# Evaluation of reproductive success and conservation strategies for *Senecio coincyi* (Asteraceae), a narrow and threatened species

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**Abstract.** *Senecio coincyi* is a threatened endemic plant of central western Spain, with a very narrow extent of occurrence. The reproductive success and germination behaviour of this species were studied. The area of occupancy, habitat types and size of 13 known subpopulations of *S. coincyi* were evaluated. The number of individuals that form all these subpopulations was counted. In addition, the number of flowers and cypselas per fruit head and the number of fruit heads per individual plant were recorded in a subset of subpopulations. Germination tests were carried out to evaluate the effect of temperature and light regimes on, and possible intraspecific variation in, cypsela germination. Cypselas reached very high germination percentages (90–100%) from 15°C to 30°C. However, the germination decreased (19%) at 10°C. The light conditions assayed (16-h light photoperiod and complete darkness) did not significantly affect cypsela germination. In general, there was no intraspecific variability in germination patterns of *S. coincyi* cypselas. Livestock is the most important factor that can be a threat for this species. *S. coincyi* showed high reproductive success and, therefore, its conservation problems are not due to agents related to its reproduction, but rather to other factors such as alteration of its habitat caused by the presence of livestock.

## Introduction

All Mediterranean-climate regions have a high number of rare and locally endemic taxa that survive as small populations, many of which are threatened by habitat transformation (Cowling *et al.* 1996). In particular, high levels of endemic species are a significant feature of Mediterranean mountains (Gavilán *et al.* 2002; Väre *et al.* 2003; Giménez-Benavides *et al.* 2005). Evidence of global warming in the mountains of central Spain (Gavilán *et al.* 2001; Sanz-Elorza *et al.* 2003) suggests that mountain species are at serious risk from the upward advance of lowland plants, as widely predicted (Grabherr *et al.* 1994; Peñuelas *et al.* 2002). There is almost no basic biological information for conservation of these plants, despite the fact that Mediterranean mountains are considered one of the most threatened systems in Spain and the European Union (Gómez-Campo 1987).

*Senecio coincyi* Rouy (*Asteraceae*) is a threatened endemic species of the Iberian Peninsula, with a distribution restricted to a very small area of the Sierras of Villafranca and Gredos (Ávila province, central-western Spain) (see Fig. 1). This taxon has been included, since 1985, in successive catalogues of threatened species of Spain (Barreno 1985; Gómez-Campo 1987; Domínguez 2000; Moreno 2008). The species was included in the *In danger of extinction* category in the protected flora catalogue of the Autonomous Community of Castile and León (Spain) (Anonymous 2007). It was later catalogued as *Vulnerable* (VU) in the Red List 2008 of Threatened Spanish Vascular Flora (Martínez-García *et al.* 2008). Despite its status as a threatened and protected species, very little is known on the biology of this species.

*Senecio coincyi* was first described by Rouy (1890) from plants herborised by Coincy at Pinar Hoyocasero (Ávila province, Spain), a location where this species is now considered to be extinct. It belongs to the *S. integrifolius* (L.) Clairv. group (Tutin *et al.* 1964–1980), which is widely distributed in the mountains of Europe. *S. coincyi* is a hygrophilous, hemicryptophyte, perennial taxon, the leaves of which form a basal rosette that emits a floriferous stem up to 1.5 m high. This flower scape blooms in June and fruits from late June to July. Its flowers are grouped into capitula-forming corymbs, with ~10–20 thick capitula, and they are pollinated by several insect species. The fruit is a ribbed fusiform cypsela between 3 and 5 mm in length and <1 mm in diameter, with a pappus that facilitates its dispersion by wind. Therefore, it is an entomophilous and anemochorous species.

Most of the subpopulations (according to IUCN terminology; IUCN 2010) of *S. coincyi* are found at an altitude between 1500 and 1800 m, although there are some subpopulations located at

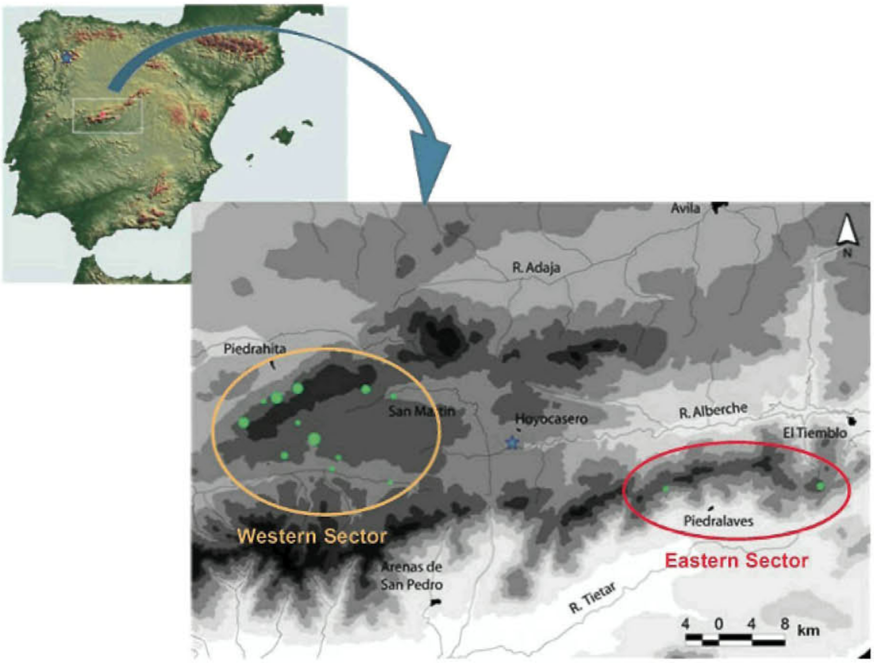


**Fig. 1.** Distribution of the known subpopulations of *Senecio coincyi* (green circles). The blue start indicates an extinct subpopulation.

1300 m and others at 1900 m. The species inhabits wet siliceous soils with a permanently high level of edaphic humidity throughout the year. For this reason, it grows exclusively on the edges of streams, (mostly) abandoned hay meadows, peaty meadows and small peat bogs (Martínez-García 2008; Martínez-García *et al.* 2008). Mean annual precipitation in areas where *S. coincyi* grows ranges from 800 to 970 mm. Mean annual temperature is ~8–11°C, with mean maximum temperature of the warmest month of 19–21°C and mean minimum temperature of the coldest month of 0.3–2.2°C (Ninyerola *et al.* 2005).

There are relatively few studies on germination behaviour of mountain endemic species from the Iberian Peninsula (Pérez-García *et al.* 1995; Albert *et al.* 2002; Giménez-Benavides *et al.* 2005; Lorite *et al.* 2007; Copete *et al.* 2011*a*, 2011*b*). Similarly, little information is available on the seed germination of *Senecio* species (Fernández *et al.* 2011; Kirmizi *et al.* 2011). The present study is the first about germinative characteristics of *S. coincyi*.

In the present work, the results of explorations of *S. coincyi* subpopulations over 2 years are presented. The aims of the study were to (1) provide information on germination conditions of *S. coincyi* cypselas, (2) determine the existence or not of inter-population variation in seed germinability and (3) evaluate the degree of threat and protection measures for the conservation of *S. coincyi*.

## Materials and methods

### *Chorology and census of the subpopulations*

Data on the area of occupancy (see IUCN 2010), altitude, habitat types, size and conservation status were collected for each of the 13 known subpopulations of *S. coincyi*. All of these subpopulations were located in the Sierras of Villafranca and Gredos (Ávila province, central-western Spain) (Fig. 1, Table 1). In total, 62 UTM squares (1 × 1 km) were visited to evaluate the number of individuals per subpopulation (Table 1). Groups of individuals that were separated from each other by a distance of 1 km were considered as belonging to different subpopulations, assuming that gene flow was sporadic or negligible (Iriondo 2003).

### *Number of flowers and fruits per capitulum*

The flowers are grouped into capitula (fruit heads hereafter) and there is only one floriferous stem per individual plant. Unripe fruit heads from individuals chosen at random from seven subpopulations were collected (see Table 2) in June 2007. The number of fruit heads collected was proportional to the subpopulation size. Then, in the laboratory, the number of ligulate, tubular and total flowers per fruit head was recorded.

Ripe fruit heads were chosen at random and collected from several individuals in six subpopulations (see Table 3) in July 2007. All fruit heads collected contained ripe fruits (cypselas hereafter). Then, in the laboratory, the number of cypselas per single fruit head was recorded. In addition, the total number of ripe fruit heads per individual plant was recorded for five subpopulations (see Table 4). The number of plants sampled at random from each subpopulation ranged from 6 to 45, depending on the subpopulation size.

At the beginning of June 2007, several unripe fruit heads from three subpopulations were chosen at random and bagged in fine paper bags (see Table 3). This was done to ensure that any cypselas (having a pappus, for wind dispersal) that fell from the fruit head would not be lost. The bags were very thin and they allowed transpiration and the transmission of light. Bagged fruit heads were collected when mature in late July 2007. Then in the laboratory, the number of cypselas of these fruit heads was recorded. The number of cypselas per single fruit head was compared with the number of flowers per fruit head.

**Table 1. Subpopulation code, location, UTM coordinates, altitude, surface occupied and population size related to the *Senecio coincyi* locations known**
All subpopulations are located in the Sierras of Villafranca and Gredos (Ávila province, central-western Spain, see Fig. 1)

| Subpopulation code | Location | Sector | Altitude (m asl) | UTM square of reference | No. of UTM squares | Surface (ha) | No. of individuals |
|---|---|---|---|---|---|---|---|
| NAM | Navamuñana | Western | 1700–1750 | 30TTK9974 | 3 | 4.1 | 3570 |
| NAL | Navalmahillo | Western | 1450–1500 | 30TUK0177 | 2 | 0.2 | 391 |
| PIE1 | Piedrahita | Western | 1750–1850 | 30TUK0377 | 2 | 0.5 | 1895 |
| PIE2 | Piedrahita | Western | 1750–1900 | 30TUK0679 | 4 | 1.7 | 4217 |
| SAN | San Martín | Western | 1580–1600 | 30TUK1478 | 3 | 2.4 | 1927 |
| HER1 | La Herguijuela | Western | 1650–1800 | 30TUK0574 | 3 | 0.1 | 43 |
| HER2 | La Herguijuela | Western | 1500–1700 | 30TUK0873 | 5 | 11.2 | 10 179 |
| ORT | Ortigosa | Western | 1450–1570 | 30TUK0470 | 2 | 1.5 | 1211 |
| NAV1 | Navacepeda | Western | 1600 | 30TUK1069 | 1 | 0.1 | 113 |
| NAV2 | Navacepeda | Western | 1300–1600 | 30TUK1071 | 2 | 4.3 | 304 |
| HOY | Hoyos del Espino | Western | 1470 | 30TUK1767 | 3 | 1.7 | 436 |
| PEV | Piedralaves | Eastern | 1360–1400 | 30TUK4967 | 1 | 0.9 | 500 |
| TIE | El Tiemblo | Eastern | 1550 | 30TUK6867 | 2 | 1.2 | 827 |

**Table 2. Number (mean ±s.e.) of ligulate, tubular and total flowers per fruit head for seven subpopulations of *Senecio coincyi***
*n* = number of fruit heads sampled from each subpopulation

| Subpopulation code | Ligulate flowers | Tubular flowers | Total flowers | *n* |
|---|---|---|---|---|
| HER1 | 19 ± 0.83 | 119 ± 12.05 | 138 ± 12.56 | 11 |
| HER2 | 19 ± 0.48 | 134 ± 5.09 | 153 ± 5.60 | 43 |
| HOY | 15 ± 0.81 | 131 ± 12.40 | 146 ± 12.90 | 8 |
| NAM | 19 ± 0.89 | 140 ± 7.58 | 159 ± 8.31 | 12 |
| PIE1 | 21 ± 0.68 | 160 ± 7.78 | 181 ± 7.71 | 7 |
| PIE2 | 20 ± 0.65 | 138 ± 6.60 | 158 ± 7.09 | 22 |
| TIE | 17 ± 1.06 | 124 ± 9.57 | 141 ± 11.44 | 11 |

**Table 3. Number (mean ±s.e.) of cypselas per a single fruit head for six subpopulations of *Senecio coincyi***
The number of cypselas was recorded for fruit heads with (bagged) or without (non-bagged) paper bag. *n* = number of fruit heads sampled from each subpopulation

| Subpopulation code | Bagged | *n* | Non-bagged | *n* |
|---|---|---|---|---|
| HER2 | 145 ± 7.27 | 19 | 159 ± 7.43 | 17 |
| HOY | 160 ± 10.88 | 5 | 154 ± 9.15 | 9 |
| NAV1 | 166 ± 21.43 | 4 | – | – |
| NAV2 | 132 ± 8.02 | 14 | – | – |
| PIE1 | 145 ± 8.42 | 17 | 180 ± 13.30 | 15 |
| SAN | 152 ± 14.43 | 9 | – | – |

**Table 4. Number (mean ±s.e.) of fruit heads per individual plant for five subpopulations of *Senecio coincyi***
*n* = number of individuals sampled from each subpopulation

| Subpopulation code | Mean ± s.e. | *n* |
|---|---|---|
| HER2 | 15 ± 0.78 | 45 |
| HOY | 8 ± 0.55 | 24 |
| NAV1 | 12 ± 1.36 | 6 |
| PIE1 | 13 ± 1.66 | 32 |
| SAN | 13 ± 2.19 | 20 |

**Table 5. Final germination percentage (mean ±s.e.) and mean ±s.e. germination time (MGT) of *Senecio coincyi* cypselas belonging to six subpopulations**
The number of empty cypselas in each replicate was excluded for calculating the final germination percentage. Results after 20 days of incubation under a 16-h light photoperiod at 25/15°C. Means followed by the same letter in a column are not significantly different according to the l.s.d. test at 0.05 level

| Subpopulation code | Empty cypselas (%) | Germination (%) | MGT (days) |
|---|---|---|---|
| HER2 | 19 ± 7.19b | 88 ± 5.36a | 7.50 ± 0.31ab |
| NAV1 | 15 ± 6.81ab | 97 ± 1.99ab | 6.93 ± 0.23a |
| PIE1 | 11 ± 3.42ab | 93 ± 3.75ab | 7.52 ± 0.61ab |
| PEV | 1 ± 1.00a | 98 ± 1.66b | 8.11 ± 0.29bc |
| SAN | 0a | 99 ± 0.87b | 8.45 ± 0.15bc |
| TIE | 42 ± 9.02c | 88 ± 4.37a | 8.91 ± 0.23c |

### *Cypsela collection and storage*

Ripe cypselas from mature fruit heads taken in July 2007 for evaluating the number of fruits per head were used for the germination experiments. Cypselas belonging to subpopulations HER2, NAV2 and SAN were cleaned manually (immature cypselas or cypselas attacked by parasites were eliminated), placed in paper bags and stored dry under laboratory conditions at ~23°C, until the start of the germination trials in December 2007 and January 2008. For each subpopulation, cypselas belonging to fruit heads from different individuals were bulked. Cypselas collected in July 2007 were used for studying the effect of different temperature regimes and light conditions on the germination behaviour of *S. coincyi*.

Likewise, ripe fruit heads containing mature cypselas were taken from six subpopulations in July 2008 (see Table 5). These subpopulations were chosen from the two sectors in the distribution of this species, namely, western and eastern sectors (Fig. 1, Table 1). The cypselas from all selected fruit heads showed a similar degree of ripeness, as observed from their colour and hardness. As above, all fruit samples were cleaned manually, placed in paper bags and stored dry under laboratory conditions (~23°C) until use in October 2008. Cypselas collected in July 2008 were used for studying the possible intraspecific germination variability of *S. coincyi*.

### Germination trials

Four replicates of 25 cypselas each were tested for germination on top of two sheets of filter paper (previously moistened with 3.5 mL distilled water) in 7-cm-diameter glass Petri dishes. Filter papers were rewetted regularly with distilled water as required. Dishes were checked three times a week over a total 20-day test period and germinated cypselas were counted and removed. The criterion for germination was visible radicle protrusion. In all trials, at the end of the incubation period, ungerminated cypselas were checked by cutting open each cypsela, to see whether an embryo was present and looked healthy. Final germination percentages were based on the number of intact cypselas in each replicate.

### Effect of temperature and light regimes on the cypsela germination

The aim of this trial was to determine the optimal temperature and light requirements for radicle emergence. In December 2007 and January 2008, germination of cypselas belonging to the subpopulations HER2 and NAV2 collected in July 2007 was tested at different constant temperatures (10°C, 15°C, 20°C, 25°C and 30°C), with a 16-h light photoperiod (provided by cool white fluorescent tubes with an irradiance of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and the alternate temperatures of 25°C/15°C (25°C for 16 h in light and 15°C for 8 h in dark). For the lowest incubation temperature assayed (10°C), the germination period was extended up to 40 days. In addition, cypselas belonging to the subpopulation SAN collected in July 2007 were tested for germination at alternate temperatures of 25°C/15°C under a 16-h light photoperiod and, additionally, under constant darkness. The darkness treatment was obtained by wrapping Petri dishes in a double layer of aluminium foil.

### Intraspecific variation in cypsela germination

Germination trials were performed to detect germination differences among subpopulations. In October 2008, germination of cypselas belonging to six subpopulations (see Table 5) collected in July 2008 was tested at 25°C/15°C under a 16-h light photoperiod. In every subpopulation, the cypselas from all fruit heads collected from different individuals were bulked.

Germination experiments were also carried out to detect germination differences among individuals belonging to the same subpopulation. Ripe fruit heads from several individuals chosen at random belonging to the subpopulations HER2 (13 individuals) and SAN (12 individuals) were collected in July 2008 and kept separately before cypsela extraction. Then, for each individual plant, cypselas from all fruit heads harvested were bulked. In October and November 2008, cypselas from individuals were set to germinate at 25°C/15°C under a 16-h light photoperiod. In the same way, ripe fruit heads (from 3 to 5) belonging to several individuals randomly chosen from the subpopulations HER2 (4 individuals) and PEV (3 individuals) were harvested in July 2008 and kept separate before cypsela extraction. Then, the cypselas from each fruit head were collected and kept separately (without bulking). In November and December 2008, the cypselas from the different fruit heads were tested for germination at 25°C/15°C under a 16-h light photoperiod.

### Data analysis

At the end of the germination period, the final germination percentage (mean value ± s.e.) and the mean germination time (MGT, mean value in days ± s.e.) were calculated. In all germination trials, the number of empty cypselas in each replicate was excluded when calculating the final germination percentage.

The values of final germination percentages were arcsine transformed and then subjected to ANOVA (untransformed data appear in Tables). One-way factorial ANOVA was used to test the effects of the different temperature regimes and light conditions (light/dark and constant darkness) on seed germination capacity. Similarly, to determine differences in germination among cypselas belonging to different subpopulations, individuals and single fruit heads, data were analysed by means of one-way ANOVA. Where ANOVA indicated a significant effect, a comparison of mean values was carried out through the least significant difference test (l.s.d.). The statistical analysis of MGT was also carried out with one-way factorial ANOVA.

## Results

### Habitat types where *S. coincyi* grows

Characteristics and number of individuals of 13 known subpopulations of *S. coincyi* are shown in Table 1. Approximately half of the areas occupied by *S. coincyi* are along streams (Table 6). These habitats range from 1–2-m-wide streams at the bottom of valleys that are relatively open (treeless), to small creeks descending from the upper parts of watersheds with steep slopes. Hay meadows represent 30% of the total area occupied by *S. coincyi*, many of which are now abandoned but still contain herbaceous communities. About 20% of the total area of occupation consists of naturally flooded meadows that were never used as hay meadows. This is a highly fragmented habitat type, consisting of very small strips of land, most of them barely reaching a half hectare. The water pipes that were built for agricultural and livestock purposes represent a very small percentage of *S. coincyi* habitats, occupying only 3%. Around these channels, soil maintains high moisture and it allows the growth of small groups of individuals of this species (usually not more than 4 or 5 individuals together). Peat bogs represent only 1% of habitats occupied by this species.

**Table 6. Surface estimated of the different habitat types where *Senecio coincyi* inhabits**

| Habitat type | Surface (ha) | % of the total surface |
|---|---|---|
| Edges of streams | 15.1 | 50.7 |
| Hay meadows | 8.6 | 28.8 |
| Peaty meadows | 5.0 | 16.8 |
| Water pipes | 0.8 | 2.7 |
| Peat bogs | 0.3 | 1.0 |
| Total surface | 29.8 | 100 |

### Number of mature individuals

The total number of mature individuals is ~26 000, distributed in 13 subpopulations over almost 30 ha (Table 1). The number of individuals ranged widely, from ~10 000 for the biggest subpopulation (11.2 ha) to less than 50 for the smallest subpopulation (0.1 ha) (Table 1). Subpopulations were located in two different sectors (Table 1) clearly differentiated by the distance between them; the western sector is mainly located in the Sierra of Villafranca, and the eastern sector on the southern slope of the Sierra of Gredos.

### Number of flowers per fruit head

Sexually mature individuals produced a floriferous stem that usually has between 10 and 20 fruit heads. The proportion of adult individuals flowering observed in June 2007 was very high, ranging from 80% to 90% for most subpopulations. However, a lower percentage of flowering individuals was recorded in some subpopulations; for example, HOY and TIE subpopulations had 70% and 15%, respectively, of adult individuals flowering. Flowering occurs throughout June and it starts at the apical tip of the stem.

The mean number of flowers (both ligulate and tubular flowers) per single fruit head ranged from 138 to 181 (Table 2). The mean number of ligulate and tubular flowers per fruit head ranged from 15 to 21 and from 119 to 160, respectively (Table 2). No significant differences in the number of tubular flowers ($P = 0.1268$) and total number of flowers ($P = 0.1135$) per fruit head were found among subpopulations. However, significant ($P = 0.0012$) differences in the number of ligulate flowers per fruit head were found among subpopulations. In addition, no significant differences ($P > 0.05$) in the number of ligulate, tubular or total flowers per fruit head were found among individuals within a subpopulation. The mean value of flowers per fruit head was 153.7 for 114 fruit heads collected belonging to seven subpopulations. The mean values of ligulate and tubular flowers per fruit head were 18.6 (12.1%) and 135.1 (87.9%), respectively.

### Number of cypselas per fruit head

The fruiting period occurs between late June and July. The mean number of cypselas per fruit head for all subpopulations sampled was 146 (71 minimum, 237 maximum) for 68 heads collected without a paper bag, and 165 (102 minimum, 290 maximum) for 41 heads collected with paper bags (Table 3). The mean number of flowers and the mean number of cypselas per fruit head were very similar (see Tables 2, 3). Therefore, the proportion of flowers setting fruits is very high, indicating that most flowers are fertilised. Percentages of sterile flowers and/or aborted cypselas observed were very low, indicating that *S. coincyi* produces a large amount of ripe cypselas per year. For all subpopulations sampled, no significant ($P > 0.05$) differences in the number of cypselas per fruit head were found among individuals within a subpopulation. Similarly, no significant ($P > 0.05$) differences in the number of cypselas per fruit head were found among subpopulations.

### Number of fruit heads per individual plant

The mean number of fruit heads per individual was 13 (3 minimum, 44 maximum) for 127 individuals sampled belonging to five subpopulations (Table 4). Significant ($P = 0.0008$) differences in the number of fruit heads per individual plant were found among subpopulations. However, no significant ($P > 0.05$) differences were found among individuals within a subpopulation.

### Effect of incubation temperatures

The effect of incubation temperature on the germination of cypselas from subpopulations HER2 and NAV2 is shown in Table 7. Significant ($P < 0.05$) differences were found among temperature regimes assayed for the final germination percentages reached by cypselas belonging to HER2. The highest germination percentage (100%) was reached at 25°C and the lowest (19%) at 10°C. However, when the lowest temperature was not considered, no significant ($P > 0.05$) differences were found among the other three temperatures. Similarly, no significant ($P > 0.05$) differences were found among the three temperatures assayed for NAV2. MGT values showed significant ($P < 0.05$) differences for HER2, and no significant differences were found for NAV2. The cypselas of HER2 germinated faster at 25°C, 20°C and 25/15°C than at 15°C and 30°C. Germination was significantly delayed at the lowest temperature assayed (10°C). Both subpopulations exhibited very high germination percentages (90–100%) and high rates of germination (5.87–7.96 days) in all incubation temperatures tested (except at 10°C). Cypselas from HER2 reached lower MGT values at 20°C, 25°C and 25/15°C than did those from NAV2.

### Effect of light conditions

Final germination percentages reached by cypselas belonging to the SAN subpopulation under photoperiod and complete darkness were very similar (94% and 91%, respectively). Germination percentage was not significantly ($P = 0.8448$) affected by light conditions. However, significant ($P = 0.0108$)

**Table 7. Effect of different temperature regimes on the final germination percentage (mean ±s.e.) and mean ±s.e. germination time (MGT) of *Senecio coincyi* cypselas belonging to two subpopulations**

The number of empty cypselas in each replicate was not taken into account for calculating the final germination percentage. Results after 20 days of incubation (40 days at 10°C) under a 16-h light photoperiod. For each subpopulation, means within a column followed by the same letter are not significantly different according to the l.s.d. test at 0.05 level

| Subpopulation code | Incubation temperature (°C) | Germination (%) | MGT (days) |
|---|---|---|---|
| HER2 | 10 | 19 ± 3.79a | 13.45 ± 0.26c |
| | 15 | 98 ± 2.00b | 7.96 ± 0.14b |
| | 20 | 98 ± 1.18b | 6.11 ± 0.10a |
| | 25 | 100b | 5.87 ± 0.10a |
| | 30 | 92 ± 3.90b | 6.68 ± 0.44a |
| | 25/15 | 98 ± 1.20b | 6.10 ± 0.04a |
| NAV2 | 20 | 98 ± 1.00a | 7 0.77 ± 0.32a |
| | 25 | 90 ± 3.75a | 7.12 ± 0.44a |
| | 25/15 | 91 ± 5.92a | 7.55 ± 0.43a |

differences were found between photoperiod and darkness for the MGT values. Cypselas reached lower MGT values under a 16-h light photoperiod (8.82 days) than under constant darkness (9.99 days).

### *Intraspecific variation in cypsela germination*

Germination of cypselas belonging to six subpopulations is shown in Table 5. Significant differences ($P < 0.05$) were found among subpopulations for the final germination percentages (from 88% to 99%) and MGT values (from 6.93 to 8.91 days). Similarly, the number of empty cypselas varied significantly among subpopulations (from 0% to 42%).

Germination of cypselas belonging to different individuals of the subpopulations HER2 and SAN are shown in Table 8. In both subpopulations, the number of empty cypselas varied significantly ($P = 0.0004$ and $P = 0.0094$, respectively) among individual plants (from 0% to 46% for HER2 and 0% to 44% for SAN). Similarly, MGT values showed significant ($P = 0.0001$ for both subpopulations) differences. However, in every population no significant ($P = 0.0747$ and $P = 0.2821$, respectively) differences were found among individuals for the final germination percentages (from 84% to 100% for HER2 and from 90% to 100% for SAN).

**Table 8. Final germination percentage (mean ±s.e.) and mean ±s.e. germination time (MGT) of *Senecio coincyi* cypselas belonging to 13 individual plants from the subpopulation HER2 and 12 plants from the subpopulation SAN**

The number of empty cypselas in each replicate was excluded for calculating the final germination percentage. Results after 20 days of incubation under a 16-h light photoperiod at 25/15°C. Significant *P*-values are given in bold

| Subpopulation code | Empty cypselas (%) | Germination (%) | MGT (days) |
|---|---|---|---|
| HER2 | 0 | 100 | 10.30 ± 0.26 |
| | 46 ± 2.00 | 96 ± 3.85 | 10.50 ± 0.50 |
| | 0 | 98 ± 2.00 | 6.57 ± 0.19 |
| | 8 ± 0.00 | 98 ± 2.17 | 10.23 ± 0.23 |
| | 0 | 100 | 10.54 ± 0.46 |
| | 4 ± 2.83 | 100 | 8.48 ± 0.44 |
| | 2 ± 1.41 | 100 | 8.08 ± 0.24 |
| | 20 ± 5.66 | 84 ± 10.86 | 10.37 ± 0.81 |
| | 0 | 100 | 7.54 ± 0.00 |
| | 14 ± 7.07 | 100 | 9.65 ± 0.03 |
| | 4 ± 2.83 | 94 ± 2.35 | 9.35 ± 0.73 |
| | 18 ± 1.41 | 95 ± 5.00 | 8.08 ± 0.08 |
| | 14 ± 1.41 | 91 ± 4.44 | 10.39 ± 0.39 |
| *P*-value | **0.0004** | 0.0747 | **0.0001** |
| SAN | 44 ± 5.66 | 90 ± 10.00 | 7.87 ± 0.37 |
| | 8 ± 2.83 | 100 | 8.02 ± 0.48 |
| | 2 ± 1.41 | 94 ± 2.35 | 9.71 ± 0.05 |
| | 0 | 96 ± 0.00 | 10.46 ± 0.62 |
| | 0 | 98 ± 0.00 | 7.60 ± 0.15 |
| | 2 ± 1.41 | 100 | 7.65 ± 0.31 |
| | 10 ± 4.24 | 100 | 7.68 ± 0.09 |
| | 10 ± 6.00 | 98 ± 2.17 | 8.93 ± 0.12 |
| | 2 ± 1.41 | 100 | 7.89 ± 0.11 |
| | 2 ± 1.41 | 98 ± 2.17 | 8.14 ± 0.28 |
| | 0 | 100 | 6.50 ± 0.10 |
| | 8 ± 5.66 | 95 ± 0.11 | 9.75 ± 0.84 |
| *P*-value | **0.0094** | 0.2821 | **0.0001** |

Table 9 shows the germination characteristics of cypselas belonging to different fruit heads of several individuals from the subpopulations PEV and HER2. No significant differences were found in most cases studied. For only one individual plant of PEV, the final germination percentage varied significantly ($P = 0.0001$) among fruit heads. Similarly, significant ($P = 0.0219$) differences were found among the MGT values reached by cypselas of single heads from just one individual plant of this same population. The number of empty cypselas did not vary significantly ($P > 0.05$) among fruit heads from individuals of PEV, but significant ($P = 0.0492$ and $P = 0.0095$) differences were found for two individuals from HER2.

**Table 9. Final germination percentage (mean ±s.e.) and mean ±s.e. germination time (MGT) of *Senecio coincyi* cypselas belonging to a single fruit head**

Fruit heads (3–5) were taken from three individual plants of the subpopulation PEV and four plants of HER2. The number of empty cypselas in each replicate was not taken into account for calculating the final germination percentage. Results after 20 days of incubation under a 16-h light photoperiod at 25/15°C. Significant *P*-values are given in bold

| Subpopulation code | Individual plant | Fruit head | Empty cypselas (%) | Germination (%) | MGT (days) |
|---|---|---|---|---|---|
| PEV | 1 | 1 | 8 ± 2.83 | 89 ± 1.70 | 9.68 ± 0.18 |
| | | 2 | 2 ± 1.41 | 100 | 10.09 ± 0.37 |
| | | 3 | 6 ± 4.24 | 96 ± 0.27 | 9.97 ± 0.18 |
| | | 4 | 0 | 100 | 9.34 ± 0.02 |
| | | 5 | 16 ± 0.00 | 83 ± 2.38 | 9.17 ± 0.00 |
| *P*-value | | | 0.1551 | **0.0001** | 0.0847 |
| | 2 | 1 | 4 ± 0.00 | 96 ± 4.17 | 7.98 ± 0.44 |
| | | 2 | 0 | 92 ± 4.00 | 8.84 ± 0.43 |
| | | 3 | 0 | 100 | 9.98 ± 0.10 |
| | | 4 | 8 ± 2.83 | 98 ± 2.08 | 9.51 ± 0.10 |
| | | 5 | 4 ± 2.83 | 92 ± 0.35 | 9.66 ± 0.08 |
| *P*-value | | | 0.1384 | 0.2332 | **0.0219** |
| | 3 | 1 | 0 | 100 | 9.14 ± 0.90 |
| | | 2 | 2 ± 1.41 | 98 ± 2.08 | 9.88 ± 0.08 |
| | | 3 | 10 ± 4.24 | 93 ± 7.14 | 9.30 ± 0.09 |
| | | 4 | 0 | 98 ± 2.00 | 8.79 ± 0.09 |
| | | 5 | 0 | 94 ± 2.00 | 9.30 ± 0.12 |
| | | *P* | 0.0742 | 0.6035 | 0.5244 |
| HER2 | 1 | 1 | 2 ± 1.41 | 98 ± 2.00 | 7.71 ± 0.50 |
| | | 2 | 16 ± 8.48 | 100 | 7.75 ± 0.75 |
| | | 3 | 18 ± 4.24 | 95 ± 4.55 | 8.03 ± 0.33 |
| | | 4 | 6 ± 1.41 | 100 | 6.63 ± 0.38 |
| *P*-value | | | 0.2914 | 0.6021 | 0.3551 |
| | 2 | 1 | 20 ± 2.83 | 87 ± 8.15 | 10.08 ± 0.28 |
| | | 2 | 46 ± 9.90 | 95 ± 5.00 | 10.97 ± 0.14 |
| | | 3 | 4 ± 0.00 | 96 ± 0.00 | 10.57 ± 0.04 |
| *P*-value | | | **0.0492** | 0.5711 | 0.0877 |
| | 3 | 1 | 14 ± 1.41 | 100 | 11.81 ± 0.33 |
| | | 2 | 62 ± 1.41 | 94 ± 5.56 | 12.38 ± 0.13 |
| | | 3 | 16 ± 5.66 | 100 | 12.20 ± 0.36 |
| | | 4 | 38 ± 1.41 | 70 ± 23.54 | 12.03 ± 0.17 |
| *P*-value | | | **0.0095** | 0.2145 | 0.5417 |
| | 4 | 1 | 34 ± 10.00 | 92 ± 7.89 | 10.67 ± 0.11 |
| | | 2 | 32 ± 2.83 | 78 ± 22.22 | 11.60 ± 1.60 |
| | | 3 | 24 ± 15.56 | 93 ± 7.14 | 11.96 ± 0.29 |
| | | 4 | 6 ± 1.41 | 100 | 8.52 ± 0.65 |
| *P*-value | | | 0.3584 | 0.7491 | 0.1516 |

## Discussion

*Senecio coincyi* grows in a very small area within supra and oromediterraneous bioclimatic stages of the Sierras of Villafranca and Gredos. All subpopulations known are located in Ávila province (central-western Spain). The locations of the species can be grouped into five different habitat types, including edges of streams, hay meadows, peaty meadows, peat bogs and water pipes, indicating habitats that maintain a high level of humidity throughout the year (Martínez-García *et al.* 2008).

Results from the present study have highlighted the high reproductive capacity of *S. coincyi*, on the basis of a high production of fruit heads, flowers and cypselas. Their cypselas germinate readily at a wide temperature range, both under light and darkness. Multiplying the total number of individuals recorded in 2007 (25 613) by the approximate ratio of flowering individuals of this year (0.8) by the mean number of fruit heads per individual plant (13) by the mean number of cypselas per fruit head (165), we get an output of more than 40 million cypselas at fruiting time.

Very high germination percentages were reached under laboratory conditions, even higher than those reached by other species of the genus *Senecio* that also grow in mountain habitats, such as *S. integrifolius* (Widén 1987), *S. boissieri* and *S. pyrenaicus* (Giménez-Benavides *et al.* 2005). Moreover, the ability to germinate over a wide range of temperatures frequently occurs in many *Asteraceae* species (Baskin and Baskin 1998; Schütz *et al.* 2002). Cypselas reached germination percentages (≥90%) at temperatures between 15°C and 30°C. However, final germination percentage was <20% at 10°C. Light was not a crucial factor for germination of this species. The incapacity to germinate in darkness is a trait generally associated with the ability to form a soil seed bank especially of small seeds (Pons 1991). The high germination percentage reached by *S. coincyi* cypselas under darkness indicated that this species may not form a persistent soil seed bank. *S. coincyi* cypselas showed opportunistic germination behaviour and they were able to germinate under a wide range of temperature and light conditions. The evident lack of seed dormancy showed by *S. coincyi* could be a constraint for the maintenance of some populations of this species over time.

Soil type and edaphic moisture are parameters that remain constant throughout the year in the habitats where the species grows. Therefore, the main environmental factor limiting cypsela germination and subsequent seedling survival under natural conditions would be low temperatures (<10°C). *S. coincyi* cypselas readily germinated in all temperatures tested (except at 10°C), as reported, similarly, for many temperate climate species around the world (Baskin and Baskin 1998; Vandelook and Van Assche 2008). Because mountain ecosystems are typically subjected to a short growing season, this strategy could ensure enough time for seedling establishment (Chambers *et al.* 1987; Körner 1999; Grime 2001).

Assuming 20% empty cypselas and a mean germination of 85% for viable cypselas (at temperatures higher than 10°C), from those 40 million cypselas produced in a year, nearly 30 million will germinate and produce seedlings. These figures, although approximate and subject to possible wide margins of error, would indicate that if only the reproductive characteristics of this species had been taken into account, the total number of individuals would be much closer to 30 million than the 30 000 individuals recorded in 2007.

The results of population demography are not very encouraging in contrast to the very positive results concerning reproductive biology. Altitude, precipitation and temperature conditions, and, above all, high soil moisture needed for germination and normal development of *S. coincyi* occur in a high number of potential sites that, taken together, could add a much larger area than actually occupied by this species. We have witnessed the disappearance of two populations of this species located in Ávila and Zamora provinces (central-western Spain). These two populations were present two decades ago (García López and Roa Medina 1988; Luceño and Vargas 1991). Beside, its extent of occurrence represents a moderate fragmentation and some populations have a very small number of individuals, which could make them demographically unviable in a short time. Specific studies to verify the percentage of seedlings attacked by fungi, eaten by animals, damaged by early or late frost, or victims of strong competition for resources with other plant species, should be carried out to better understand the regeneration capacity of the species.

Pyrrolizidine alkaloids are the most characteristic secondary metabolites present in the genus *Senecio* and several of these compounds are hepatotoxic to mammals (Mattocks 1989; Hartmann and Witte 1995). Suau *et al.* (2002) found a high alkaloid content in three Spanish *Senecio* species (*S. elodes*, *S. granatensis* and *S. malacitanus*). These toxic compounds could also be present in aerial parts of *S. coincyi*, so that livestock avoid eating its plants. Therefore, the danger of livestock to this species does not seem to be the result of direct consumption of plants or parts of them, but rather of ecological disturbance caused by livestock on habitats where *S. coincyi* grows. Livestock trampling would favour the development of caespitose species with vegetative reproduction, such as grasses of genus *Festuca* or *Nardus stricta*, instead of sexually reproducing species, such as *S. coincyi*. This replacement of species typical of peat meadows by caespitose species is happening throughout many occurrences of *S. coincyi* and could explain, at least in part, the absence of this species at sites that would satisfy their ecological requirements (humidity, altitude, temperature). Because of this, the species grows mainly at sites with difficult or impossible access for cattle.

*Senecio coincyi* is a species linked to permanently wet soils, so a little change in the hydrological regime of streams where it grows could be detrimental for its survival. More than half of the area of occupancy of this species is represented by edges of streams, and this implies that any change involving a reduction or elimination of the flow of these waterways is a serious threat to the conservation of *S. coincyi*.

*Senecio coincyi* was catalogued as *Vulnerable* (VU) species in the 2008 Red List (Martínez-García 2008). Although *S. coincyi* is not in danger of imminent extinction, we consider it necessary to implement, as soon as possible, several management measures for conservation of this species. In June 2007, the Official Gazette of Autonomous Community of Castile and León (Spain) published the List of Protected Flora of Castile and Leon and the legal form of protection called 'Flora Microreserves'. *S. coincyi* has the highest degree of protection that this List provides, being included in the '*In danger of extinction*' category. We propose the inclusion of a part of the subpopulation HER2 area in the legal figure of 'Flora Microreserves'. This is the largest known subpopulation of the species, with more than 10 000 individuals, representing ~34% of all individuals of this taxon. Therefore, if this site was included as microreserve, it would be preserving a high percentage of the total individuals of *S. coincyi*.

Moreover, *ex situ* conservation of *S. coincyi* cypselas could be very effective for preservation of this species. The long-term storage of orthodox seeds is the most widely used method for *ex situ* preservation of plant genetic resources. Therefore, *S. coincyi* cypselas collected from all subpopulations were stored in the Plant Germplasm Bank of the Universidad Politécnica de Madrid (UPM) at temperature between −5°C and −10°C in flame vials containing dehydrated silica gel. This preservation method based on silica gel (ultra-dry storage) and low temperatures has proved highly effective for long-term storage of orthodox seeds (see e.g. Pérez-García *et al.* 2009; González-Benito *et al.* 2011). Data on the germination conditions of *S. coincyi* could be used for *ex situ* conservation management of this threatened species.

In conclusion, *S. coincyi* is a narrow and threatened endemic species for which conservation has become a necessary strategy. The results in the present study highlight the reproductive success of this species, and therefore, show that its rarity is not due to factors related to its reproductive biology *per se*. However, alteration of its habitats caused by the continuous and intense presence of livestock is a major problem. Moreover, any changes in hydrologic regimes might be a problem because the species requires relatively wet habitats. This is the first report on germination behaviour of *S. coincyi* cypselas.

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