
EXPERIMENTAL EVIDENCE
OF POTENTIAL FOR
PERSISTENT SEED BANK
FORMATION AT A
SUBANTARCTIC ALPINE SITE
IN TIERRA DEL FUEGO,
CHILE¹

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ABSTRACT

Seed burial experiments were conducted for 15 alpine species derived from 15 genera and 12 plant families from a subantarctic location on Cerro Riñón, Tierra del Fuego, 54°S, Chile. Batches of seed buried at 5 cm depth at 550 m elevation in February 1996 were exhumed after 335, 363, and 755 days, and their status and viability determined. Thirteen species (87%), one marginally (*Acaena magellanica*, *Anemone multifida*, *Azorella lycopodioides*, *Bolax gum-mifera*, *Calceolaria biflora*, *Caltha appendiculata*, *Draba magellanica*, *Empetrum rubrum*, *Gentianella magellanica*, *Gunnera magellanica*, *Luzula alopecurus*, *Oreomyrrhis hookeri* (marginal), *Pernettya pumila*), were judged as having potential to form a persistent soil seed bank, while two (*Senecio magellanicus*, *Berberis buxifolia*) only showed evidence of a transient seed bank. Seed viability in residual intact seeds was high and showed little tendency to diminish over the two-year experimental period, indicating optimal conditions for seed survival in the alpine soil. Fits of the number of remaining viable seeds at each exhumation date to the negative exponential model gave maximum seed longevity estimates of 1623 days in *Draba magellanica*, 2779 days in *Empetrum rubrum*, 3026 days in *Gunnera magellanica*, 777 days in *Oreomyrrhis hookeri*, and 5983 days in *Pernettya pumila*. Estimated seed longevities for some species at the subantarctic alpine site are among the highest reported for alpine species to date.

Key words: Chile, persistent seed bank, seed burial experiments, seed viability, Tierra del Fuego, transient seed bank.

Examination of soil cores for viable and/or germinable seeds has revealed the existence of extensive soil seed banks in arctic and alpine vegetation at several Northern Hemisphere locations (e.g., McGraw & Vavrek, 1989, and references therein; Ebersole, 1989; Hatt, 1991; Ingersoll & Wilson, 1993; Chambers, 1993, 1995; Diemer & Prock, 1993; Molau & Larsson, 2000). McGraw and Vavrek (1989) estimated that up to one half or more of the species present in the standing vegetation in alpine and arctic sites could be found in the seed bank.

Although the study of soil cores has been useful in ascertaining that sexual reproduction is a common feature in the cold and harsh alpine and arctic habitats, few studies have been carried out in such a way as to be able to distinguish clearly between the transient and persistent components of the soil

seed bank (e.g., Roach, 1983; Ebersole, 1989; Ingersoll & Wilson, 1993). The soil core method has an additional disadvantage. Because soil cores contain seeds produced over a number of years, even with careful sampling so as to exclude seeds corresponding to the present year's seed rain, very little can be said about the longevity of seeds beyond one year. Longevity of seeds in the soil constitutes the vital information required for understanding the relevance of a persistent seed bank in the context of life-history theory (Brown & Venable, 1986; Rees, 1996), and for the successful restoration of degraded areas in many alpine and arctic sites (e.g., Urbanska, 1997).

An alternative approach is to experimentally bury batches of seeds of known age in the soil, retrieving and examining samples for viability and/or germinability at a series of programmed future

¹ Research supported by CONICYT Grant No. 1950461 and Grant P99-103 F ICM supporting the Centre for Advanced Studies in Ecology and Research in Biodiversity. Fieldwork in Tierra del Fuego was conducted on the private property of Forestal Trillium Ltda., to whom we express gratitude for logistic support. We are grateful to Manuel Arroyo Kalin for collaboration with collecting and preparing seeds and digging the burial sites.

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dates (Baskin & Baskin, 1998). This method has been employed for single or small numbers of species from several habitats (e.g., grassland: Pavone & Reader, 1982; tropical: Lonsdale et al., 1988; sand dunes: Zhang & Maun, 1994) including high mountains (Guariguata & Azocar, 1988; Spence, 1990; Cavieres & Arroyo, 2001; Schwienbacher & Erschbamer, 2001). Nevertheless, the majority of these studies have been focused on single species. For example, Guariguata and Azocar (1988) buried seeds of the long-lived, dominant paramo species *Espeletia timotensis* Cuatrec. (Asteraceae) in its natural habitat for a period of one year. Spence (1990) performed a seed burial experiment of just over one year's duration on *Chionochoa macra* Zotov (Gramineae), dominant in the low alpine region in the Craigieburn Range in New Zealand. Cavieres and Arroyo (2001) performed a seed burial experiment of 3 years' duration with the perennial herb *Phacelia secunda* J. F. Gmel. at different elevations in the Andes of central Chile. Only Schwienbacher and Erschbamer (2001) have experimentally evaluated the persistence of seeds in the soil for nine species in the Austrian Alps at 2400 m a.s.l. However, they evaluated the fraction of seeds that remain viable in the soil only after eight months' burial, reporting that six species showed high percentages of seeds that remain ungerminated; in only three of those species was there certainty that the remaining ungerminated seeds were viable.

In this paper, we report the results of seed burial experiments of 2 years' duration carried out at a subantarctic alpine location in Tierra del Fuego (54°S), Chile, in order to detect the potential for persistent seed bank formation. Persistent seed banks, by definition (Thompson et al., 1998), are those in which a fraction of the seeds of a species not only remain in the soil, but are also viable for at least one year after production. First, we determine whether 15 alpine species individually exhibit potential to form a persistent seed bank. Second, we attempt to fit mathematical functions to our seed burial results in order to estimate the length of time seeds of the species studied could potentially remain viable in the soil. Third, we assessed whether the persistence of seeds in the soil is related with other characteristics of the seed such as the size and shape, as it has been shown in other ecosystems (e.g., Thompson et al., 1993).

MATERIALS AND METHODS

STUDY SITE

Work was conducted in the alpine belt immediately above the *Nothofagus pumilio* (Poepp. &

Endl.) Krasser tree line lying at 450–500 m elevation, on Cerro Riñón (54°S), located in the Patagonian Andean complex on the edge of the forest-steppe boundary in Tierra del Fuego (Fig. 1). As is found on inland mountains throughout Tierra del Fuego, the dominant species are cushion plants (*Bolax gummifera* (Lam.) Spreng. (Apiaceae), *Azorella lycopodioides* Gaud. (Apiaceae)) and the prostrate shrub *Empetrum rubrum* Vahl ex Willd. (Empetraceae), these being accompanied by many caespitose and rosette perennial herbs and slender grasses (Moore, 1983; Arroyo et al., 1996; Mark et al., 2001). Flowering in the eastern mountains of Tierra del Fuego occurs from close to snow melt in early October through to early March. Although not quantified, peak fruiting occurs in the interval of February to early March.

Available climatic data for the general area of Cerro Riñón are limited. Annual precipitation on the forest-steppe boundary, based on a climatic station at Cameron (53°40'S, 69°53'W), is 503 mm with more than 50% of precipitation falling in summer. In the windy, arid climate of inland Tierra del Fuego (based on field observations from 1995, 1996, and 1997), permanent snow was present above the tree line on Cerro Riñón every year for five months (May through September). Mean annual temperature at Pampa Guanaco (200 m a.s.l.) located some 19 km west of Cerro Riñón is 2.7°C, while mean monthly temperature during the late spring to late summer (October–April) is ca. 6.0°C.

SEED SOURCES

Bulk seed collections were made on Cerro Riñón during March 1996. Species included perennial herbs, cushion species, shrubs, and one annual-biennial herb. The choice of species was made so as to maximize the taxonomic coverage of a manageable number of species, while at the same time representing a mixture of dominant and subdominant taxa. Mature seeds at the point of being dispersed or seeds recently dispersed around a mother plant were collected from some 30 or more individuals. When we collected seeds on the soil surface or lodged within the foliage of a mother plant, we made a special effort to distinguish between the current and earlier years' seeds. The field seed collections were carefully checked under a binocular microscope for the presence of any aborted and immature seeds, the latter being discarded. Adequate amounts of filled seed were amassed for 15 species (Table 1). For each species, 30 seeds were randomly selected and measured under the binocular microscope in order to obtain their lengths and

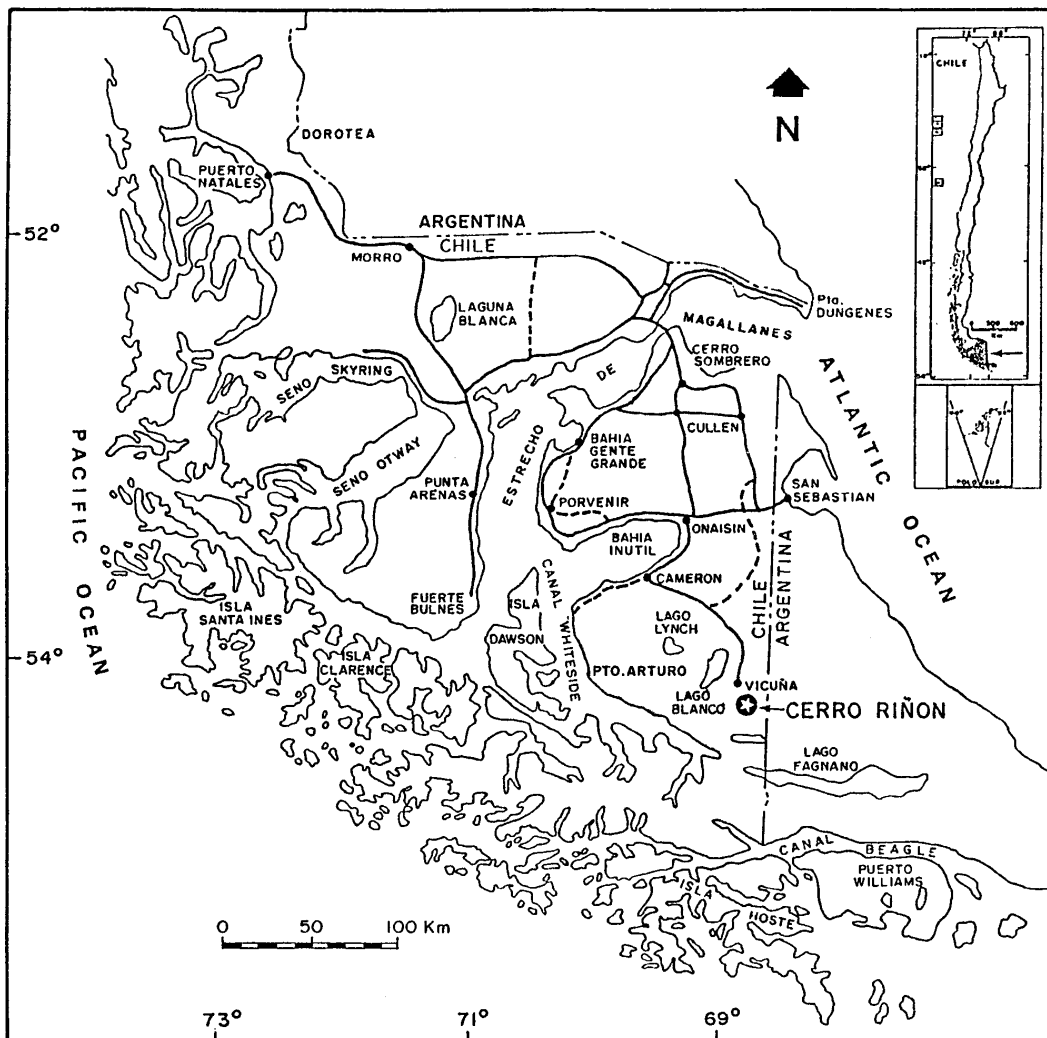


Figure 1. Location of Cerro Riñón in central Tierra del Fuego, Chile.

widths; after that seeds were weighed in a digital balance (OHAUS). In species with extremely small seeds (e.g., *Calceolaria biflora*), these were weighed in groups of 20 seeds each.

Within 1 to 2 days of collection in the field, 50 seeds of each species were subjected to the Tetrazolium chloride test (Moore, 1973) in order to assess their initial viability.

Over the period 12–14 March 1996 three sets of six batches per species of 30–39 seeds (total of 18 batches per species) were counted out and introduced into 10 × 10 cm nylon mesh envelopes of three sizes, electing a smaller mesh size than the introduced seeds. The envelopes containing the seeds for each of the 15 species were separated into three sets, each of six replicates. For every set of

species in a given replicate, the species was assigned a number between 1 and 15 randomly, for later positioning in the buried seed cages.

SEED BURIAL

On 15 March 1996 seeds were transported back to the field to a burial location on a southeast-facing slope at 550 m, where six randomly placed replicate burial sites within an area of approximately 50 × 50 m were previously established. The general burial area, which was flatter than the surrounding slopes on average, was dominated by *Bolax gumifera* and *Azorella lycopodioides* with some lichen and moss growth at the soil surface, good soil development, and moderate drainage. At each of the

Table 1. Life-form and seed characteristics of the species studied in assessment of potential persistent seed bank formation in the alpine zone on Cerro Riñón, Tierra del Fuego. A-B = annual to biennial herb; PH = perennial herb; CU = cushion-forming perennial herb; SH = shrub.

Species	Family	Life-form	Weight (mg)	Length (mm)	Width (mm)	Seed shape
<i>Acaena ovalifolia</i> Ruiz & Pav.	Rosaceae	PH	1.49	3.7	1.7	cupule obconical
<i>Anemone multifida</i> Poir.	Ranunculaceae	PH	1.34	4.0	2.0	achene asymmetrically ovate
<i>Azorella lycopodioides</i> Gaud.	Apiaceae	CU	0.60	1.7	2.0	ovoid
<i>Berberis buxifolia</i> Lam.	Berberidaceae	SH	6.33	5.0	2.0	lunate, smooth
<i>Bolax gummifera</i> (Lam.) Spreng.	Apiaceae	CU	1.29	3.0	2.0	ovoid
<i>Calceolaria biflora</i> Lam.	Scrophulariaceae	PH	0.03	0.7	0.3	navicular, reticulate
<i>Caltha appendiculata</i> Pers.	Ranunculaceae	PH	0.33	1.7	0.9	ovoid, ellipsoid
<i>Draba magellanica</i> Lam.	Cruciferae	PH	0.15	1.0	0.8	ovoid, smooth
<i>Empetrum rubrum</i> Vahl ex Willd.	Empetraceae	SH	0.25	1.8	1.2	ovoid
<i>Gentianella magellanica</i> (Gaud.) Fabris ex D. M. Moore	Gentianaceae	A-B	0.15	0.8	0.5	ovoid
<i>Gunnera magellanica</i> Lam.	Gunneraceae	PH				ovoid
<i>Luzula alopecurus</i> A. N. Desv.	Juncaceae	PH	0.25	1.3	0.7	cylindrical
<i>Oreomyrrhis hookeri</i> Mathias & Const.	Apiaceae	PH	0.87	3.5	1.5	oblong
<i>Pernettya pumila</i> (L.f.) Hook.	Eriacaceae	SH	0.11	1.0	0.7	ovoid
<i>Senecio magellanicus</i> Hook. & Am.	Asteraceae	PH	1.35	4.0	2.0	achene cylindrical

six replicate sites, three rectangular areas separated by 5–10 cm of untouched turf were excavated to allow for the introduction of three wire cages (70 cm long \times 40 cm wide \times 5 cm tall). A layer of sieved soil was placed in the open cages after they had been introduced into the burial sites. One envelope of seeds per each of the 15 species was placed over the sieved soil, respecting the previously established randomized order. The seed envelopes were covered with additional sieved soil, and the cages carefully adjusted in the ground such that the envelopes lay 5 cm below the soil surface. Roach (1983) found that the great majority of the seeds fail to penetrate further than 5 cm depth in soil of arctic and alpine sites. The cages were wired closed and covered with additional sieved soil and the original excavated turf, which had been carved thinner so as to lie at the level of the surrounding vegetation. The wire cages served to prevent any downslope movement of the seed envelopes and as deterrents for the small burrowing mammal *Ctenomys magellanicus*, which has been observed by us to occur above tree line on nearby mountains in Tierra del Fuego. The position of each replicate burial site was marked, and the three cages per each of the six burial sites identified as to number. We randomly assigned positions 1–3 to each of the three cages at each of the six sites for later exhumation on three separate dates.

RETRIEVAL OF BURIED SEEDS

In accordance with the previously randomized order for retrieval, at each replicate site a set of six cages was exhumed on the following dates: (1) 14 February 1997; (2) 14 March 1997; (3) 10 April 1998. The first exhumation date was planned so as to be fairly sure that any current year's seed germination taking place after the winter period would have already occurred. The second date was planned toward the end of the summer–autumn season so as to assess the viability of any remaining seeds after one year's burial and to assess any late summer seed mortality. The third date was planned so as to allow the seeds to remain in the soil for at least 2 full calendar years, thus allowing us to judge the potential for the formation of a persistent seed bank. Remaining intact ungerminated seeds retrieved on each of the exhumation dates were subjected to the Tetrazolium chloride test, using the same protocol as outlined earlier so as to determine the seed viability.

DATA ANALYSIS

The recovered intact seeds demonstrated as viable on the basis of the Tetrazolium test, expressed

as a percentage of initial buried seed number, provided a measure of the “potential” persistent seed bank (Zhang & Maun, 1994). To assess loss of total viable seeds over time (and hence depletion of the potential soil seed bank) and estimate maximum seed ages, we used the negative exponential model (Guariguata & Azocar, 1988; Lonsdale et al., 1988), linearized for statistical application: $\ln N_t = \ln N_0 - kt$, where N_t = mean number of viable seeds at time t days; N_0 = mean initial number of viable seeds. The accepted significant level for the linear regressions was $P < 0.05$. The age of the last viable seed for a species was determined by the equation $t = \ln N_0/k$.

Relationships between seed persistence and seed size and shape were assessed through linear regressions between the percentage of ungerminated seeds after 2 years’ burial (arcsin transformed for normalization) with the seed weight (log transformed) and the quotient width/length as suggested in Schwienbacher and Erschbamer (2001).

RESULTS

Seeds of most of the study species were very small. Seed size ranged from 1.3 mg weight, 4 mm long, and 2 mm wide in *Senecio magellanicus*, to 0.03 mg, 0.7 mm long, and 0.3 mm wide in *Calceolaria biflora* (Table 1). The most common seed shape was ovoid, followed by cylindrical achene (Table 1).

The percentage of viable seeds in relation to initial buried seed number (Table 2) reveals a wide range of situations among the 15 species studied after being buried 2 years. By the end of the 2-year period, not a single seed of *Acaena ovalifolia*, *Calceolaria biflora*, *Caltha appendiculata*, *Luzula alopecurus*, or *Azorella lycopodioides* had germinated or decayed. For these same species, terminal seed viability as deduced from the Tetrazolium chloride test was over 80%, and indeed close to 100% in a number of cases (Table 2). Similar, but less rigid, behavior was seen in *Anemone multifida* and *Gentianella magellanica*, where a small percentage of the buried seeds had either germinated or were decayed, with again a very high percentage of the remaining ungerminated seed fraction remaining viable (Table 2). For a third group of species, composed of *Gunnera magellanica* and *Pernettya pumila*, around half of the seeds had germinated after the 2-year burial period. However, remaining seeds continued to exhibit high viability. In three species (*Bolax gummifera*, *Draba magellanica*, and *Empetrum rubrum*) around 2/3 to 3/4 of the seeds had exited the seed bank through germination or other causes

Table 2. Status of exhumed seeds (mean ± SE) in the alpine zone on Cerro Riñón 335, 363, and 755 days after burial date. N = 6 replicates.

Species	Buried 335 days		Buried 363 days		Buried 755 days	
	Ungerminated (% intact seeds)	Viable seeds (as % of intact seeds)	Ungerminated (% intact seeds)	Viable seeds (as % of intact seeds)	Ungerminated (% intact seeds)	Viable seeds (as % of intact seeds)
<i>Acaena ovalifolia</i>	100 ± 0	98.9 ± 1.0	100 ± 0	78.3 ± 6.6	100 ± 0	87.8 ± 3.4
<i>Anemone multifida</i>	100 ± 0	90.6 ± 5.9	93.3 ± 4.4	77.2 ± 5.9	96.7 ± 1.4	95.8 ± 3.8
<i>Azorella lycopodioides</i>	100 ± 0	78.5 ± 13.2	100 ± 0	74.7 ± 5.9	100 ± 0	81.2 ± 2.8
<i>Berberis buxifolia</i>	10.0 ± 7.5	83.3 ± 6.8	0 ± 0	—	0 ± 0	—
<i>Bolax gummifera</i>	100 ± 0	95.4 ± 2.0	56.0 ± 12.1	81.9 ± 8.7	37.5 ± 4.8	57.6 ± 11.9
<i>Calceolaria biflora</i>	100.0 ± 0	79.6 ± 6.7	100 ± 0	81.7 ± 5.9	100 ± 0	98.9 ± 0.6
<i>Caltha appendiculata</i>	100 ± 0	82.3 ± 6.0	98.9 ± 1.0	9.1 ± 4.5	100 ± 0	96.8 ± 1.3
<i>Draba magellanica</i>	60.2 ± 12.9	100 ± 0	44.6 ± 4.7	98.6 ± 1.3	27.4 ± 2.5	57.8 ± 12.9
<i>Empetrum rubrum</i>	74.4 ± 11.1	95.1 ± 2.9	85.0 ± 4.7	94.0 ± 2.5	36.8 ± 2.5	98.7 ± 1.2
<i>Gentianella magellanica</i>	93.9 ± 3.1	53.2 ± 7.2	88.2 ± 2.1	57.1 ± 8.3	89.9 ± 2.3	94.3 ± 2.6
<i>Gunnera magellanica</i>	100 ± 0	76.5 ± 8.3	93.6 ± 5.9	77.8 ± 8.5	50.9 ± 8.3	78.5 ± 9.0
<i>Luzula alopecurus</i>	100 ± 0	100 ± 0	100 ± 0	45.0 ± 9.4	100 ± 0	98.9 ± 1.0
<i>Oreomyrhis hookeri</i>	32.8 ± 5.7	73.8 ± 6.6	25.3 ± 3.6	98.2 ± 1.7	0 ± 0	—
<i>Pernettya pumila</i>	92.7 ± 6.7	96.4 ± 1.2	100 ± 0	77.8 ± 8.5	46.9 ± 16.8	93.7 ± 3.1
<i>Senecio magellanicus</i>	15.4 ± 5.8	53.7 ± 16.3	0 ± 0	—	0 ± 0	—

Table 3. Potential to form a persistent seed bank in the subantarctic alpine zone, Tierra del Fuego, 54°S, as adduced from seed burial experiments. Species are arranged according to their potential to form a persistent seed bank versus a transient seed bank. Species showing potential to form a persistent seed bank are arranged along a gradient from long to short persistent seed banks.

Long	⇐ Persistent seed banks ⇒	Short	Transient seed banks
<i>Acaena ovalifolia</i>	<i>Anemone multifida</i>	<i>Bolax gummifera</i>	<i>Berberis buxifolia</i>
<i>Calceolaria biflora</i>	<i>Gentianella magellanica</i>	<i>Draba magellanica</i>	<i>Senecio magellanicus</i>
<i>Caltha appendiculata</i>	<i>Pernettya pumila</i>	<i>Empetrum rubrum</i>	
<i>Luzula alopecurus</i>			<i>Oreomyrrhis hookeri</i>
<i>Azorella lycopodioides</i>			

Table 4. Significant adjustment ($P < 0.05$) to the negative exponential model for loss of viable seed with time in species from subantarctic alpine zone in Tierra del Fuego, 54°S.

Species	R ²	Predicted maximum seed longevity (days)
<i>Draba magellanica</i>	0.874	1623
<i>Empetrum rubrum</i>	0.881	2779
<i>Gunnera magellanica</i>	0.932	3026
<i>Oreomyrrhis hookeri</i>	0.923	777
<i>Pernettya pumila</i>	0.890	5983

within 2 years, with less than 1/3 of the original seed number remaining viable. In the remaining species (*Oreomyrrhis hookeri*, *Berberis buxifolia*, and *Senecio magellanicus*) no seeds remained intact and viable after 2 years. In *O. hookeri*, although a fraction of the seed (25%) had not germinated and remained highly viable at the end of the first summer season, none remained in this state by the end of the second autumn. In *Berberis buxifolia* and *Senecio magellanicus* practically all seeds had germinated or had decayed by late summer the first year, and all had germinated or decayed by the time a full calendar year had expired. Summarizing these results (Table 3), 13 species (87%) on the subarctic alpine site showed some potential to form a persistent seed bank, with seven showing no decay on seed viability after 2 years' burial.

Table 4 shows the species with viable seeds after 2 years' burial in which there was a statistically significant fit for number of viable seeds over time with the negative exponential models. We only obtained significant fits for five species, where estimates of the longevity of seeds buried for these species revealed a range from 16.4 years (*Pernettya pumila*) to 4.2 years (*Draba magellanica*). We failed to obtain significant fits in other species because percent viability in residual physically intact seeds among the three exhumation dates showed that there were statistically higher terminal viability (755 days' burial) in relation to seeds exhumed in early autumn the previous year (363 days' burial). This was the case of *Gentianella magellanica*, *Anemone multifida*, *Caltha appendiculata*, and *Luzula alopecurus*. Such a result could accrue if physically less apt (more prone to loss of viability) seeds were more abundant among the intact seeds at the time of the earlier exhumation date and had been progressively weeded out with time. However, the percentage of seeds in the germinated and decayed category stayed relatively constant in these same species over time, such that there was little change

in the proportion of intact seeds over the 2-year period (Table 2). One-Way ANOVA performed with arcsin transformed for percentage of seed remaining intact showed no significant differences in *A. multifida* ($F = 1.663$, NS), *C. appendiculata* ($F = 1.000$, NS), and *G. magellanica* ($F = 2.411$, NS) throughout the length of the experiment. A similar result was obtained in *O. hookeri* ($t = 0.9204$, NS). The percentage of intact seeds in *L. alopecurus* remained at 100% over the entire experimental period (Table 2).

Linear regressions showed that neither seed size nor seed shape were significantly related with the percentage of seeds that remained ungerminated after 2-year burial. That is, among the studied species, independent of the seed size and shape, a high percentage of seeds remained ungerminated in the soil after 2 years' burial.

DISCUSSION

As far as we are aware, our study would appear to constitute the first experimental seed burial study for an alpine habitat in which a taxonomically diverse set of species (representing 15 genera and 12 families) has been studied simultaneously and under identical conditions for more than one year. Including the marginally persistent seed bank species, *Oreomyrrhis hookeri*, we have found that 87% of the species studied showed potential to form a persistent seed bank because they maintained an important fraction of viable ungerminated seed after 2 years' burial. If we consider that the total number of species just above tree line in the alpine region of Tierra del Fuego is ca. 25–30 species (Mark et al., 2001), according to our results at least 50% have the potential to form a persistent seed bank. Thus, considering the definition of persistent seed bank, our results suggest a fairly generalized potential for persistent seed bank formation in the subantarctic alpine habitat, with significant seed longevity in a number of species.

Results obtained for alpine and arctic habitats with the use of the soil core method have found a variety of results in terms of percentage of species present in the persistent component of the seed banks. For instance, Roach (1983) found 5 out of 6, and 13 out of 35 species represented in the standing vegetation to be present in the persistent seed bank at two tundra sites in the Brooks Range, 790 m in Alaska (68°20'N). Ebersole (1989) studied four tundra communities at Oumalik (69°N) on the edge of Alaska's Arctic Coastal Plain, and registered persistent seed banks for 37–58% of the species in the surrounding vegetation. Ingersoll and

Wilson (1993) studied a treeless high subalpine site on Mount Jefferson (44°45'N) in Oregon, reporting a persistent seed bank comprised of 12 (55%) of the 22 standing vegetation species. Arroyo et al. (1999) detected persistent soil seeds representing 31% of the species in the standing vegetation at a high alpine site (3250 m) in the central Chilean Andes.

Percentages of persistent seed bank species obtained with the soil core method are generally lower than found in the present experimental study. The fewer persistent seed bank species detected in general surveys with the soil core method may partially be a result of a high probability of missing rare species in the persistent seed bank. It should also be borne in mind that in our study, burying seeds 5 cm beneath the soil surface might be somewhat artificial in terms of light penetrance, nutrients, and drainage. Roach (1983) found a striking reduction in seedling emergence comparing the second and first 5-cm layers of soil in Alaskan tundra, suggesting that the great majority of the seeds fail to penetrate further than 5 cm. As Chambers et al. (1991) pointed out, however, seed entrapment in alpine areas is highly dependent upon such factors as particle size, soil porosity, and seed size itself. In general, substrates in the South American Andes are strongly porous with much coarse material found at the surface as a result of wind erosion, which, added to the small size of seeds found in this study, could favor the formation of persistent seed bank in this habitat.

Exclusion of predators in our experimental burials could also be an additional contributory factor for the difference, although no evidence of burrowing animals was seen at the particular site. By excluding large seed predators, we have possibly overestimated real species richness in relation to the local persistent soil seed bank.

While alpine seeds may remain viable for long periods under cold laboratory storage conditions (Billings & Mooney, 1968; Chambers, 1989), little comparative data are available on seed longevities in the natural habitat. The prolonged seed longevities predicted on the basis of our experiments thus are very interesting. Using a similar extrapolation technique and the same negative exponential model we have employed here, Guariguata and Azocar (1988) estimated that seeds of the giant tropical Andean rosette species *Espeletia timotensis* could potentially remain viable in the soil for 4–5 years. *Chionochloa macra* turned out to have a non-persistent seed bank in the alpine region of New Zealand (Spence, 1990). Cavieres and Arroyo (2001) reported that in the Andes of central Chile, seeds

of the perennial herb *Phacelia secunda* remained viable in the soil for more than 3 years, estimating a longevity of 1782 days (> 4 years) in seeds buried at 3400 m a.s.l. The maximum predicted ages for seeds of several Tierra del Fuego alpine species fall well beyond that of *E. timotensis* and *P. secunda*. However, species with shorter longevities were also revealed for our site.

It should be recalled that mathematical fits were only possible for 4 of the 12 species showing potential to form a persistent seed bank. Most of those excluded belong to a group of species in which percent seed viability in the penultimate exhumation date was significantly lower than on the last date, resulting in U-shaped seed viability curves. Notwithstanding, it also turns out that several of the same species in the group that showed anomalous behavior in the Tetrazolium test showed little or no depression in final total viable seeds as measured on the final exhumation date. Consequently, the maximum seed longevities estimated on the basis of curve fitting are probably lower than in several species for which curves could not be developed.

That significant reductions in viability of physically intact seeds were registered mostly on a single observation date (363 days) might indicate a technical problem. However, this is unlikely because many species assayed on that date were not affected, and the experimental protocol was identical for all species. It is well known that some seeds experience an annual dormancy cycle when buried (Baskin & Baskin, 1998) involving profound changes in seed physiology. Changes in basic seed physiology over an annual cycle determining variation in sensitivity to the Tetrazolium test could also be expected. The depression in seed viability registered on the second exhumation date, which was the driest of the three exhumation dates, followed by recuperation of the viability reaction at the later autumn date, is perhaps determined by some aspect of seed physiology eliciting the Tetrazolium reaction that might be altered by late summer–early autumn environmental conditions.

One aspect that cannot be evaluated satisfactorily on the basis of the present information is potential phylogenetic effects. A growing body of evidence has demonstrated strong phylogenetic constraints in flowering phenology (Kocher & Handel, 1986), fruit traits (Herrera, 1992), seed number and mass (Mazer, 1989), and seed longevity (Thompson et al., 1998). Nevertheless, we have tried to avoid the confounding effect of possible phylogenetic constraints on seed longevity in our study by eliminating taxonomic redundancy as much as possible without compromising the need

to include the dominant alpine species on the study site. Our data set has no taxonomic redundancy at the generic level, and only two sources of redundancy at the familial level, where the Apiaceae are represented by three genera (*Bolax*, *Azorella*, and *Oreomyrrhis*) and the Ranunculaceae are represented by two genera (*Caltha* and *Anemone*). Perusal of the results suggests that the seed longevities of representatives of Apiaceae are almost as diverse in their seed bank characteristics as the total set of genera considered. The representatives of *Caltha* and *Anemone*, nevertheless, show fairly similar behavior.

Finally, although it is clear that there are still many unknowns surrounding the persistent seed bank biology in alpine habitats in general, our results suggest an important potential to form long-term persistent seed banks (cf. Thompson et al., 1998) in many species of the alpine region of the subantarctic zone of southern Chile. This is in agreement with recent studies (e.g., Cavieres, 1999), which suggest that factors such as the strong inter-annual variation in the length of growing season and the small-scale disturbances in the soil will favor the formation of persistent seed banks in alpine habitats (see also Forbis, 2003). Additionally, the low diversity of both seed predators and pathogenic fungi (McGraw & Vavrek, 1989), and the ambient low temperatures that are associated with low embryonic metabolic rates and slow consumption of seed reserves, favor the seed longevity observed in alpine soils.

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