LAZAROA 34: 43-53. 2013
doi: 10.5209/rev_LAZA.2013.v34.n1.42253 ISSN: 0210-9778

Seed germination capability of four endemic species in the Central Apennines (Italy): relationships with seed size

Anna Rita Frattaroli (*), Luciano Di Martino (**), Valter Di Cecco (*), Rosangela Catoni (***), Laura Varone (***), Marco Di Santo (**) & Loretta Gratani (***)

Abstract: Frattaroli, A.R., Di Martino, L., Di Cecco, V., Catoni, R., Varone, L., Di Santo, M. & Gratani, L. *Seed germination capability of four endemic species in the Central Apennines (Italy): relationships with seed size*. *Lazaroa 34: 43-53 (2013)*.

Seed germination capability of *Adonis distorta, Androsace mathildae, Aquilegia magellensis* and *Campanula fragilis* subsp. *cavolinii* endemic species of the Central Apennines (Italy) were analyzed. Seed traits varied significantly among the considered species. In particular, seed volume was the largest in Adonis (91.642 ± 16.851 mm^3) and the lowest in *Campanula* (0.029 ± 0.008 mm^3). The seed coat thickness ranged from 31 ± 10 μm in *Adonis* to 9 ± 1 μm in *Campanula*. Pretreatments were carried out to improve seed germination. Seed germination did not happen in *Adonis* and *Androsace* in response to the applied treatments (i.e., 0, 250, 500 ppm gibberelic acid, GA3) and the cold-wet stratification. A 65 % increase of germination was observed after the pre-treatment with 500 ppm GA3 in *Aquilegia* which could be justified by an endogenous non-deep physiological dormancy. The final germination percentage increased by 26% in *Aquilegia* and decreased by 89% in *Campanula* after the cold-wet stratification treatment. The obtained results were used to define germination protocols which could be used in reinforcement projects for the wild populations of the considered endemic species as a means of reducing their extinction risk.

Keywords: endemics, seed germination, seed size, seed dormancy.

Resumen: Frattaroli, A.R., Di Martino, L., Di Cecco, V., Catoni, R., Varone, L., Di Santo, M. & Gratani, L. *Capacidad germinativa de especies endémicas de los Apeninos centrales (Italia): relaciones con el tamaño de la semilla*. *Lazaroa 34: 43-53 (2013)*.

En este trabajo se ha analizado la capacidad germinativa de cuatro especies endémicas de los Apeninos centrales: *Adonis distorta, Androsace mathildae, Aquilegia magellensis* y *Campanula fragilis* subsp. *cavolinii*. Los rangos de las semillas variaron significativamente entre las especies, particularmente se encontró que el volumen de semillas en *Adonis* (91.642 ± 16.851 mm^3) era el más alto y en *Campanula* el más bajo (0.029 ± 0.008 mm^3). El grosor de la cubierta seminal se encontraba desde 31 ± 10 μm en *Adonis* hasta 9 ± 1 μm en *Campanula*. Se realizaron algunos pretratamientos para mejorar la germinación como fue la adición de distintas concentraciones de ácido giberélico (i.e., 0, 250, 500 ppm gibberelic acid, GA3) o la estratificación frío-caliente. Sin embargo no detectamos germinación ni en *Adonis* ni en *Androsace*. En el caso de *Aquilegia* se observó un aumento del 65 % de la germinación después de pretratarla con 500 ppm GA, lo cual queda justificado por una dormancia fisiológica endógena no muy profunda. El porcentaje de germinación final se incrementó en *Aquilegia* en un 26%, mientras que decreció un 89% en *Campanula* después del tratamiento de estratificación frío-caliente. Los resultados obtenidos fueron utilizados para definir los protocolos que deben ser utilizados para mejorar la conservación de especies endémicas en riesgo de extinción.

Palabras clave: especies endémicas, germinación, tamaño de semilla, dormancia

* Department of Life Health and Environmental Sciences. University of L'Aquila. Via Vetoio loc. Coppito. 67100, L'Aquila, Italy.

** Majella Seed Bank. Majella National Park. loc. Colle Madonna. 66010, Lama dei Peligni (CH), Italy.

*** Department of Environmental Biology. Sapienza University of Rome. P.le A. Moro, 5 00185 Rome, Italy. Corresponding author: Loretta Gratani. Fax: 0649912358. Email: loretta.gratani@uniroma1.it

INTRODUCTION

Knowledge of rare species life-cycle and reproductive traits is essential for identifying limits to population growth and persistence (BEVILL & LOUDA, 1999) especially in threatened wild species. Seed germination is a critical stage for the establishment of the plant (YOUSSEF & *al*., 2012) as its success is determinant for plant species propagation (RAJJOU & *al*., 2012). Each species has specific requirements for seed germination (HARPER & *al*., 1970; MEYER & MONSEN, 1991; SCHÜTZ & MILBERG, 1997) which involves particular features of seed and environmental factors (BASKIN & BASKIN, 1998). Many authors (JAKOBSSON & ERIKSSON, 2000; BONITO & *al*., 2011; LÖNNBERG & ERIKSSON, 2013) underline the relationship between seed germination and seed size. Seed size represents the amount of maternal investment in the individual offspring (LEISHMAN & *al*., 2000). Generally, large seeds have a greater germination success than small seeds (PIZO & *al*., 2006). Seed coat plays an important role in embryo nutrition during seed development and against detrimental agents from the environment (MOHAMED-YASSEEN & *al*., 1994; WEBER & *al*., 1996). Seed coat-imposed dormancy is part of the seed survival strategy of many species (WERKER, 1981; KELLY & *al*., 1992; SCHÜTZ, 2000). Moreover, seed coat exerts a germination-restrictive action most of the time by being impermeable to water and/or oxygen or by its mechanical resistance to radical protrusion. Seed dormancy is a trait that has been acquired by many species through selection for the ability to survive in unfavourable environments (BEWLWY & *al*., 2013). Seed dormancy can be viewed as an adaptive mechanism for survival in a seasonally variable environment (WESTOBY, 1981). Under natural conditions, dormant seeds are exposed to changes in environmental factors (e.g. light, temperature, moisture) which lead to cyclical changes in the dormancy state (FINKELSTEIN & *al*., 2008). Many high mountain plants produce seeds with different types of dormancy to avoid germination in the year of seed dispersal and favour rapid emergence after snowmelt (BILLINGS & MOONEY, 1968; BASKIN & BASKIN, 1998; SHIMONO & KUDO, 2005). Several germination traits have been claimed to be specific to high-altitude species (i.e. rapid onset of germination after snowmelt and high seed viability; GIMÉNEZ-BENAVIDES & MILLA, 2013).

Endemic species are a significant feature of the Mediterranean mountains (GÓMEZ-CAMPO, 1985; VÄRE & *al*., 2003; SAÍNZ & MORENO, 2002; GAVILÁN & *al*., 2002; ESCRIBÁ & *al*., 2007; FUENTE & *al*., 2011) because of the high number of speciation events that have occurred (MARTÍN-BRAVO & *al*., 2010). Mediterranean mountains are characterized by a high genetic diversity with many populations being genetically unique (RUIZ-LABOURDETTE & *al*., 2012). Predictions of climate change indicate that this genetic diversity could be disturbed significantly in the future (THUILLER & *al*., 2005). Moreover, Mediterranean mountains are considered one of the most threatened ecosystems in the European Union (GÓMEZ-CAMPO, 1987; European Community, 1992). Thus, many efforts should be addressed to improve the conservation strategies for Mediterranean mountain species considering that the survival of endemic and threatened species is based on different and complementary conservation approaches and techniques (IUCN, 2002). The definition of germination protocols, in particular for species characterized by small populations and for which data are missing, could be an important step in this direction.

The aim of this work focused on seed germination capability of four endemic species growing in the Central Apennines, in Italy. In particular, we analysed the influence of different pre-treatments on seed germination for *Adonis distorta* Ten., *Androsace mathildae* Levier, *Aquilegia magellensis* F. Conti & Soldano and *Campanula fragilis* Cirillo subsp. *cavolinii* (Ten.) Damboldt. The results may be used for conservation projects of the wild populations of these endemic species.

MATERIALS AND METHODS

STUDY SITE AND SPECIES

Experiments were carried out in the Majella Seed Bank within the Botanical Garden Michele

Tenore (42° 2' 59" N; 14° 11' 34"E; 650 m a.s.l., Italy) on seeds collected from the wild populations of *Adonis distorta, Androsace mathildae, Aquilegia magellensis* and *Campanula fragilis* subsp. *cavolinii* growing on Mount Majella. Hereafter, to facilitate reading, each species will be referred to the name of Genus. All the species are endemic of the Central Apennines and are included in the Regional Red List (Conti & *al*., 1997). In particular, *Adonis* (Ranunculaceae) grows in the Marche, Umbria, Lazio and Abruzzo regions (Conti & *al*., 2005) on high-altitude screes (2000-2500 m a.s.l.) characterized by small clasts (Pignatti, 1982). In Abruzzo, populations of few individuals grow in the Gran Sasso Massif, Velino Mount, Sirente Mount and Majella Mount. *Adonis* is included in the II and IV Annex of the Habitat Directive (Habitat Natura 2000) and categorized as DD (Data Deficient) in the IUCN Red List. *Androsace* (Primulaceae) is endemic of the Abruzzo region (Conti & *al*., 2005) where it grows at Gran Sasso Massif and Majella Mount (2500-2900 m a.s.l.) (Pignatti, 1982) on cracks of limestone cliffs, mainly in the northern exposure. It is included in the II and IV Annex of the Habitat Directive (Habitat Natura 2000) and categorized as DD (Data Deficient) in the IUCN Red List. *Aquilegia* (Ranunculaceae) grows in Abruzzo and Molise regions (Conti & *al*., 2005) on dripping limestone walls from 1000 to 1500 m a.s.l. (Pignatti, 1982). *Campanula* (Campanulaceae) grows in Lazio, Abruzzo, Molise and Campania regions (Conti & *al*., 2005) on limestone cliffs from 200 to 1800 m a.s.l. (Pignatti, 1982). To date, there are no studies regarding the conservation strategies and seed germination capability of the selected species.

The climate of the Mount Majella, is characterized by a mean minimum air temperature (T_{min}) of -3.9 ± 2.2 °C (February), a mean maximum air temperature (T_{max}) of 22.3 ± 0.1 °C (July-August) and a mean annual air temperature (T_{mean}) of 7.6 ± 6.5 °C. Total annual rainfall is 1343 mm. Snow fallen from December to April (Meteorological Station of Passo Lanciano, Ch, 42° 18' 62"; 14° 09' 87", data for the period 2000-2012, cetemps.aquila.infn.it).

Seed collection

Freshly-matured seeds of *Adonis, Androsace, Aquilegia* and *Campanula* were collected from the small wild populations growing on Mount Majella, from August to September 2010, in the fruiting period and immediately before dissemination, according to Hay & Smith (2003). The mother plants were randomly selected, according to Marshall & Brown (1983) (Table 1). In particular, seeds of *Adonis* were collected at 2675 m a.s.l, on the north-northeast facing of Mount Focalone (42°6'18" N; 14°7'10" E); seeds of *Androsace* at 2760 m a.s.l. in the north facing of Mount Amaro (42°5'12" N; 14°7'14" E); seeds of *Aquilegia* at 1225 m a.s.l on the south-west facing of the Eremo di San Giovanni (42°9'14" N; 14°4' 50" E), and seeds of *Campanula* at 750 m a.s.l. in the north-east facing of Lama dei Peligni (42° 0'09" N; 14° 8' 44" E). Seeds (n = 300 for *Adonis, Androsace* and *Aquilegia*; n = 2000 for *Campanula*) were immediately transported to the Botanical Garden.

Table 1
Characteristics of the considered species and of their natural environments at Mount Majella are shown. Abbreviations are: HScap., scapose hemicryptophyte; ChPulv., pulvinate chamaephyte; ChSuffr., suffruticose chamaephytes. Altitude (m.a.s.l.). End. App. Abr., Endemic Appenine Abruzzo; End. App. C., Endemic Appenine Campania.

Species	Family	Life Form	Chorotype	Altitude	Exposure
Androsacea mathildae	*Primulaceae*	ChPulv	End.App.Abr.	2760.	NNE
Adonis distorta	*Ranunculaceae*	HScap	End.App.C.	2675	NNE
Aquilegia magellensis	*Ranunculaceae*	HScap	End.App.C.	1225	SW
Campanula fragilis subsp. *cavolinii*	*Campanulaceae*	ChSuffr	End.App.C.	750	NE

Seed traits measurements

At the Majella Seed Bank seeds were surface-sterilized in a 1% sodium hypochlorite solution for 1-10 minutes, according to the seed coat type (Bacchetta & *al*., 2006), then rinsed in distilled water. The seed coat after the imbibition in distilled water was dried and then the experiment was carried out. Seeds were placed in paper bags at 5°C until the germination experiment started. Three groups of 100 seeds each for *Adonis, Androsace* and *Aquilegia*, and two groups of 1000 seeds each for *Campanula* (seeds < 0. 010 mg) were weighed to measure seed fresh mass (S_M), according to Cerdà & García-Fayos (2002). The reduced number of seeds used was justified by the small number of the wild populations of the considered species in Majella National Park. In particular, there were few *Adonis* populations for a total number of ca. 1500 plants, and few *Androsace* populations for a total number of ca. 400 plants (non-published data from the Park). Despite *Aquilegia* being largely distributed in respect to the other two species (van Gils & *al.,* 2012), there are no data about the number of populations and the number of plants per population. No data about number and size of populations are available for *Campanula*.

Seed length (L, longest axis), width (W, intermediate axis) and thickness (T, shortest axis) (20 seeds per species) were measured, according to Cerdà & García-Fayos (2002); from these data, seed surface (S = L x W), volume (V = L x W x T), density ($D = S_M/V$) and the ratio S/S_M (surface/mass) were calculated. To characterize seed shape, the Eccentricity Index (E.I. = L/W) was used (Balkaya & Odabas, 2002). Seed coat thickness was measured at 3 or 4 points of each seed by a stereo-microscope (Leica Wild M10), according to Tunjai & Elliott (2012).

Germination experiment

The seed germination experiment was carried out on seeds of the considered species which were transferred to an agar medium (7 g l^{-1}) (Morgan & *al*., 1997). Media was integrated with 4.4 g l^{-1} of Murashige Skoog salts (MS; Murashige & Skoog, 1962) because it provided nutrients to allow the seedlings growth (Pence, 1999). We followed this procedure because we wanted to cultivate the seedlings in the Botanical Garden and use them in reinforcement programs of wild populations in Majella National Park.

The pH was stabilized at 5.5 and autoclaved at 120 °C and 2 atm for 20 min (Cerabolini & *al*., 2004). Seeds were transferred to Petri dishes (90 x 10 mm each) and sown under a laminar flow hood. Petri dishes were transferred to the growth chamber for 30-d incubation (Bacchetta & *al*., 2006). Germination tests were performed in a light and temperature controlled growth chamber (Angelantoni Ekoch 700, Italy) at 20° C constant temperature and photoperiod of 12 h in the light and 12 h in the dark (Bacchetta & *al*., 2006). The chamber was equipped with cool-white fluorescent tubes providing a photon flux density (PFD) of 22 μmol (photon) m^{-2} s^{-1}. Seeds showing radicle emergence were recorded as 'germinated' (Côme, 1970).

The following treatments were carried out for the considered seed types: control treatment (0 ppm GA3 treatment); 250 ppm GA3treatment; 500 ppm GA3 treatment; cold-wet stratification treatment. Each of the considered treatments consisted of two replicate of 25 seeds each (Cerabolini& *al*. 2004) for *Adonis, Androsace* and *Aquilegia*, and 50 seeds for *Campanula*. The number of germinated seeds was counted every day for 30 days to evaluate the dynamics of germination, according to Bacchetta & *al*. (2006). The low number of seeds per replicate and the low number of replicates in each experiment was due to the limited seeds availability because of the species were rare and there were few populations (Mattana & *al*., 2012).

Pre-germination seed treatment

Gibberellic acid (GA3)

Seeds were imbibed for 24 h in either 250 ppm and 500 ppm GA3 and distilled water (0 ppm, control) (Rodríguez Pérez,1993).

Cold-wet stratification treatment

The seeds were subjected to a cold–wet stratification treatment to simulate chilling conditions under snow-pack typical of high elevation moun-

tain areas (GIMÉNEZ-BENAVIDES & MILLA, 2013). The pots were filled with sand and soil and wetted with distilled water to ensure humid (BACCHETTA & *al*., 2006). The pots were wrapped in aluminium foil and stored in a refrigerator at 5 °C for 3 months before germination tests. The refrigerator (Angelantoni EKOFRIGOLAB 1500) was equipped with a display that show continuously the temperature inside, and a microprocessor control system with audible and visual alarm systems.

The cold-wet treatment was extended up to 9 months for *Adonis* and *Androsace* seeds.

DATA ANALYSIS

The dynamics of germination was determined by the Weibull function (WEIBULL, 1951; JOHNSON & KOTZ, 1970) from the following formula:

$$y = M \{ 1- e^{[k(t-z)]c}\}$$

where y was the germination percentage at time t (days); M the final germination at 30 d; z the germination delay; c the curve shape parameter (ranging from 0 to 3) obtained by optimising the sum of the squared differences.

The relative germination rate (k) was determined from the following equation:

$$k = 1 / (T_{50} - z)$$

where T_{50} was the half-germination time (i.e. number of days in reaching 50% of final germination) calculated from the formula of COOLBEAR & *al*. (1980) modified by THANOS & DOUSSI (1995):

$$T_{50} = [(N/2) - N_1) \times (T_2-T_1)] / N_2-N_1$$

where N was the final percentage of germinated seeds; N_1 the percentage of seeds germinated slightly lower than N/2; N_2 the percentage of seeds germinated slightly higher than N/2; T_1 the number of days that correspond to N_1; T_2 the number of days that correspond to N_2.

Pearson's correlation analysis was performed to evaluate the correlation among the considered seed traits (L, W, T, S, V, D, Ratio S/S_M, E.I.).

One way ANOVA was performed to analyze differences in seed traits among the considered species followed by a post-hoc Tukey's test to compare differences among means (Statistica, Stasoft, USA). Moreover, in order to test the interactive effect of the treatments and species on M, T_{50} and z, a 2×4 factorial design was performed by a generalized linear model (GLM) in R (R DEVELOPMENT CORE TEAM, 2011).

RESULTS

SIZE SEED

Seed traits of the considered species are shown in table 2. L ranged from 7.1 ± 0.4 mm in *Adonis* to 0.5 ± 0.1 mm in *Campanula*. W ranged from 3.92 ± 0.55 (*Adonis*) to 0.24 ± 0.02 mm (*Campanula*) and T from 3.31 ± 0.30 mm (*Adonis*) to 0.24 ± 0.02 mm (*Campanula*). S varied from 0.12 ± 0.02 mm^2 in *Campanula* to 27.62 ± 4.68 mm^2 in *Adonis* and V from 0.029 ± 0.008 mm^3 in *Campanula* and to 91.642 ± 16.85 mm^3 in *Adonis*. The mean value of D was 0.45 ± 0.16 mg mm^{-3} with *Androsace* having the highest value (0.62 ± 0.10 mg mm^{-3}) and *Adonis* the lowest one (0.30 ± 0.05 mg mm^{-3}).

Table 2
Seed traits of the studied species
Abbreviations are: L, length; W, weight; T, thickness; S, surface; V, volume; D, density; Ratio S/SM, ratio surface/ mass; E.I., Eccentricity Index; mean ± st.dev, n = 20. Within each column mean with the same letter are not significantly different (ANOVA, Tuckey test, $p > 0.05$).

Species	L (mm)	W (mm)	T (mm)	S (mm^2)	V (mm^3)	D (mg mm^{-3})	Ratio S/ S_M	E.I.	Seed coat T (μm)
Adonis distorta	7.1±0.4a	3.92±0.55a	3.31±0.30a	27.62±4.68a	91.642±16.851a	0.30±0.05a	1.0±0.2a	1.82±0.24a	31±10a
Androsacea mathildae	2.3±0.2b	1.52±0.14b	0.70±0.06b	3.53±0.54b	2.486±0.49b	0.62±0.10b	2.4±0.4b	1.52±0.11b	27±5a
Aquilegia magellensis	1.9±0.1b	0.88±0.07c	0.79±0.07b	1.64±0.16c	1.298±0.169c	0.56±0.07b	2.3±0.2b	2.12±0.23c	26±2a
Campanula fragilis subsp. *cavolinii*	0.5±0.1c	0.24±0.02d	0.24±0.01c	0.12±0.02d	0.029±0.008d	0.33±0.08a	13.2±2.5c	2.04±0.18ac	9±1b

Table 3
Results of the Pearson's correlation analysis among the considered seed variables. Abbreviations are: Ratio S/S_M, Ratio surface/ mass; E.I., Eccentricity Index. Bold type indicate significant correlation ($P < 0.05$).

	Weight	Thickness	Surface	Volume	Density	Ratio S/S_M	E.I.
Length	**0.995**	**0.992**	**0.984**	**0.968**	-0.644	-0.500	-0.286
Weight		**0.975**	**0.975**	**0.952**	-0.652	-0.472	-0.381
Thickness			**0.993**	**0.988**	-0.576	-0.582	-0.172
Surface				**0.996**	-0.500	-0.644	-0.218
Volume					-0.440	-0.698	-0.147
Density						-0.334	0.392
Ratio S/S_M							-0.203
E.I.							

The significantly highest ($p<0.05$) S/S_M ratio was found in *Campanula* (13.2 ± 2.5) and the lowest ratio in *Adonis* (1.0 ± 0.2). As regards to seed shape, E.I. ranged from 1.52 ± 0.11 in *Androsace* to 2.12 ± 0.23 in *Aquilegia*. The seed coat thickness was 31 ± 10 μm in, *Adonis*, 26 ± 2 μm in *Aquilegia*, 27 ± 5 μm in *Androsace* and 9 ± 1 μm. in *Campanula*.

A significant correlation was verified among L, W, T, S and V (Table 3).

Seed germination dynamic

The seed germination dynamic of *Aquilegia* and *Campanula* in the control treatment and after 250 ppm GA3, 500 ppm GA3 and cold-wet treatments are shown in figure 1. Values of M, T_{50} and z for *Aquilegia* and *Campanula* are show in figure 2.

Control treatment

In the control treatment M was 46 ± 8 % and 93 ± 1% in *Aquilegia* and *Campanula*, respectively, T_{50} was 12 ± 0 and 5.5 ± 0.7 days in *Aquilegia* and *Campanula*, respectively. The z was 9 ± 1 days in *Aquilegia* and the final germination took place 20 days after sowing, while in *Campanula* z was 3 ± 0 days and the final germination took place 20 days after sowing. In *Adonis* and *Androsace* M was 0%.

250 ppm GA3 treatments

Compared with the control M increased by 35% and 1% in *Aquilegia* and *Campanula* when the 250 ppm GA3 treatment was applied. The final germination took place 30 and 26 days after sowing, respectively. With respect to the control, T_{50} decreased by 4% in *Aquilegia* while in *Campanula* it did not vary significantly. In *Aquilegia*

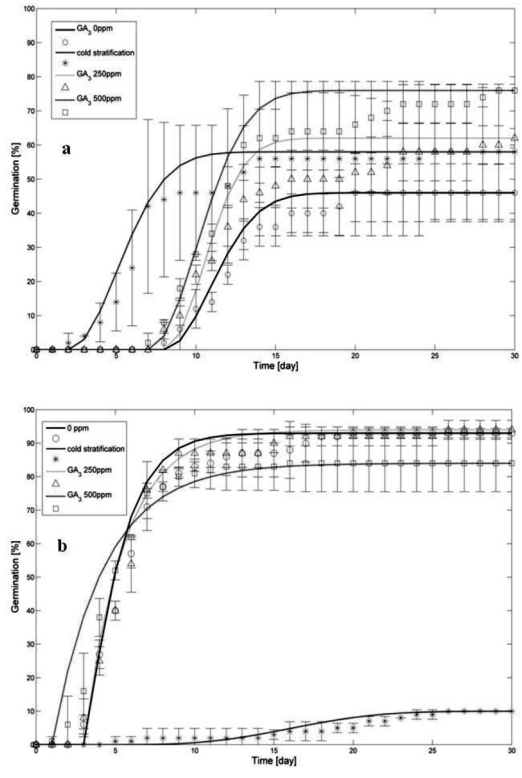

Figure 1. – Germination trend in seeds of *Aquilegia* (a) and *Campanula* (b) in the control treatment, 250 ppm GA3 treatment, 500 ppm GA3 treatment and cold-wet treatment. Continuous and dashed lines show fitted Weibull functions which was calculated using germination parameters.

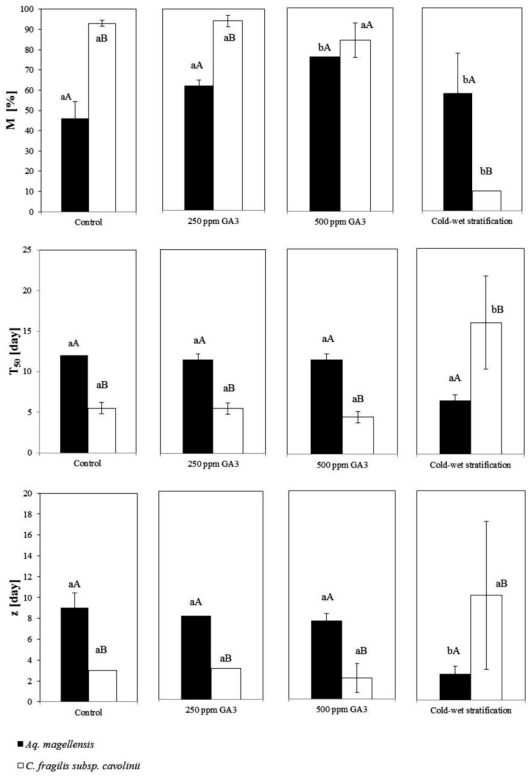

Figure 2. – Percentage of the final germination (M), half-germination time (T50) and germination delay (z) for Aquilegia and Campanula. Mean with the same letters are not significantly different (result of GLM model). For each parameter lowercase letters indicate the intra-specific different in each treatment and capital letters indicate the inter-specific different in each treatments.

and *Campanula*, z was 8 ± 0 and 3 ± 0 days, respectively. In *Adonis* and *Androsace* M was 0 % after the treatment.

500 ppm GA3 treatments

After 500 ppm GA3 treatment, M increased by 65 % in *Aquilegia* compared to the control, while it decreased by 10 % in *Campanula*. The final germination took place 28 and 16 days after sowing, respectively. T_{50} was 11 ± 0 and 4.5 ± 0.7 days in *Aquilegia* and *Campanula*, respectively. In *Aquilegia* and *Campanula*, z was 7.5 ± 0.7 and 2 ± 1 days, respectively. In *Adonis* and in *Androsace* M was 0 % after the treatment.

Cold- wet stratification

In the cold-wet stratification treatment M increased by 26 % in *Aquilegia* while it decreased by 89 % in *Campanula* compared to the control. The final germination happened 25 and 26 days after sowing, respectively. T_{50} decreased by 46 % in *Aquilegia* with respect to the control while it increased more than 100 % in *Campanula*. Results also showed that z was 2.5 ± 0.7 and 10 ± 7 days, respectively. In *Adonis* and *Androsace* M was 0 % after the treatment. A low M (< 10 %) was observed 9 months after the cold-wet treatment.

DISCUSSION

On the whole, the results of this study show significant differences in seed traits and germination capability in response to the considered treatments among the species.

The Pearson's correlation analysis underlines a significant correlation among L, W, T, S, V and D.

In particular, L, W, T, S and V are the largest in *Adonis* and the lowest in *Campanula*, while in *Androsace* and *Aquilegia* have intermediate values. Kikuzawa & Koyama (1999) underline that small seeds have a faster water absorption capacity than large seeds, since they have a larger surface area to mass ratio. Our results underline that S/S_M ratio is the highest in *Campanula* (13.25 ± 2.5) and the lowest in *Adonis* (1.03 ± 0.17).

As concerns germination it does not occur in *Adonis* and *Androsace* in response to treatments (control treatment, GA3 treatment, cold-wet treatment). Nevertheless, increasing the time of cold-wet stratification from three to nine months, the germination capability is below 10%. This result suggests that seeds from higher altitudes, such as *Androsace*, have a stronger dormancy than those from lower altitudes (i.e. *Aquilegia* and *Campanula*). We hypothesize that *Androsace* seeds have a deep physiological dormancy (*sensu* Nikolaeva, 1969) because dormancy is not completely broken by the stratification treatment. The same results are obtained for *Adonis*, in agreement with those of Godefroid & *al.* (2010) for other species of the same genus, who underline the lack of kno-

wledge with regard to germination and dormancy for rare and threatened species. A morphological dormancy cannot be excluded for *Adonis* and *Androsace*; in particular, *Adonis* belonging to *Ranunculaceae* family is characterized by a rudimentary underdeveloped embryo of mature seeds (MARTIN, 1946; FINCH-SAVAGE & LEUBNER-METZGER, 2006), while *Androsace* is characterized by a linear underdeveloped embryo of mature seeds (MARTIN, 1946; FINCH-SAVAGE & LEUBNER-METZGER, 2006). The presence of an underdeveloped embryo of mature seeds suggests for these species both a morphological and physiological dormancy according to BASKIN & BASKIN (2004), FINCH-SAVAGE & LEUBNER-METZGER (2006). Nevertheless, in the present study we have not investigated this type of dormancy because a large amount of seeds would have been necessary, and considering that CRAWFORD & *al*. (2007) suggest that only one germination treatment should be done when there are small seed amounts available for endemic species. Thus, in this case it is important to define an efficient protocol to enhance the germination success (GODEFROID & *al*., 2010).

Our results underline that treatment, species and their interaction significantly affect M, T_{50} and z as shown by GLM analysis. In particular, the interaction effect between treatment and species on M differs significantly for cold ($t = -0.072$, $p < 0.001$) and 500 ppm GA3($t = -3.314$, $p < 0.01$). With regards to T_{50} and z, the interaction effect between treatment and species differ significantly only for the cold-wet treatment ($t = 7.260$, $p < 0.01$ and $t = 4.095$, $p < 0.05$, respectively).

DEBEAUJON & KOORNNEEF (2000) show the role of gibberellins in promoting seed germination. Exogenous application of GA3 overcomes seed dormancy in several species (BASKIN & BASKIN, 1998) promoting germination in some species that normally require cold stratification, light, or afterripening (BEWLWY & *al*., 1994). GA promotes the production of enzymes such as endo-b-mannanase, which loosen cell walls in the endosperm, thereby reducing resistance to radicle emergence (BEWLWY, 1997; GROOT & KARSSEN, 1987; YAMAGUCHI & KAMIYA, 2002). Nevertheless, our results underline a different behaviour for *Aquilegia* and *Campanula* in response to GA3 treatments. In particular, *Aquilegia* is more responsive to GA3 treatments than *Campanula* which in turn shows a significant response only to the cold-wet treatment. In fact, the 500 ppm GA3 treatment increases the final germination by 65 % in *Aquilegia* compared to the control and it can be justified by an endogenous non-deep physiological dormancy, according to the results of NIKOLAEVA (1969). On the contrary, the 250 and 500 ppm GA3 treatments do not significantly affect the final germination in *Campanula* compared to the control.

MEYER & MOSEN (1991) suggest that populations normally encountering long periods with snow cover and adverse winter conditions require longer periods of cold stratification for germination than those exposed to milder winters. The cold-wet stratification improves germination in many high mountain species of eastern Europe and North America (BASKIN & BASKIN, 1998) and it is indicative of a physiological dormancy (BASKIN & BASKIN, 2005). Our results show a different response to the cold-wet treatment for the considered species. In particular, the 89% decrease in the final germination and the more 100% increase in the T_{50} more in *Campanula* compared to the control can be also related to the low seed size of as suggested by SCHLORHAUFER (2006) who underlines that small seeds are more subjected to viability loss. This result can be interpreted as an adaptive consequence of the short period of exposure to snow, considering that this species grows at 750 m a.s.l. and snow covers the soil only for 15 days for one month during the year. On the contrary, *Adonis* and *Androsace* grow at a higher altitude (2675 m a.s.l. and 2760 m a.s.l., respectively) where snow persists for 7 to 9 months during the year. Moreover, the 93 % germination percentage in the controls suggests a low level of physiological dormancy in *Campanula*, according to the results of GODEFROID & *al*. (2010) for the species of the same genus. This result shows that cold-wet treatment does not necessarily promote germination.

The observed differences in seed dormancy of the considered species could also be related to the seed coat thickness, as suggested by UUBANSKA & *al*. (1979), ZUUR-ISLER (1982) and SCHÜTZ (2000). Seed coat thickness is larger in *Adonis, Aquilegia* and *Androsace* (28 ± 3, mean value) and the lo-

west in *Campanula* (9 ± 1). Large-seeded species invest proportionately greater resources into physical defences, such as a thick endocarp or seed coat, in response to high predation risks (FENNER, 1983; BLATE & *al*., 1998; MOLES & *al*., 2003). Nevertheless, the presence of a thick seed coat may delay germination by limiting oxygen exchange or by acting as a physical constraint to embryo growth (NORDEN & *al*., 2009).

Seed shape is an important determinant of seed dispersal, probable loss and moisture imbibitions (CERDÀ & GARCÍA-FAYOS, 2002; BALKAVA & ODABAS, 2002). The results show that seed shape is elliptic for *Androsace*, egg-shaped for *Adonis* and long-shaped for *Aquilegia* and *Campanula*. Differences in seed shape determine variations of the surface area that provides contact with the external environment (GRUNDY & *al*., 2003) and influence the response to burial depth in a different way. There is a significant correlation between the optimum emergence depth and seed shape (THOMPSON & *al*., 1993) where small and rounded seeds tend to persist in soil, while large and elongate or flattened seeds are transient in the soil (THOMPSON & *al*., 1994; BEKKER & *al*., 1998). TTHOMPSON & *al*. (1993) suggest that ease of burial and rates of predation could be the mechanism underlying the relationship between seed size and shape and persistence in the soil.

On the whole our results give information on the relationship between seed traits and germination capability of the considered endemic species underlining the importance of the selected treatments to favour germination capability. Thus, based on the obtained results, germination protocols for the considered species can be suggested. In particular, the germination protocol for *Campanula* forecasts seed imbibition for 24 hours in distilled water, then moving seeds to Petri dishes with agar medium (7 g l^{-1}) integrated with 4.4 g l^{-1} of Murashige Skoog salts and pH stabilized at 5.5. Petri dishes must be put into the growth-chamber at 20°C with 12/12 hour light and dark period.

With regards to *Aquilegia*, the protocol is the same as that for *Campanula* except carrying out the imbibition for 24 hours in 500 ppm GA rather than in distilled water.

We recommend a cold-wet treatment at least for 9 months to improve the germination capability for *Adonis* and *Androsacea*. Nevertheless, for these two species further research regarding their germination behaviour should be carried out. The recommended protocols may be used in reinforcement projects of the wild populations as a means of reducing the extinction risk of these endemic species.

REFERENCES

Bacchetta, G., Fenu, G., Mattana, E., Piotto, B., Virevaire, M. — 2006— Manuale per la raccolta, studio, conservazione e gestione ex situ del germoplasma — APAT. Ag. Prot. Amb. Serv. Tec.

Balkaya, A. & Odabas, M.S. —2002—Determination of seed characteristics in some significant snap bean varieties grown in Samsun, Turkey — Pak. J. Biol. Sci. 5(4): 382-387.

Baskin, C.C. & Baskin, J.M. (Eds.) —1998—Seeds: ecology, biogeography, and evolution of dormancy and germination — Academic Press, San Diego.

Baskin, J.M. & Baskin, C.C. —2004—A classification system for seed dormancy — Seed Sci. Res. 14: 1-16.

Baskin, C.C. & Baskin, J.M. —2005— Seed dormancy in wild flowers — In: McDonald, M.B. & Kwong, F.Y. (Eds.). Flower seeds: Biology and technology. Pp. 163-185. CABI Publ., Cambridge, MA.

Bekker, R.M., Bakker, J.P., Grandin, U., Kalamees, R., Milberg, P. —1998— Seed size, shape and vertical distribution in the soil: indicators of seed longevity — Funct. Ecol. 12: 834-842.

Bevill, R.L. & Louda, S.M. —1999— Comparisons of related and common species in the study of plant rarity — Cons. Biol. 13: 493-498.

Bewley, J. D. —1997— Seed germination and dormancy — Plant Cell 9: 1055-1066.

Bewley, J. D. & Black, M. (Eds.) —1994— Seeds: Physiology of development and germination — Plenum Press, New York.

Bewley J.D., Bradford, K., Hilhorst, H., Nonogaki, H. (Eds.) —2013— Seeds: Physiology of Development, Germination and Dormancy, 3rd ed. — Springer, New York

Billings, W.D. & Mooney, H.A. —1968— The ecology of arctic and alpine plants — Biol. Rev. 43: 481-529.

Blate, G.M. Peart, D. R. & Leighton, M. —1998— Post-dispersal predation on isolated seeds: a comparative study of 40 tree species in a Southeast Asian rainforest — Oikos, 82: 522-538.

Bonito, A., Varoni, L. & Gratani, L. —2011— Relationship between acorn size and seedling morphological and physiological traits of Quercus ilex L. from different climates — Photosynthetica 49(1): 75-86.

Cerabolini, B., De Andreis, R., Ceriani, R.M., Pierce, S. & Raimondi, B. —2004— Seed germination and conservation of endangered species from the Italian Alps: Physoplexis comosa and Primula glaucescens — Biol. Conserv. 117: 351-356.

Cerdà, A. & García-Fayos, P. —2002 — The influence of seed size and shape on their removal by water erosion — Catena 48: 293-301.

Côme, D. (Eds.)— 1970— Les Obstacles a la Germination — Ed. Masson, Paris.

Conti, F., Manzi, A. & Pedrotti, F. —1997— Liste rosse regionali delle piante d'Italia — Soc. Bot. Ital., Ass. Ital. WWF, Camerino. 139 pp.

Conti, F., Abbate, G., Alessandrini, A., Blasi, C., Bonacquisti, S. & Scassellati, E. —2005— An annotated checklist of the Italian vascular flora — Palombi Ed., Roma.

Coolbear, P., Grierson, D. & Heydecker, W. —1980— Osmotic pre-sowing treatments and nucleic acid accumulation in tomato seeds (Lycopersicon lycopersicum) — Seed Sci. Technol. 8: 289-303.

Crawford, A.D. Steadman, K.J., Plummer, J.A., Cochrane, A., Probert, R.J. —2007— Analysis of seed-bank data confirms suitability of international seed-storage standards for the Australian flora — Aust. J. Bot. 55:18-29.

Debeaujon, I & Koornneef, M. —2000— Gibberellin requirement for Arabidopsis seed germination is determined both by testa characteristics and embryonic Abscisic Acid — Plant Physiol. 122: 415-424.

Escribá Baeza, M.C., Arregui, J.M. & Laguna, E. —2007— Germinación de Cistus heterophyllus Desf. subsp. carthaginensis (Pau) M.B. Crespo & Mateo, taxon gravemente amenazado en la Comunidad Valenciana — Lazaroa 28: 101-107.

European Community —1992— Conservation of natural habitats and of wild fauna and flora — Council Directive 92/43/EEC.

Fenner, M. —1983— Relationships between seed weight, ash content and seedling growth in twenty-four species of Compositae — New Phytol. 95: 697-706.

Finkelstein, R., Reeves, W., Ariizumi, T., Steber, C. —2008— Molecular aspects of seed dormancy — Ann. Rev. Plant Biol. 59: 387- 415.

Finch-Savage, W.E. & Leubner-Metzger, G. —2006— Seed dormancy and the control of germination — New Phytol. 171: 501-523.

Fuente, V., Rufo Nieto, L. & Sánchez-Mata, D. —2011— Sarcocornia hispanica (Chenopodiaceae), a new species from the Iberian Peninsula — Lazaroa 32: 9-13.

Gavilán, R.G., Sánchez-Mata, D., Escudero, A., & Rubio, A. —2002— Spatial structure and interspecific interactions in Mediterranean high mountain vegetation (Sistema Central, Spain) — Isr. J. Plant Sci. 50: 217-228.

Giménez-Benavides, L. & Milla, R. — 2013— Comparative germination ecology of two altitudinal vicariant Saxifraga species endemic to the north of Spain — Plant Biol. 15: 593-600.

Godefroid, S. & al. —2010— Germination capacity and viability of threatened species collections in seed banks — Biodiv. Cons. 19: 1365-1383.

Gómez-Campo, C. —1985— The Iberian Peninsula. Plant conservation in the Mediterranean area — Junk Publishers, Dordrecht.

Gómez-Campo, C. (Eds.) —1987— Libro rojo de las especies vegetales amenazadas de España peninsular e Islas Baleares — ICONA, Madrid

Groot, S.P.C. & Karssen, C.M. —1987— Gibberellins regulate seed germination in tomato by endosperm weakening: a study with gibberellins-deficient mutants — Planta 171: 525-531.

Grundy, A.C. & al. —2003— Modelling the emergence response of weed seeds to burial depth: interactions with seed density, weight and shape — J. Appl. Ecol. 40: 757-770.

Harper, J.L., Lovell, P.H. & Moore, K.G. —1970— The shapes and sizes of seeds— Ann. Rev. Ecol. Syst. 1: 327-356.

Hay, F.R. & Smith, R.D. (Eds.) —2003— Seed maturity: when to collect seeds from wild plants. Seed conservation: turning science into practice — Kew Royal Botanic Gardens, London.

IUCN —2002— IUCN red list of threatened species— IUCN, Gland.

Jakobsson, A. & Eriksson, O. —2000— A comparative study of seed number, seed size, seedling size and recruitment in grassland plants — Oikos 88: 494-502.

Johnson, N.L. & Kotz, S. (Eds.) —1970— Distributions in Statistics: Continuous Univariate Distributions Vol. 2. — Houghton Mifflin, New York.

Kelly, K.M., Van Staden, J. & Bell, W.E. —1992— Seed coat structure and dormancy— Plant Growth Regul. 11: 201-209.

Kikuzawa, K. & Koyama, H. —1999— Scaling of soil water absorption by seeds: an experiment using seed analogues — Seed Sci. Res. 9:171-178.

Leishman, M.R., Wright, I.J., Moles, A.T. & Westoby, M. —2000— The evolutionary ecology of seed size — In: Fenner M. (Ed.). Seeds, the Ecology of Regeneration in Plant Communities. Pp. 31-58. CABI Publishing, New York.

Lönnberg, K. & Eriksson, O. —2013— Relationships between intra-specific variation in seed size and recruitment in four species in two contrasting habitats — Plant Biol. 15: 601- 606.

Marshall, D.R. & Brown, A.H.D. —1983— Theory of forage plant collection — In : McIvor J.G. & Bray, R.A. (Ed.). Genetic resources of forage plants. Pp. 135-148. CSIRO Publishing, Melbourne.

Martin, A.C. —1946— The comparative internal morphology of seeds — Am. Midl. Nat. 36: 513-660.

Martín-Bravo, S., Valcárcel, V., Vargas, P. & Luceño, M. —2010— Geographical speciation related to Pleistocene range shifts in the western Mediterranean mountains — Taxon 59: 466-482.

Mattana, E., Pritchard, H.W., Porceddu, M., Stuppy, W.H., Bacchetta G. —2012— Interchangeable effects of gibberellic acid and temperature on embryo growth, seed germination and epicotyl emergence in Ribes multiflorum ssp. sandalioticum (Grossulariaceae) — Plant Biol. 14: 77–87.

Meyer, S.E. & Monsen, S.B. —1991— Habitat-correlated variation in mountain big sagebrush (Artemisia tridentata ssp. vaseyana) seed germination patterns — Ecology 72: 739-742.

Mohamed-Yasseen, Y. & al. —1994— The role of seed coats in seed viability — Bot. Rev. 60: 426-439.

Moles, A.T., Warton D.I. & Westoby, M. —2003— Do small-seeded species have higher survival through seed predation than large-seeded species? — Ecology 84: 3148-3161.

Morgan, E.R., Butler, R.M., Bicknell, R.A. —1997— In vitro propagation of Gentiana cerina and Gentiana corymbifera — New Zealand J. Crop Hort. Sci. 25: 1-8.

Murashige, T. & Skoog, F. —1962— A revised medium for rapid growth and bioassays with tobacco tissue cultures — Physiol. Plantarum 15: 473-497.

Nikolaeva, M.G. (Ed.) —1969— Physiology of deed dormancy in seed — lzdatel'stvo 'Nauka' Leningrad.

Norden, N., Daws M.I., Antoine, C., Gonzalez, M.A., Garwood, N.C. & Chave, J. —2009— The relationship between seed mass and mean time to germination for 1037 tree species across five tropical forests — Funct. Ecol. 23: 203-210.

Pence,V. —1999— The application of biotechnology for the conservation of endangered plants — In: Benson, E.E. (Ed.). Plant Conservation Biotechnology, Chapter 15. Pp. 227– 241. Taylor and Francis, London.

Pignatti, S. (Ed.) —1982— Flora D'Italia— Edagricole, Bologna.

Pizo, M.A., Von Allmen C.L. & Morellato, P.C. —2006— Seed size variation in the palm Euterpe edulis and the effects of seed predators on germination and seedling survival — Acta Oecol. 29: 311-315.

Rajjou, L., Duval, M., Gallardo, K., Catusse, J., Bally, J., Job, C. & Job, D. —2012— Seed Germination and Vigor — Annu. Rev. Plant Biol. 63: 507-33.

Rodriguez Perez, J.A. —1993—Effects of treatment with gibberellic acid on germination of Protea cynaroides, P. eximia, P. nerifolia and P. repens (Proteaceae) — III International Protea Research Symposium 387: 85-90.

Ruiz-Labourdette, D., Nogués-Bravo, D., Sáinz Ollero, H., Schmitz, M.F., Pineda, F.D.—2012— Forest composition in Mediterranean mountains is projected to shift along the entire elevational gradient under climate change— J. Biogeogr. 39: 162-176.

Sáinz, H. & Moreno, J.C. —2002 Flora vascular endémica Española — In: Pineda, F.D. & al. (Eds.). La diversidad biológica de España. Pp. 175-195. Pearson, Madrid.

Shimono, Y. & Kudo G. —2005— Comparisons of germination traits of alpine plants between fellfield and snowbed habitats — Ecol. Res. 20: 189-197.

Schlorhaufer, D. —2006— Alpine Garden Patscherkofel Innsbruck. Alpine Plants in their Environment — University of Innsbruck, Innsbruck.

Schütz, W. & Milberg, P. —1997— Seed dormancy in Carex canescens: regional differences and ecological consequences — Oikos 78: 420-428.

Schütz, W. —2000— Ecology of seed dormancy and germination in sedges (Carex) — Pers. Plant Ecol. Evol. Syst. 3/1: 67-89

Thanos, C.A. & Doussi M.A. —1995— Ecophysiology of seed germination in endemic Labiates of Crete — Isr. J. Plant Sci. 43: 227-237.

Thompson, K., Band S.R. & Hodgson, J.G. —1993— Seed size and shape predict persistence in the soil — Funct. Ecol. 7: 236-241.

Thompson, K., Green, A. & Jewelsh, A.M. —1994— Seeds in soil and worm casts from a neutral grassland — Funct. Ecol. 8: 29-35.

Thuiller, W., Lavorel, S., Araújo, M.B., Sykes, M.T. & Prentice, I.C. —2005— Climate change threats to plant diversity in Europe — Proc. Natl. Acad. Sci. U.S.A. 102: 8245-8250.

Tunjai, P. & Elliott, S. —2012— Effects of seed traits on the success of direct seeding for restoring southern Thailand's lowland evergreen forest ecosystem — New Forests 43: 319-333.

Urbanska, K.M., Schwank, O. & Fossati, A. —1979— Variation within Lotus corniculatus L. s. l. from Switzerland, II. Reproductive behaviour of L. alpinus (DC) Schleicher — Berlin Geobot. Inst. ETH 46: 62-85.

Väre, H., Lampinen, R., Humphries, C., Williams, P. —2003— Taxonomic diversity of vascular plants in the European alpine areas — In: Nagy, L. & al. (Eds.). Alpine biodiversity in Europe. Ecological studies. Vol 167. Pp. 133-148. Springer, New York.

Weibull, W. —1951— A statistical distribution function of wide applicability— J. Appl. Mech. 18: 293-297.

Weber, H., Borisjuk, L. & Wobus, U. —1996— Controlling seed development development and seed size in Vicia faba: a role for seed coat-associated invertases and carbohydrate state — Plant J. 10: 823-834.

Werker, E. —1981— Seed dormancy as explained by the anatomy of embryo envelopes — Israel J. Bot. 29: 22-44.

Westoby, M. —1981— How diversified seed germination behavior is selected— Amer. Naturalist 118: 882-885.

Yamaguchi, S. & Kamiya, Y. —2002— Gibberellins and light-stimulated seed germination — J. Plant Growth Regul. 20: 369-376.

Youssef, S. & al. —2012— Breaking seed dormancy in Cupressus atlantica Gaussen, an endemic and threatened coniferous tree in Morocco — J. For. Res. 23(3): 385-390.

Zuur-Isler, D. —1982— Germinating behaviour and early life phase of some species from alpine serpentine soils — Berlin Geobot. Inst. ETH 49:76-107.

Received: 21 May 2013
Accepted: 30 July 2013