Comparative germination ecology of the endemic *Centranthus amazonum* (Valerianaceae) and its widespread congener *Centranthus ruber*

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Abstract

*Centranthus amazonum* Fridl. & A. Raynal (Valerianaceae) is a critically endangered narrow endemic to Central–Eastern Sardinia (Italy), where it occurs in rocky habitats. In the present study, the germination ecology of this species was investigated and compared with the relatively widespread species *Centranthus ruber* (L.) DC. subsp. *ruber*. In particular, the effects of a range of constant temperatures (5–25°C) and two alternating temperature regimes (25/10 and 30/15°C) on seed germination were analyzed. For the two species, cardinal temperatures were determined as the reciprocal of time to 50% germination (t50). In addition, seed persistence in the soil was assessed by experimental seed burials. Seeds of both species were non-dormant; however, *C. amazonum* produced more empty seeds and germination was slower and reached lower final percentages than *C. ruber*. Moreover, seed and seedling mass of *C. ruber* were higher than for *C. amazonum* and the lack of a persistent soil seed bank detected for *C. amazonum* increases vulnerability to extinction for this species. The present study provides new data on the germination ecology of the previously unstudied *C. amazonum* that may explain the rarity of this species when compared with the commonness of *C. ruber*.

Keywords: cardinal temperatures, rarity, Sardinia, seed size, soil seed bank.

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Introduction

*Centranthus* DC. (Valerianaceae) is a circum-Mediterranean genus that includes 10 species with Mediterranean and European distribution, with nine recognized by Richardson (1975) and one additional species described more recently by Fridlender and Raynal-Roques (1998). Species in this genus are glabrous, usually glaucous, annual or rhizomatous perennial herbs (Richardson 1975). Two perennial species belonging to this genus are reported for the flora of Sardinia (Italy): *Centranthus amazonum* Fridl. & A. Raynal and *Centranthus ruber* (L.) DC. ssp. *ruber* (Conti et al. 2005). *Centranthus amazonum* is a narrow endemic occurring predominately (less than 100 individuals) in Central–Eastern Sardinia at 1300 m a.s.l. on a limestone karstic mountain (Monte Corrasi, Oliena—NU). A few individuals (2–5) have also been found in the locality of Codula di Luna (Urzulei—OG) at 140 m a.s.l. (Fridlender & Raynal-Roques 1998; Bacchetta et al. 2008). This rare endemic species is included in the IUCN Red Lists as CR Blab(iii, iv)+2ab(iii, iv); D (Fridlender 2006). *Centranthus ruber* is probably native only in the Balkan Peninsula and parts of the Central Mediterranean region (Richardson 1975). However, this species has been cultivated for ornamental purposes and is now naturalized in many regions of Western and Central Europe, South-Western Asia, Macaronesia and Australia, as well as in North and South America, where it grows in disturbed habitats and rocky places (López Martinez & Devesa 2007). Currently in Sardinia this species is only found in the south part of the island.

Species in the Valerianaceae have either non-dormant (ND) or physiologically dormant (PD) seeds (Baskin & Baskin 1998). Branca (1998) reported over 78%
germination at temperatures ranging between 10 and 20°C for C. ruber, suggesting that this species has ND seeds. Fridlender et al. (1999) compared the germination ecology of C. amazonum and C. trinervis (Viv.) Bég. (a Corsican endemic). They found that C. trinervis seeds germinated faster and to a higher level than seeds of C. amazonum. However, their experimental design and common garden approach do not enable conclusions to be deduced regarding final germination percentages or the optimal temperature ranges for the two species.

Among environmental factors, temperature is arguably the single most important factor governing the maximum germination percentage and rate of germination (Heydecker 1977), as well as the success or failure of plant establishment (Kader & Jutzi 2004). Seed mass also affects seedling survival: larger seeds generally result in larger seedlings, which often have a higher probability of survival (Daws et al. 2005) as a result of a greater ability to withstand either low levels of resources or various hazards (Leishman et al. 2000). Seed size is also related to persistence in the soil seed bank, with persistent seeds generally being smaller and more spherical than transient seeds (Cerabolini et al. 2003). The ability to form a persistent soil seed bank (PSB) is crucial to the survival of many rare or declining species (Quilichini & Debussche 2000; Eckstein et al. 2006). For example, British species that are increasing in abundance are twice as likely to have a PSB as those that are declining (Hodgson & Grime 1990).

In their review, Bevill and Louda (1999) found that pair-wise comparison studies between rare and common species provide a method of gathering intensive information on key similarities and differences between closely related species that differ in abundance. A range of reproductive attributes such as seed size, seed viability, germination rate, seedling growth and the presence of a soil seed bank (PSB) is crucial to the survival of many rare or declining species (Quilichini & Debussche 2000; Eckstein et al. 2006). For example, British species that are increasing in abundance are twice as likely to have a PSB as those that are declining (Hodgson & Grime 1990). In the present study, reproductive traits such as germination requirements, seed and seedling size and seed persistence in the soil of the narrow endemic species C. amazonum were investigated and compared with closely related species C. ruber subsp. ruber. The aims of this study were: (i) to investigate the germination ecology and provide new data for the previously unstudied, in terms of seed biology, C. amazonum; and (ii) to identify differences in seed biology, with respect to the widespread congener C. ruber, that might contribute to both explaining their respective rarity and commonness.

Materials and methods

Seed lot details

Achenes (hereafter referred to as seeds) were collected in late spring and summer 2007 after obtaining permits as required by European and national laws for the species listed in the appendices of the Habitat Directive (DIR.92/43/EEC). Seeds were collected at the point of natural dispersal by placing fine mesh nylon bags over the infructescences to avoid seed dispersal by wind. Seeds of C. amazonum were collected in July 2007 from the natural population in the Monte Corrasi of Oliena (Central Eastern Sardinia, 1300 m a.s.l., 40°14′N, 09°25′E), whereas C. ruber ssp. ruber (hereafter C. ruber) seeds were collected from May to July 2007 from plants cultivated in the Botanic Gardens of Cagliari (South Sardinia, 50 m a.s.l., 39°13′N, 09°06′E). Seeds were stored at the Sardinian Germplasm Bank (BG-SAR) where, once cleaned by removing the pappus, they were placed in a dry room at 15°C and 15% relative humidity (RH). For the germination tests, three replicates of 25 seeds per treatment were sown on the surface of 1% solid water agar, which provided a solid, non-sterile medium for germination, in 90 mm plastic Petri dishes and incubated at both constant (5, 10, 15, 20 and 25°C) and alternating temperatures (25/10 and 30/15°C) in November 2007. In all treatments, seeds were exposed to irradiance for 8 h per day (the ratio of red : far
red light was $-2.0$ and the light quantity was $7 \mu \text{mol/m}^2/\text{s}$. In the alternating temperature regimes the 8 h light period coincided with the elevated temperature period. Low numbers of seeds per replicate (25) were used because of limited seed availability for \textit{C. amazonum} (i.e. this species is rare with a small population). Germination was considered to have occurred at the time of visible radicle emergence. Germination was scored daily for 85 days and any seeds that had germinated were removed. During germination scoring minimal microbial growth was observed. To verify the effect of light on germination, a preliminary test was carried out by sowing three extra replicates at 15°C in the dark, achieved by wrapping the dishes in aluminum foil. Seeds in this experiment were only scored once, at the end of the test, to avoid any exposure to irradiance. At the end of the germination tests, a cut-test was carried out to determine the viability of the remaining seeds and the final germination percentage was calculated on the basis of the total number of filled seeds as the mean of the three replicates ± standard deviation (SD).

**Seed mass and seedling vigor**

After visible germination, seedlings were incubated on 1% water agar under the same conditions as the germination test for a further 8 days. Subsequently, seedling dry mass was calculated by drying 10 8-day-old seedlings, for each germination condition at constant temperatures, in an oven at 103°C for 17 h. Seedlings mass was then determined to seven decimal places (Mettler Toledo, UMT 2, Greifensee, Switzerland). To avoid any possible damage to the seedlings when the seed coat was still attached to the cotyledons, the seedlings were weighed with the seed coats in place. Data were then corrected by subtracting the mean dry mass of three replicates of five seed coats. To compare the data, the dry weight of three replicates of five intact seeds was also determined. Subsequently, the ratio of the mass of covering structures to the mass of the total dispersal unit (SCR) was determined (Grubb & Burslem 1998).

**Experimental seed burial**

Experimental seed burials were carried out at the time of natural seed dispersal, following a modification of the protocol in Arroyo et al. (2004). Sets of five replicates containing 10 seeds each were introduced into fine grain nylon mesh envelopes that were placed into plastic nets. The envelopes were filled with sieved local soil and buried so that the seed envelopes were at a depth of 5 cm. The burial sites were the four vertices and the center of a square (1 m sides). \textit{Centranthus amazonum} plants have only been found on the top of rocky cliffs surrounded by dark crevices. Consequently, it was not possible to carry out burials at the site of the original population. Therefore, both trials were conducted in the Botanic Gardens of Cagliari on a limestone substrate (similar to the collection site of \textit{C. amazonum}), where, during the experiments, the average air temperature was 18.3°C (maximum 39.8°C, minimum $-0.2°C$). After 1 year, the replicates were exhumed. Any remaining, intact, non-germinated seeds were sown immediately at 15°C in the light to check their viability and germination capacity.

**Data analysis**

Germination percentages were arcsine transformed and analyzed statistically by ANOVA; subsequently, a post-hoc Fisher’s least significant difference test was conducted. A non-parametric Mann–Whitney U-test was also carried out to analyze seedling dry mass using MINITAB release 11.21 (Minitab, State College, PA, USA). Theoretical cardinal temperatures were evaluated by determining the seed germination rate (GR), defined as the reciprocal of \textit{tb} (time to reach 50% of the maximum germination in one replicate) for the germination tests carried out at constant temperatures (5–25°C). The data for each species were regressed using a linear model to estimate the base temperature (\textit{tb}) and the ceiling temperature (\textit{tc}) at which the germination rate is equal to zero, by averaging the x-intercept for both suboptimal and supra-optimal temperature ranges (Ellis et al. 1986). The optimum temperature (\textit{to}) was calculated as the intercept of the suboptimal and supra-optimal temperature response functions (Hardegree 2006), using SigmaPlot 2002 for Windows version 8.0 (SPSS, Chicago, IL, USA). Regression lines were also statistically analyzed using an analysis of covariance (ANCOVA) in MINITAB release 11.21 (Minitab) to test for differences in the response between the two species.

**Results**

**Germination ecology**

The preliminary germination test carried out at 15°C both in the light (8 h of irradiance per day) and in the dark showed that the seeds of the two species have different light requirements for germination ($P < 0.05$ by one-way ANOVA; Fig. 1). Although \textit{C. ruber} seeds germinated equally well in the light (93.3 ± 6.1%) and in the dark (96.5 ± 3.0%) (one-way ANOVA, $P > 0.05$), \textit{C. amazonum} seeds achieved higher germination in the light (84.7 ± 3.7%) than in the dark (53.2 ± 14.1%), and this difference was statistically significant (one-way ANOVA, $P < 0.05$).
Both species reached high germination percentages (Fig. 2), which were not statistically different (one-way ANOVA, \( P > 0.05 \)) at constant temperatures between 10 and 20°C. However, at the two extreme constant temperatures (5 and 25°C) there were significant differences between the species (one-way ANOVA, \( P < 0.05 \)); only 60.1 ± 2.8% of *C. amazonum* seeds germinated at 5°C, whereas 93.3 ± 8.3% of *C. ruber* seeds germinated. Similarly, at 25°C both species achieved low, but significantly different, levels of germination (\( P < 0.05 \)) of 21.3 ± 2.3% and 43.1 ± 3.7% for *C. ruber* and *C. amazonum*, respectively. The two-way ANOVA carried out on the final germination percentages at constant temperatures highlighted that the effect of species was not significant (\( P > 0.05 \)); however, the effect of temperature and the interaction between species × temperature were both highly significant (\( P < 0.001 \)). In addition, there were no significant differences between germination at constant and alternating temperatures (\( P > 0.05 \), one-way ANOVA; Fig. 2).

Both species showed differences in both GR and cardinal temperatures. The maximum GR (Table 1; Fig. 3) for *C. amazonum* was 0.15/day, which corresponds to an optimal temperature \( (t_o) \) of 12°C; the base \( (t_b) \) and ceiling \( (t_c) \) temperatures were 3.75 and 24.75°C, respectively. Following the same method, *C. ruber* had a higher GR (5 and 25°C) there were significant differences between the species (one-way ANOVA, \( P < 0.05 \)); only 60.1 ± 2.8% of *C. amazonum* seeds germinated at 5°C, whereas 93.3 ± 8.3% of *C. ruber* seeds germinated. Similarly, at 25°C both species achieved low, but significantly different, levels of germination (\( P < 0.05 \)) of 21.3 ± 2.3% and 43.1 ± 3.7% for *C. ruber* and *C. amazonum*, respectively. The two-way ANOVA carried out on the final germination percentages at constant temperatures highlighted that the effect of species was not significant (\( P > 0.05 \)); however, the effect of temperature and the interaction between species × temperature were both highly significant (\( P < 0.001 \)). In addition, there were no significant differences between germination at constant and alternating temperatures (\( P > 0.05 \), one-way ANOVA; Fig. 2).

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Fig. 1 Germination percentages (±standard deviation) for both species at 15°C in the light (8/16) and in the dark (0/24). Data are the means of three replicates (ns, \( P > 0.05 \); *P < 0.05\), one-way ANOVA followed by post-hoc Fisher's least significant difference test).

Fig. 2 Final germination percentages (±standard deviation) for each condition in the light (8 h of irradiance per day). Data are the means of three replicates. Bars with the same letters above them are not significantly different at \( P > 0.05 \) (post-hoc Fisher's least significant difference test).

Fig. 3 Germination rates for the two species calculated on the basis of the reciprocal of the \( t_0 \) values. \( t_b \) base temperature; \( t_o \) optimal temperature; \( t_c \) ceiling temperature. Points correspond to the actual data and solid lines indicate the fitted lines from the linear regressions. \( P < 0.001 \) for the suboptimal models and \( P < 0.05 \) for the supra-optimal models by ANCOVA. Data are the means of three replicates (±standard deviation).

<table>
<thead>
<tr>
<th>Species</th>
<th>Function type</th>
<th>Model</th>
<th>( r^2 )</th>
<th>( t_b )</th>
<th>( t_o )</th>
<th>( t_c )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. amazonum</em></td>
<td>Suboptimal</td>
<td>( y = -0.060 + 0.017x )</td>
<td>0.98</td>
<td>3.75</td>
<td>12.00</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Supra-optimal</td>
<td>( y = 0.289 - 0.012x )</td>
<td>0.95</td>
<td>—</td>
<td>—</td>
<td>24.75</td>
</tr>
<tr>
<td><em>C. ruber</em></td>
<td>Suboptimal</td>
<td>( y = 0.008 + 0.014x )</td>
<td>0.97</td>
<td>-0.50</td>
<td>15.00</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Supra-optimal</td>
<td>( y = 0.69 - 0.031x )</td>
<td>0.97</td>
<td>—</td>
<td>—</td>
<td>22.25</td>
</tr>
</tbody>
</table>

\( t_b \), base temperature; \( t_o \), optimal temperature; \( t_c \), ceiling temperature.

Table 1 Regression equations, \( r^2 \) values and cardinal temperatures (°C) estimated by linear suboptimal and supra-optimal models for germination rates of the two species.
(0.22/day at 15°C of t₀) and the temperature range between t₀ and t₁ was wider than that for C. amazonum, ranging from −0.50 to 22.25°C. The analysis of covariance (ANCOVA) applied to the fitted lines in Figure 3 showed significant differences, both in the suboptimal \((P < 0.001)\) and supra-optimal ranges \((P < 0.05)\), between the regression equations for the two species.

**Seed viability**

The cut-test carried out at the end of the germination tests (Fig. 4) showed a considerable percentage \((18.5 \pm 9.5\%)\) of empty seeds for C. amazonum compared with C. ruber \((1.3 \pm 2.3\%)\). These percentages were significantly different \((P < 0.001)\) as were the percentages of germinated seeds, with a mean germination across all the tested temperatures of 62.7 ± 16.6% for C. amazonum and 76.4 ± 25.3% for C. ruber \((P < 0.05, \text{one-way ANOVA})\), whereas the percentages of dead and non-germinated viable seeds did not show differences between the two taxa.

**Seed and seedling size**

Centranthus amazonum seeds, with a dry mass of 0.55 ± 0.02 mg, were significantly smaller \((P < 0.05, \text{Mann–Whitney U-test})\) than C. ruber seeds (dry mass 2.01 ± 0.06 mg). Centranthus amazonum seeds had a lower SCR \((0.36 \pm 0.06)\) than C. ruber seeds \((0.41 \pm 0.05)\), although this difference was not statistically significant \((P > 0.05, \text{one-way ANOVA})\). Seedling dry mass was significantly different \((P < 0.001, \text{Mann–Whitney U-test})\) between the two taxa (Fig. 5); in fact C. ruber seedlings \((1.28 \pm 0.54 \text{ mg})\) were more than twice as heavy as C. amazonum seedlings \((0.43 \pm 0.16 \text{ mg})\).

**Seed persistence in the soil**

No buried seeds of C. amazonum were retrieved because they had decayed completely in the soil before being exhumed. In contrast, only 57.8 ± 24.6% of C. ruber seeds decayed in the soil and the remaining intact seeds, sown immediately after being exhumed, germinated to 83.3 ± 33.3% at 15°C in the light. The cut-test carried out at the end of the germination test showed that all of the non-germinated seeds were still viable, suggesting that this species can form a PSB. No viable seedlings were retrieved for either species.

**Discussion**

**Germination ecology**

The high germination percentages achieved for both species in the present study, across a wide temperature range, mean that it is unlikely that seeds of either species exhibit dormancy (sensu Baskin & Baskin 1998, 2004). Consequently, in agreement with Branca (1998) and Fridlender et al. (1999) they can be classified as ND.

The two species showed different responses, in terms of germination percentages, at the extreme temperatures tested \((5 \text{ and } 25°C)\). Centranthus ruber showed ‘typical’ germination behavior for Mediterranean plants, with an optimal range of germination temperatures of 5–15°C (Thanos et al. 1989, 1995). This confirms the results of Branca (1998), who detected low germination at warm environments.
temperatures (<6% at 25°C and no germination at 30°C). *Centranthus amazonum* seeds germinated better at higher temperatures (20–25°C) than those of *C. ruber*, confirming the findings of Giménez-Benavides *et al.* (2005), who found that Mediterranean orophytic plants generally show temperature indifference or warm-preference for germination, but not cold-adaptation unlike ‘typical’ Mediterranean species. This adaptation may limit the distribution of this species to the top of mountains (~1300 m a.s.l.) by a requirement for water for germination/seeding emergence during the warmer season that is not available at lower altitudes under typical Mediterranean climate conditions.

Rapid germination has been suggested to be an adaptation for rapid establishment in advance of soil drying in unpredictable environments (Daws *et al.* 2002). This is likely to be of particular importance for species growing in exposed rocky outcrops where soil drying will occur rapidly after rainfall, particularly in a karstic habitat such as Monte Corrasi, where *C. amazonum* grows. Consequently, our data suggest that *C. ruber*, whose seeds germinated more quickly than *C. amazonum* seeds, is better adapted for rapid establishment in harsh conditions. The application of alternating temperature regimes did not improve the germination percentages of either species. This is consistent with the findings of Pearson *et al.* (2002), who found, for neotropical pioneer species, that seeds that responded to alternating temperatures were generally greater than 2 mg in mass, that is, larger than the seed mass of both species examined in the present study. *Centranthus ruber* seeds germinated well both in the light and in the dark, whereas *C. amazonum* seeds had higher germination in the light. As found in several studies, both in tropical and temperate climates (Milberg *et al.* 2000; Pearson *et al.* 2002), small seeds are more likely to require light to germinate. In particular, Pearson *et al.* (2002) found that the maximum seed mass of species likely to show a significant positive germination response to irradiance was approximately 1.0 mg, therefore, larger than *C. amazonum* seeds and smaller than *C. ruber* seeds. The low germination percentage achieved by *C. amazonum* in the dark may limit the distribution of this species to the top of the rocky cliffs, which are surrounded by deep dark crevices.

**Seed viability**

The endemic *C. amazonum* had a higher proportion of empty seeds. A greater production of empty seeds in a narrow endemic could be related to inbreeding depression and reduced fitness resulting from homozygocity, which can be severe enough to affect the viability of small and isolated wild plant populations (Keller & Waller 2002): at the last census the population of *C. amazonum* was less than 100 individuals (Bacchetta *et al.* 2008). Chauhan *et al.* (2008) in their study on the endangered *Nardostachys jatamansi* DC. (Valerianaceae) found that the species is self-pollinated, but dependent on pollinators for optimum pollination. Cross-pollination may have therefore an adaptive value for the species because it compensates for failure of autogamy, maintaining genetic variability in the population. Pollination studies should be carried out to confirm the presence of inbreeding phenomena for the population of *C. amazonum*. Although *C. ruber* is not currently considered an invasive in Sardinia (Celesti-Grapow *et al.* 2009) and no naturalized populations have been detected close to the Monte Corrasi, the broader ecological range detected for this species confirms the potential threat resulting from the possible expansion of *C. ruber* on the Island and the risk of hybridization of this species with *C. amazonum*, as suggested by Fridlender (2006) and detected by Montmollin and Strahm (2005) for the closely related endemic species *C. trinervis*.

**Seed mass and seedling vigor**

The differences in seed mass between the two species may explain the widespread distribution of *C. ruber* with respect to *C. amazonum*. Large-seeded species establish over a wide range of conditions, whereas smaller-seeded species are more dependent on disturbance (Burke & Grime 1996): *C. ruber* seeds were approximately fourfold heavier than those of *C. amazonum* and the seedlings were more than twice as heavy as those of *C. amazonum*. Seedlings from larger seeds are typically more tolerant of a range of hazards (e.g. drought and herbivory; Leishman *et al.* 2000), suggesting that seedlings of *C. ruber* will be both more robust and more likely to establish than seeds of *C. amazonum*.

**Seed persistence in the soil**

Small size and light requirement for germination have been related to the ability to form a PSB (e.g. Probert 1992; Cerabolini *et al.* 2003), which leads to the prediction that *C. amazonum* is more likely to form a PSB than *C. ruber*. However, in the field burial experiment the reverse pattern was observed: all of the *C. amazonum* seeds decayed before being exhumed and approximately half of the *C. ruber* seeds were still viable, achieving a high germination percentage (~80%) in the laboratory experiments. Thompson *et al.* (1998) found that increasing habitat disturbance selects for increased seed persistence, without being always associated with reduced seed size. Therefore, in species characteristic of disturbed habitats, such as *C. ruber* (López Martínez & Devesa 2007), persistence in the soil depends not only on seed size, but also on
other traits, such as the higher (although not statistically significant) SCR detected for *C. ruber* with respect to *C. amazonum* seeds, which are less defended and more likely to decay in the soil. The lack of a PSB for *C. amazonum* may explain the rarity of this species and these data are in accordance with the results found in previous comparative studies, where the ability to form a PSB has been related to commonness in comparisons of related common and rare species (e.g. Walck et al. 2001; Williams et al. 2005; Burmeier & Jensen 2008). Although it is unlikely that the substrate and environmental conditions recorded during the experiments may have negatively affected the viability of *C. amazonum* seeds compared with ‘natural’ conditions at the collection site, these results should be confirmed by further investigations.

**Conclusion**

A comparison of *C. amazonum* with the widespread congeneric *C. ruber* highlighted a potential gap in the fitness of this species. In particular, even if further studies on pollination system, seed yield, seed dispersal and seedling recruitment are needed to identify the key factors contributing to their respective rarity and commonness, *C. ruber* appears to be more adapted to harsh conditions by virtue of its rapid germination (Daws et al. 2002), larger seed and seedling size (Leishman et al. 2000) and its ability to form a PSB (e.g. Williams et al. 2005).

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**References**


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