ± 1.4	12.0 ± 1.9
		20	1	2	13.8 ± 5.5	16.0 ± 5.0	14.8 ± 5.4
		25/15	0	0	6.7 ± 2.7	17.0 ± 1.5	16.5 ± 1.5
		25/15	0	2	6.3 ± 2.4	12.3 ± 0.9	11.8 ± 0.9
		25/15	0	3	$60.0 \pm 7.2^*$	$7.5 \pm 0.5^*$	$6.3 \pm 0.3^*$
		25/15	1	2	10.0 ± 2.0	14.3 ± 0.8	13.5 ± 0.9
D	70%	25/15	0	0	8.8 ± 3.8	8.4 ± 5.5	8.0 ± 5.5
		25/15	0	2	11.3 ± 2.4	10.4 ± 3.1	9.7 ± 3.3
		25/15	0	3	$55.0 \pm 5.0^*$	8.2 ± 0.5	7.3 ± 1.3
		25/15	1	2	12.5 ± 1.4	11.5 ± 3.4	10.3 ± 3.5

Significant results within groups ($P < 0.05$) are marked with asterisks.

germination of *M. papulosa* seeds under the studied conditions.

We did not find significant differences in germination percentage between seeds collected before (June) or after (September) the summer (Figure 7). The ANOVA for the three studied treatments with respect to the different seed collecting date gave the

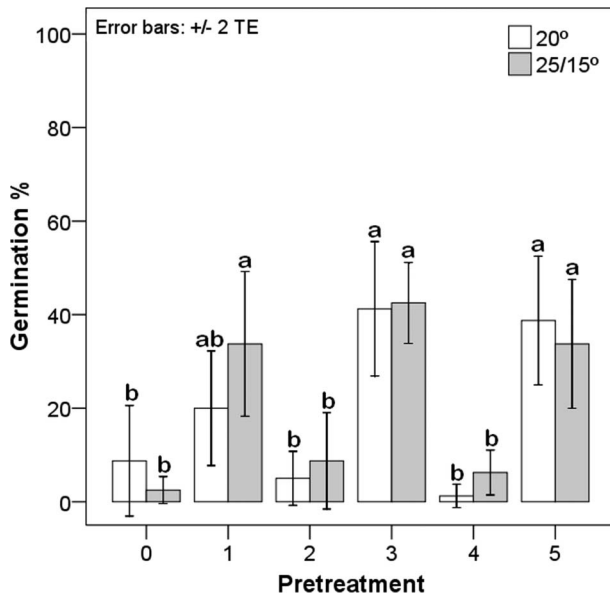


Figure 6. Effect of different pretreatments used ((0) control; (1) scarification; (2) gibberellic acid; (3) scarification and gibberellic acid; (4) six weeks cold stratification; (5) 12 weeks cold stratification) for accession B at two temperature regimes in absolute darkness (see Table I).

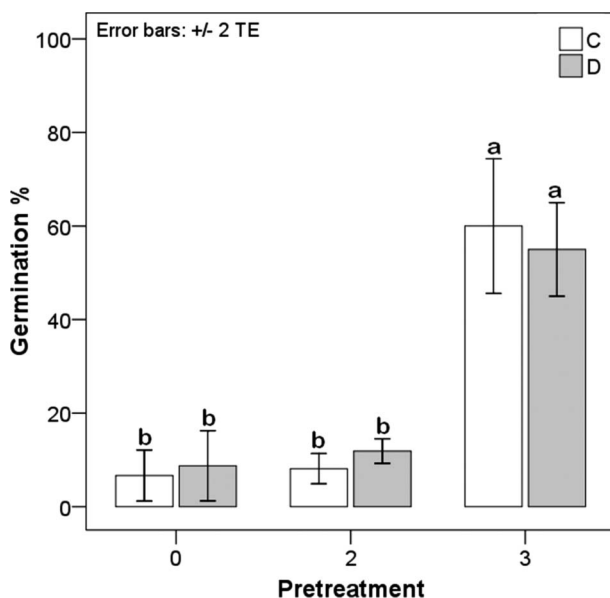


Figure 7. Comparison of germination response at 25/15°C in darkness ((0) control; (2) gibberellic acid; (3) scarification and gibberellic acid); seeds collected in different season. Accessions C and accessions D (see Table I).

following values: control ($F=0.202$; $P=0.669$), gibberellic acid pretreatment ($F=3.231$; $P=0.094$); scarification and gibberellic acid pretreatment ($F=0.325$; $P=0.589$).

The differences in MTG and T_{50} when they could be calculated were not significantly ($P < 0.05$) different between treatments.

Subpopulation differences in germination percentages were not statistically significant in any of the three tested conditions (Figure 8): control ($F=1.829$; $P=0.225$), gibberellic acid pretreatment ($F=0.147$; $P=0.707$); scarification and gibberellic acid pretreatment ($F=4.338$; $P=0.082$). Differences in MTG and T_{50} when they could be calculated were also not significant ($P < 0.05$).

Discussion

The available climatic data indicated that flowering began when the period of night temperatures below zero ended, and ended when maximum temperatures reached 25–30°C. It was also correlated with an average sunlight period longer than 7–8 h/day.

The remarkably low fruit production observed in most of the populations could be associated with a poor pollination and the specific climatic conditions of the collecting year, with below average rainfall.

Our observations suggest that long-term studies on the influence of environmental conditions on the phenology and reproductive biology of this species are necessary, in order to establish accurate conservation measures and to evaluate the impact of

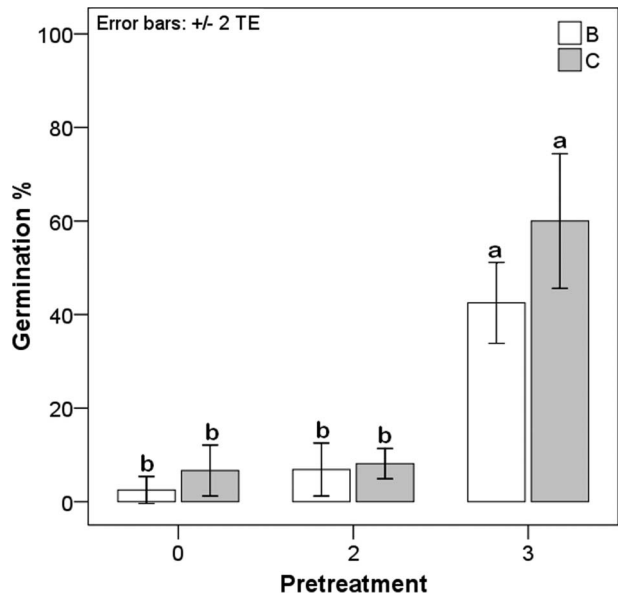


Figure 8. Comparison of germination response at 25/15°C in darkness ((0) control; (2) gibberellic acid; (3) scarification and gibberellic acid); seeds collected from different localities, accessions B and C (see Table I).

potential threats. The extinction risk for most plant species is significantly affected by limiting environmental conditions that can directly restrict reproduction (Albert et al. 2003).

Concerning the morphological seed features of *M. papulosa*, our observations are in absolute agreement with the description of Minuto et al. (2006, 2011). Seeds of this species have the most frequent color and shape of the genus; the seeds are reniform, black and shiny, with a white elaiosome. The seed dimensions are slightly above average for this taxonomic group. No interpopulation differences were detected for seed morphological features (mainly weight) that could provide some indication of possible differences in germination response or seedling survival as described by Navarro and Guitián (2003) for *Petrocoptis grandiflora*.

The elaiosome is a morpho-anatomical and biochemical element strictly correlated with the ant-mediated dispersal mechanism. In this genus, the structure of the elaiosome is directly associated with the type of habitat (Casazza et al. 2008). Field observations, carried out in the course of this study, confirm that the ant *Lasius emarginatus* Olivier 1971 is the main dispersal agent for this species.

This dispersion strategy is especially relevant for species growing in microhabitats, such as fissures, rock crevices or soil beneath overhanging rocks, the typical habitat of *M. papulosa*, in particular if they are, as in this case, a threatened or endemic species. As described by Ciccarelli et al. (2005), additional benefits are provided by this kind of strategy. The transport of seeds to the ant nests may provide protection from predation and the removal of the elaiosome could stimulate germination. This stimulation of seedling emergence is described for various Mediterranean myrmecochorous plants such as *Rhamnus alaternus* (Gómez et al. 2003), *Myrtus communis* (Ciccarelli et al. 2005), and *Euphorbia characias* (Gómez & Espadaler 1997). Present results confirm this effect in *M. papulosa*, in agreement with Casazza et al. (2008) for other species of this genus.

Enhanced germination after elaiosome removal could be explained by two different mechanisms: the presence of inhibitors in this structure and/or its functioning as a physical barrier to water absorption. Although germination was stimulated after elaiosome removal, it is evident that seeds never reached high germination rates; they evidently show additional dormancy that requires a pretreatment in order to acquire complete germination capacity.

In the present study, no improvement was obtained under the different illumination and/or temperature regimes used. By contrast, Navarro and Guitián (2003) found higher germination percentages in seeds maintained in total darkness than in those incubated under a 12/12 h photoperiod for two species of the

genus *Petrocoptis* living in the same type of habitat characteristic of *M. papulosa*. Also other authors like Vandeloos et al. (2008) also observed a positive response to fluctuating temperatures in *Moehringia trinervia*.

As it is phylogenetically expected for some Caryophyllaceae, seeds of *M. papulosa* exhibit a physiological dormancy (Finch-Savage & Leubner-Metzger, 2006). Baskin and Baskin (1998) cited physiological dormancy as one of the most common types of seed dormancy in the Caryophyllaceae. Indeed this type of dormancy has been evidenced in several members of this family, such as *Spergularia* (Carter & Ungar 2004), *Arenaria* (Baskin & Baskin 1975), *Moehringia* and *Stellaria* (Vandeloos et al. 2008). Also, physiological dormancy appears in species living in the same habitat, like *Telekia speciosissima*, another Italian endemic chasmophyte (Brusa et al. 2007).

As described by Baskin and Baskin (2004b), some species with non-deep physiological dormancy respond to a scarification pretreatment; this is the case for *M. papulosa*. In other species from this family, such as *Silene lanceolata* (Halward & Shaw 1996), scarification was shown to enhance germination in non-after-ripened seeds.

In *M. papulosa* the most reliable pretreatments for a good germination protocol are a combination of scarification and gibberellins, and 12 weeks of cold stratification. The highest percentage of germination was obtained with the former treatment after 12 days in absolute darkness at 20°C, but it should be emphasized that germination velocity was significantly higher after cold stratification.

Interestingly, there was no significant difference between germination of seeds stratified for six weeks and the control seeds, indicating that the stratification period has to be sufficiently long in order to positively affect germination. Results for *M. papulosa* differ from those of Navarro and Guitián (2003) for the endemic *Petrocoptis* from the northwest Iberian Peninsula, in which cold prior to germination had no significant effect. Also, the germination behavior of *M. papulosa* is dissimilar from other *Moehringia* species. For example, *Moehringia fontqueri*, an alpine endangered endemism from Spain, does not show seed dormancy (Lorite et al. 2007).

The available information about seed germination in the genus *Moehringia* points to a high correlation between germination requirements and habitat. Vandeloos et al. (2008) determined, for four temperate Caryophyllaceae, that contrasting germination patterns were related to differences in the habitat preferences of the species. The observed differences in seed dormancy between these closely related species living in similar habitats could be caused by differences in the amount of rains. Higher annual precipitation (ca. 950 mm) was recorded in

the *M. papulosa* localities, than in the stations of *Petrocoptis grandiflora*, *Petrocoptis viscosa* and *M. fontqueri* (ca. 600 mm). Baskin and Baskin (1975) found year-to-year variation in the proportion of dormant seeds over a three-year period within a population of *Arenaria patula* in relation to rainfall during the 30 days preceding seed maturity. Lower germination percentages were recorded in those years with higher rainfall. Water availability during the seed maturation phase seems to be determinant for the establishment of the dormancy level. This notion is in agreement with our data, and represents a future research objective for this species.

It would be interesting to continue *in situ* studies regarding phenological and demographic parameters for this species in order to follow the evolution of populations and evaluate the need to carry out reinforcement plans. Meanwhile, it would be advisable to ensure the *ex situ* long-term conservation of seeds in gene banks. García-Barriuso et al. (2011) emphasize the requirement of continued monitoring the demography of species from microhabitats inhabited by endemic plants, especially those with concrete ecological requirements, like wall and rocky areas, and those in certain isolation risk of their populations, in order to improve its management.

Pradhan and Badola (2011) indicate the relevance of using appropriate seed sources, as well as the acquisition of knowledge on germination responses addressed to establish specific propagation protocols in order to provide quality plant material for conservation programmes, especially in endangered plants which are narrowly adapted to specific microhabitats. In the context of biodiversity conservation, present data, mainly on germination behavior, can help to achieve a fuller understanding of this type of threatened habitat in order to develop future management and recovery programmes to mitigate the problems and risks generated by the alteration of chasmophytic vegetation.

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