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Research Article

New findings on seed ecology of *Ribes sardoum*: can it provide a new opportunity to prevent the extinction of a threatened plant species?

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Ribes sardoum, the most threatened endemic plant of Sardinia, is included in the Habitats Directive (92/43/EEC) and it was considered Critically Endangered in the global IUCN Red Lists. This species has been reported to have an extremely low fertility, scarce fruit production, low seed viability and a general inability to reproduce sexually. Fruits were collected for the first time from the remnant population, and the requirements for seed germination were investigated in the laboratory. Seeds were incubated at different temperatures (10, 15, and 20°C) and, in addition, they were exposed to a warm stratification (W) or a move-along treatment characterized by three cold temperature regimes (CCC). Seeds were also sown on the surface of 1% agar water with 250 mg·L⁻¹ of GA₃. At maturity, seeds have a linear underdeveloped embryo. Germination percentage between 35% and 65% were detected in the control and W groups. A low germination percentage occurred after CCC and during GA₃ treatment. W treatment speeds up seed germination. Our results demonstrate that fruits of *R. sardoum* produce viable seeds, that are able to germinate under controlled conditions, with the assumption that the seeds have morphophysiological dormancy (MPD), and that propagation from the seeds is possible. Although the ability of seed germination was demonstrated, the lack of seedlings in the natural population seems to be a consequence of unfavourable climatic conditions for recruitment. However, our results indicate that seedlings obtained under controlled conditions could be useful for future translocation reducing and/or mitigating the extinction likelihood of this highly threatened plant.

Key words: Grossulariaceae, morphophysiological dormancy, relict species, reproductive limits, unfavourable climatic conditions, warm stratification

Introduction

Relict plant species represent an important component of the Mediterranean flora and are supposed to have shown more competitive ability in the past, but have faced changing, repeated glaciation episodes and adverse environmental conditions affecting critical stages of their life cycles, except in refugia areas where environmental conditions make persistence of these plant species possible (Mejías, Arroyo, & Ojeda, 2002). Some of these species only occur in Mediterranean mountains while others represent the southernmost limit of their distribution in Europe (Picó & Riba, 2002). These species are long-lived plants whose distributions are restricted to mountainous

areas forming patches of isolated populations, and their recruitment is seriously limited due to climatic stress (García, Zamora, Hódar, Gómez, & Castro, 2000; Mattana, Pritchard, Porceddu, Stuppy, & Bacchetta, 2012; Picó & Riba, 2002).

Ribes L. (Grossulariaceae) is a genus of c. 200 taxa distributed in temperate regions of the northern hemisphere and in the Andes (Mabberley, 2008). In the Mediterranean region, Sardinia is the only island where this genus is represented exclusively by endemic taxa, while on the nearest insular systems (i.e., Tuscan Archipelago) the genus is absent or represented only by widely distributed plants (Fenu, Mattana, & Bacchetta, 2012 and references therein). Indeed, *Ribes sardoum* Martelli and *Ribes multiflorum* Kit. ex Roem. & Schult. subsp. *sandalioticum* Arri-goni are both endemic to Sardinia and currently restricted

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to refugia areas in the Supramontes and Gennargentu regions (Bacchetta et al., 2013a; Fenu, Mattana, Congiu, & Bacchetta, 2010). In general, the germplasm collection of narrow endemic plants and relict species, in order to obtain information about its seed germination ecology, is often difficult. Besides this, each plant species has particular ecological requirements for seed germination (i.e., temperatures, water, light) and can show different kinds of seed dormancy (Baskin & Baskin, 2014). As dormant seeds gain the ability to germinate at a particular time during the year, many physiological and/or morphological changes may occur in them and information is needed to understand why there is a delay in germination and how dormancy is broken (Chien et al., 2011). Growth of the underdeveloped embryo takes place after seeds have dispersed from the mother plant, and in seeds with morphological dormancy (MD) growth and germination occur in about 4 weeks or less. If germination is delayed for more longer and seeds require a dormancy-breaking treatment such as exposure to moist cold and/or to moist warm stratification to germinate, they present a morphophysiological dormancy (MPD; Baskin & Baskin, 2014). Morphophysiological dormancy has been inferred to occur in seeds of *Ribes* species (see Baskin & Baskin, 2014 and references therein). Recently, the seed ecology of *R. multiflorum* subsp. *sandalioticum* has been investigated in depth (Mattana et al., 2012); these authors reported that these seeds have MPD and germination of this taxon is positively affected both by warm stratification (25°C for 3 months) followed by cold incubation temperatures (<15°C) and by treatment with gibberellins (GA₃). These findings highlighted a high degree of specialization in seed germination and seedling establishment, which were synchronized with the Mediterranean seasonality (Mattana et al., 2012). Moreover, the requirement of this species for low temperatures for seed germination highlighted an increasing threat from global warming, which could reduce the level of natural seedling emergence in the field (Mattana et al., 2012). Conversely, to date the reproductive ecology of the threatened Sardinian congener species is poorly known, and available data are old and partially contradictory with our field observations. *Ribes sardoum* is considered a palaeoendemic species, distributed in the past throughout the boreal zone, and actually restricted to an extremely small area due to several reductions in its distribution (Valsecchi, 1977, 1981). This observation is also supported by the results of a genetic study that suggest an origin from ecologically based divergent, habitat-driven selection for *R. sardoum* (Gentili et al., 2015). In addition, the phylogenetic differentiation of *R. sardoum* from the other European *Ribes* taxa indicates a probably more ancient (palaeoendemic species) and independent origin (Gentili et al., 2015). As a consequence, *R. sardoum* is considered a relict plant species, restricted to

only one population (Fenu et al., 2012; Gentili et al., 2015). The small population size combined with the effects of human-related activities (i.e., overgrazing and tourism activities; Fenu et al., 2012) and several serious reproductive and genetic limitations make this taxon prone to extinction. For all these reasons, *R. sardoum* is included in several international regulations (i.e., the Bern Convention and the Habitats Directive 92/43/EEC), and it was considered as critically endangered on the Global and Regional IUCN Red Lists (Bacchetta, Congiu, Fenu, & Mattana, 2013b; Rossi et al., 2016). Additionally, this species has been ranked as the most threatened exclusive endemic plant of Sardinia, for which it was urgent to start conservation measures (Bacchetta, Fenu, & Mattana, 2012). *Ribes sardoum* has been reported to have a very low fertility due to low pollen production and the early dropping of ovaries and consequent scarce fruit production (Valsecchi, 1977, 1981), combined with a low seed viability (Montmollin de & Strahm, 2005). Accordingly, no seedlings were observed during the last 10 years of population monitoring (Fenu, Cogoni, Pinna, & Bacchetta, 2015), which suggests that *R. sardoum* exclusively shows a vegetative propagation, as detected in several *Ribes* species (Pfister & Sloan, 2008). This finding is consistent with the results of a genetic study demonstrating that the remnant population of this species shows a low genetic diversity and a high level of inbreeding depression, mainly related to the lack of sexual reproduction and a prevalence of vegetative propagation (Gentili et al., 2015). Given these reproductive problems, this plant species was considered unable to reproduce via sexual reproduction and in particular to produce sufficient fruits or viable seeds each year. In 2014, several fruits were observed in the natural population, allowing us to collect and conserve a sufficient number of seeds at the Germplasm Bank of Sardinia (BG-SAR – University of Cagliari). This seed collection also suggested that some mechanisms of the reproductive biology of *R. sardoum* are doubtful and in large part unknown (i.e., seed production and dispersal, seed germination and vegetative reproduction). In general, the following remarks were assumed: (1) *R. sardoum* is able to produce fruit, contrary to previous belief, and (2) fruits ripen in autumn and may contain viable seeds. As a consequence, this plant species could potentially reproduce sexually, and the possible bottleneck may be due to the limited seedling recruitment related to the peculiar ecological requirements (and for these reasons no seedlings were observed in the natural population; Fenu et al., 2015). In order to address these issues, this work focused on the *ex situ* requirements for seed germination in *R. sardoum*, evaluating the effect of different treatments and gibberellic acid (GA₃) on the promotion of germination, giving a possible solution to reducing the extinction risk of this relict and threatened taxon.

Materials and methods

Plant description and seed lot details

Ribes sardoum is a small woody shrub (0.8–2.0 m high). The flowering season is from April to the beginning of June, and fruiting from late July to September (Bacchetta *et al.*, 2013b; Fenu *et al.*, 2012). From an ecological point of view, *R. sardoum* is a mesophilous species growing on dolomitic limestone at *c.* 1160 m asl. The only population is found in Prados (*locus classicus*, Monte Corradi, Oliena) and consists of *c.* 80 reproductive plants in a surface area of *c.* 700 m² (Fenu *et al.*, 2012). Fruits of *R. sardoum* 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 fruit (and consequently seed) ripening was complete, in October 2014. A total of 546 ripe fruits were collected directly from plants. Fruit harvesting was done following the international accepted ethical criteria and methods for effective *ex situ* conservation of biodiversity, by ensuring that the natural population was not negatively affected by an excessive collection (max 20% of the fruits present in the population; Bacchetta, Fenu, Mattana, Piotto, & Virevaire, 2006). The seeds were immediately separated from the pulp by rubbing the fruits through sieves under running water. The cleaned seeds were then spread out and left to dry at room temperature ($\sim 20^{\circ}\text{C}$ and 40% relative humidity) for one week, until the experiments started. The number of seeds per fruit and percentages of developed and abortive seeds (i.e., small, shrivelled and undeveloped seeds) were determined from 50 randomly selected fruits. Average seed mass was calculated (at 20°C and 40% relative humidity) by weighing 10 replicates of 20 seeds each. In the population site, over the population monitoring (Fenu *et al.*, 2015), a temperature logger (TidbiT v2 Temp Logger, Onset Computer Corporation, Cape Cod, MA, USA) was buried in order to record the soil temperature at 90 min intervals throughout the year. Pluviometric data (monthly rainfall averages from 1922 to 2011) from the nearby climatic station (Oliena, Nuoro) were obtained from the Regione Autonoma della Sardegna website (<http://www.regione.sardegna.it/j/v/25?s=131338&v=2>).

Embryo measurement

Embryo and seed lengths at the time of dispersal were evaluated by measuring 10 longitudinally sectioned seeds. The seeds were sown on the surface of 1% agar water in 90 mm diameter plastic Petri dishes and allowed to imbibe at room temperature ($\sim 20^{\circ}\text{C}$) for 24 hours. Seeds were cut in half under a dissecting microscope and images of embryos acquired using a Zeiss SteREO Discovery.V8, with an objective Achromat S 0.63x, FWD 107mm (Carl Zeiss MicroImaging GmbH) at $8.0\times$ magnification,

coupled to a Canon (Power shot G11) digital camera. Embryo and seed lengths were measured using the image analysis software ImageJ 1.41o (National Institutes of Health, Bethesda, MA, USA). Seed length was measured excluding the thickness of the seed coat (Mattana *et al.*, 2012).

Germination tests under controlled conditions

Three replicates of 15 seeds were sown on the surface of 1% agar water in 90 mm diameter plastic Petri dishes and incubated in the light (12 h light/12 h darkness) at constant 10, 15 and 20°C (Control). At the same time, the following pre-treatments were tested: (i) a warm stratification (W) at 25°C (12 h light/12 h dark) for 3 months; (ii) a sequence ('move-along') of three different cold temperature regimes (CCC): at 10°C (12 h light/12 h dark) for one month, then moved to 5°C (12 h light/12 h dark) for 2 months, and then moved to 1°C (0/24 h light to simulate the snow cover period) for 2 months, after which the seeds were incubated at constant temperatures as detailed above. These conditions were chosen due to the limited availability of seeds, to try to reproduce the best germination protocol described for the congeneric *R. multiflorum* subsp. *sandaliticum* (Mattana *et al.*, 2012), and to test the simulate field temperatures extrapolated from data loggers buried in the soil in the natural population site. All germination tests started one week after seed collection. The experiments were conducted using different environmental test chambers of the same model (Sanyo MLR-351, SANYO Electric Co., Ltd) each equipped with white fluorescent lamps (FL40SS.W/37 70–10 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Germinated seeds were scored three times a week. Germination was defined as visible radicle emergence ($> 1 \text{ mm}$). At the end of the germination tests (for a maximum of 120 days), when no additional germination had occurred for 2 weeks, a cut test was carried out to determine the firmness of the remaining seeds and the number of empty seeds; firm seeds were considered to be viable.

Effect of gibberellic acid (GA_3) on seed germination

To determine the effects of GA_3 on seed germination, three extra replicates of 15 seeds each were sown on the surface of 1% agar water with $250 \text{ mg}\cdot\text{L}^{-1}$ GA_3 and incubated in the light (12 h light/12 h dark) at constant 10, 15 and 20°C .

Epicotyl emergence

During the germination tests, regardless of treatment, the time from germination (i.e., radicle emergence) to

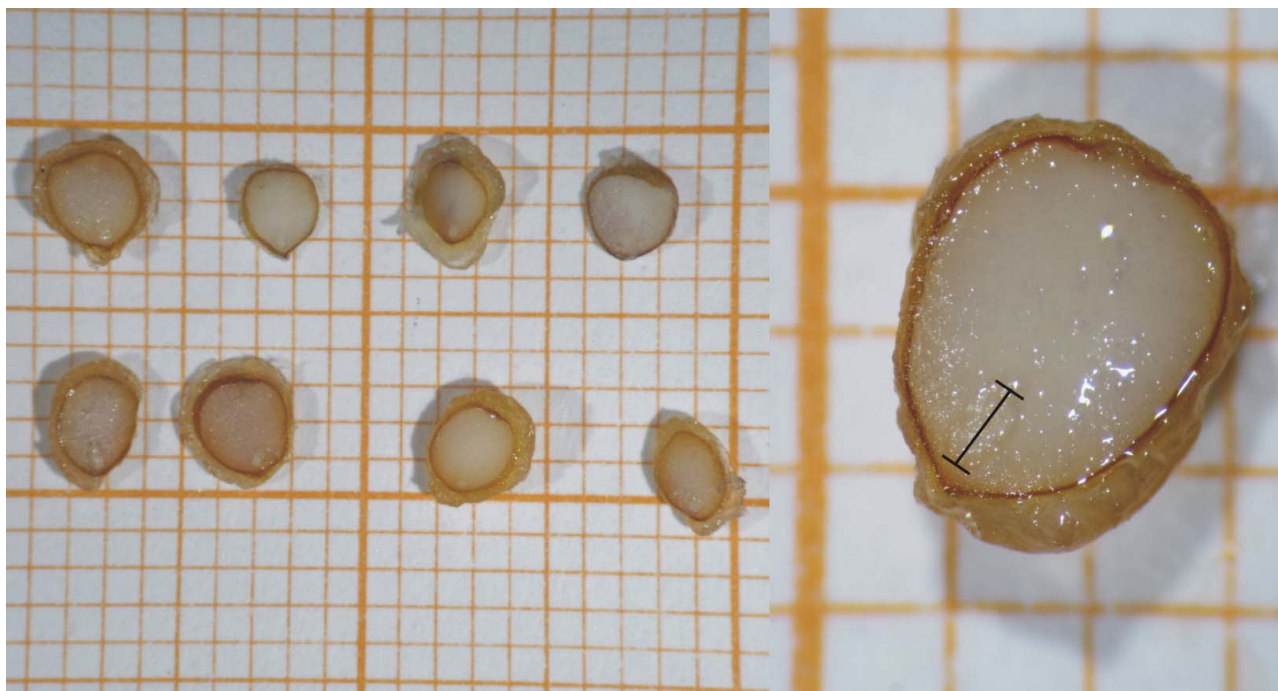


Fig. 1. Images of the longitudinally sectioned seeds of *Ribes sardoum*.

epicotyl–plumule germination (i.e., when the epicotyl or the first true leaf had emerged) was monitored and the length of hypocotyl plus root at the time of epicotyl–plumule germination was measured using a digital caliper (Alpa, Topcal 150PW, Italy).

Data analysis

The final germination percentage (FGP) was calculated as the mean of the three replicates (\pm SD) on the basis of the total number of filled seeds (empty seeds were excluded). A generalized linear model (GLM) was used to evaluate the effect of pre-treatments (i.e., Control, W, CCC, and GA₃) and temperatures (10, 15, and 20°C) on FGP. A quasi-binomial error structure with logit link function and *F*-tests with an empirical scale parameter instead of chi-squared on the subsequent ANOVA were used in order to overcome residual overdispersion (Crawley, 2007). Significant differences highlighted by GLM were then analysed by a *post-hoc* pairwise comparisons *t*-test (with Bonferroni adjustment). All statistical analyses used R v. 3.0.3 (R Development Core Team, 2014).

Results

Seed output

Ribes sardoum contains 3 ± 1 seeds per fruit. Overall, 57% of seeds were developed and 43% were abortive. The average weight of the developed seeds was $6.10 \pm$

0.60 mg. At the time of dispersal, the mean embryo length was 0.06 ± 0.01 cm and the mean seed length was 0.24 ± 0.02 cm, with an embryo:seed ratio of 0.27 ± 0.05 (see Fig. 1).

Germination results

The experimental germination test showed that seeds of *R. sardoum* are able to germinate. No germination occurred during W and the 'move-along' CCC pre-treatment. GLM highlighted a statistically significant ($P < 0.001$) effect on FGP (dependent variable) for both treatment and temperature factors, as well as for their two-way interaction (Treatment \times Temperature; $P < 0.01$; Table 1). The *post-hoc* pairwise *t*-test highlighted no statistical difference ($P > 0.05$) among W treated seeds incubated at each temperature and the untreated seeds (Control) incubated at 15 and 20°C (Fig. 2.1). Germination between c. 35 and c. 65% was detected after Control (except at 10°C) and W treatment. A low germination percentage ($< 20\%$) occurred at 15 and 20°C after simulated field temperature sequences after dispersal (CCC), while no germination was recorded at 10°C after this pre-treatment (Fig. 2.1). A low germination percentage ($< 5\%$) was observed at all tested temperatures during GA₃ treatment (Fig. 2.1). Although there were no statistical differences in the FGP achieved after Control and W pre-treatment, only W-treated seeds incubated at 15°C and untreated seeds incubated at 20°C reached at least 50% germination.

Table 1. GLMs results for the effect on seed germination (dependent variable) of the 'Treatment' (Control; W, 25°C for 3 months; move-along CCC, 10°C for one month plus 5°C for 2 months plus 1°C for 2 months; GA₃, 250 mg l⁻¹ of GA₃ in the germination substrate) and 'Temperature' (10, 15, and 20°C) factors.

	Df	Deviance	Resid. Df	Resid. Dev	F	P (>F)
Null			35	1442.01		
Treatment	3	719.50	32	722.51	26.876	7.59e-08 ***
Temperature	2	213.08	30	509.43	11.939	0.0003***
Treatment × Temperature	6	285.87	24	223.56	5.339	0.0013 **

Untreated seeds (Control) needed ~75 days to reach 50% germination, while after W treatment this time was about three times less, ~26 days (Fig. 2.2). W treatment sped up seed germination, and it also widened the temperature range for germination (germination was also observed at 10°C; Fig. 2). Considering the mean soil temperatures (Fig. 3), two favourable windows for seed germination in the field were detected during the next summer after dispersal; specifically, temperatures of 15°C and 20°C lasted 92 and 20 days, respectively (Fig. 3).

Epicotyl-plumule emergence

Of all the germinated seeds, *c.* 55% of these had the capacity to reach epicotyl-plumule germination. The remaining percentage of them died after a few days of seed germination. The mean time course from radicle emergence to epicotyl-plumule germination was 30.98 ± 13.35 days, at which point the mean hypocotyl plus root length was 22.13 ± 5.39 mm.

Discussion

Seed germination

This study demonstrates that *R. sardoum* is able to produce fruits that contain seeds capable of germinating and developing into seedlings (Fig. 4), contrary to the findings of previous studies of this threatened plant species (Montmollin de & Strahm, 2005; Valsecchi, 1977, 1981). It is well known that at the time of dispersal the seeds of *Ribes* spp. are characterized by a linear underdeveloped embryo (Baskin & Baskin, 2007; Martin, 1946; Mattana *et al.*, 2012); this internal seed morphology was confirmed also in *R. sardoum*, suggesting that the embryo needs to grow inside the seed before radicle emergence. To better understand the embryo growth during the germination process in seeds of this species, several embryo measurements and further specific tests on this topic need to be done (such as those conducted by Baskin, Milberg, Andersson, & Baskin, 2002; Phartyal, Kondo, Hoshino, Baskin, & Baskin, 2009). However, following the dichotomous key to distinguish the dormancy classes *sensu* Baskin & Baskin (2014), if the seeds with an underdeveloped embryo

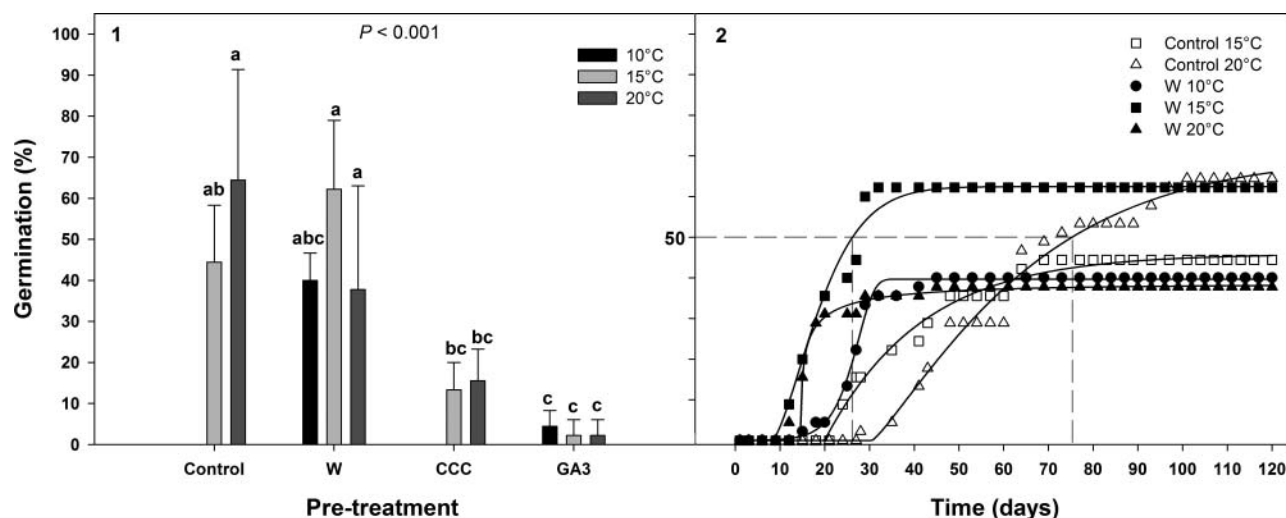


Fig. 2. (1) Final germination percentages at the end of each treatment (Control; W, 25°C for 3 months; move-along CCC, 10°C for one month plus 5°C for 2 months plus 1°C for 2 months; GA₃, 250 mg l⁻¹ of GA₃ in the germination substrate), and (2) germination rate of *Ribes sardoum* after Control and W treatment. Points correspond to mean of three replicates. Continuous lines show fitted Weibull functions, calculated using germination parameters. Dashed lines indicate the time to achieve 50% of germination. GLM was carried on FGP and different letters indicate significant differences ($P < 0.05$) by post hoc pairwise t-test comparisons.

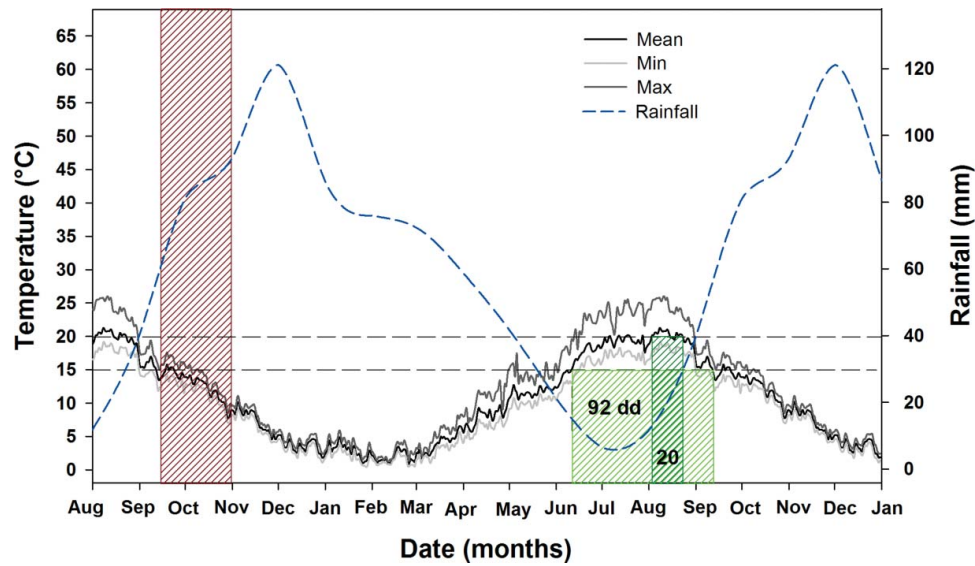


Fig. 3. Five-year average of mean, minimum and maximum daily soil temperatures recorded in Prados (*locus classicus* of *Ribes sardoum*, Monte Corradi, Oliena) and mean monthly rainfall from the nearby weather station of Oliena. The background red square corresponds to the time of fruits (seeds) natural dispersal, while the green squares correspond to the time with soil temperature of 15°C (light green) and 20°C (dark green).

germinate within about 4 weeks, the seeds have morphological dormancy (MD). *Ribes sardoum* seeds did not germinate during stratifications (i.e., during 3 months at 25°C and during move-along CCC) and, despite germinating

without any treatment at 15 and 20°C, they required more than 80 days to reach maximum germination. Therefore, we may assume that seeds of this species also have a physiological (PD) component to dormancy. After warm



Fig. 4. Flower (1), mature fruits (2), germinated seed (3), seed before cotyledon emergence (4), seedling with cotyledons (5) and developed seedling (6) of *Ribes sardoum*, cultivated in the laboratory under controlled conditions.

stratification (followed by cold temperatures), seeds germinated in ~30 days, due to increased seed germination rates. Therefore, we can conclude that seeds of *R. sardoum* have morphophysiological dormancy (MPD), as also detected in *R. multiflorum* subsp. *sandalioticum* (Mattana *et al.*, 2012). The ability of exogenous gibberellic acid to override dormancy is considered an important element in distinguishing among levels of PD (Baskin & Baskin, 2004). However, the effects of GA₃ on seed with physiological dormancy are quite variable (Baskin & Baskin, 2014). In several plant species, gibberellin treatment enhances and promotes seed germination (e.g. Mattana *et al.*, 2012; Porceddu, Mattana, Pritchard, & Bacchetta, 2016), while in other species the gibberellin treatments had no significant effect on seed germination (e.g. Chien *et al.*, 2011; Yang *et al.*, 2011). In *R. sardoum*, gibberellic acid, at the concentration tested, seems to have no effect on dormancy breaking and seed germination. In fact, a low germination percentage (< 5%) was observed at all tested temperatures during GA₃ treatment. Based on these results, we are not sure whether GA₃ is able to promote germination, and we therefore remain cautious in attributing the correct level of MPD for this plant species. A delay in shoot emergence after root emergence indicates that PD may be present in the shoot. Following the dormancy classification system (Baskin & Baskin, 2014), if the roots of warm-stratified seeds emerge at autumn temperatures but shoot emergence is delayed for 3–4 weeks (or more), and cold stratification is not required to break shoot dormancy, a non-deep simple epicotyl MPD dormancy is present. In our experiments, we observed that shoot emergence in *R. sardoum*, without applying any treatment, occurred in ~30 days. The germinated seeds of *R. sardoum* probably do not require a chilling pre-treatment for shoot dormancy release; nevertheless, the effect of cold stratification on breaking shoot dormancy was not tested (due to the low availability of germinated seeds). To confirm the level of MPD in this taxon, other specific tests on this topic therefore need to be conducted.

Ecological and reproductive thresholds

From the ecological point of view, although seed germination was demonstrated, no seedlings of *R. sardoum* were observed in the natural population during the last 10 monitored years (Fenu *et al.*, 2012, 2015). Fruits of *R. sardoum* ripen in late summer, and dispersal takes place in autumn (approximately from late September to mid-November). After dispersal, seeds are exposed to a mean soil temperature of < 20°C, without having experienced a warm stratification. In this study, we recorded a germination percentage between 35 and 65% at 15 and 20°C in the control group but, in the natural population site, after dispersal these ecological conditions do not occur, which

leads us to believe that in the field the seeds do not germinate immediately after dispersal. Moreover, the simulated field temperatures after dispersal were unfavourable for seed germination. The favourable windows for seed germination (i.e., the time with mean temperatures of 15–20°C) had a shorter duration than the time required (detected under controlled conditions) to obtain the first germination under natural conditions (Fig. 3). The results obtained under controlled conditions, lead us to believe that the seeds of *R. sardoum* stay dormant in the ground until the next summer. When the seeds were exposed to a cycle of warm temperatures (i.e., > 20°C), and the mean soil temperatures then dropped below 15°C and the water availability increased in October, the seeds were able to germinate (Fig. 3). The germinated seeds therefore go through the winter with an emerged radicle. Given the delay of about 30 days between shoot and root emergence detected in the laboratory and the likely absence of cold stratification requirement for shoot dormancy release, it makes us suppose that the seedlings that emerge at the beginning of winter are exposed to cold conditions that most likely kill them. However, further studies should be carried out, also in natural conditions, to confirm whether the seeds are able to germinate in the field and to understand the causes of the lack of saplings in the natural population.

Seed germination of *R. sardoum* appears to be adapted to the past temperate climate, supporting the results of the genetic analysis (Gentili *et al.*, 2015); the restricted distribution area of this plant species seems to be the result of successive Quaternary climate changes that favoured its marked divergence and population differentiation in refugia (Gentili *et al.*, 2015), in particular to its adaptation to the Mediterranean climatic seasonality, as also suggested by previous studies (Valsecchi, 1977, 1981); it could also explain the absence of seedlings in the natural population. The sensitivity of *R. sardoum* to low temperatures for seed germination, which puts it at an increased risk from global warming, and the absence of seedling recruitment, highlights an increasing extinction risk of this relict and threatened plant species. In addition, this taxon seems to be somewhat affected by inbreeding and low genetic diversity (Gentili *et al.*, 2015); these factors are known to further increase the extinction probability in species suffering from range contraction and habitat degradation.

Conclusions

This study unexpectedly demonstrates that *R. sardoum* is able to produce several fruits containing viable seeds, which are able to germinate under laboratory conditions. These results provide new and useful information for implementing plant conservation strategies of an extremely threatened plant species. The propagation of

this taxon from seeds therefore seems possible. In fact, the first stages of seedling development under controlled conditions did not manifest any evident problems. This picture contrasts with observations of the wild population, where the lack of seedlings seems to represent the major bottleneck for long-term population persistence. At present, the future survival of this relict plant species in its natural environment cannot be predicted; however, the information presented in this study may be useful in reducing and/or mitigating the extinction likelihood of *R. sardoum*, indicating that seedlings obtained under controlled conditions could be useful for translocation programmes (e.g. Cogoni, Fenu, Concas, & Bacchetta, 2013). The remnant population of *R. sardoum* needs a programme of absolute protection, and a translocation programme should be implemented urgently in order to conserve this plant of priority European interest.




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Disclosure statement

No potential conflict of interest was reported by the authors.

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