RESEARCH NOTE

Longevity of seeds in a glacier foreland of the Central Alps – a burial experiment

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Summary

1 The seed germination of alpine species and the persistence of their seeds in the soil of a recently deglaciated area were studied to evaluate the potential importance of seed dormancy and persistent seed banks as survival strategies of plant populations in glacier forelands.

2 Seeds of *Achillea moschata, Anthyllis vulneraria* ssp. *alpestris, Artemisia genipi, Geum reptans, Linaria alpina, Oxyria digyna, Trifolium pallescens, Saxifraga aizoides,* and *Saxifraga oppositifolia* were collected on the glacier foreland in autumn 2000 and subdivided into four groups: 'fresh seeds' were stratified for 10 weeks at 4 °C, and their germination ability was examined in a growth chamber. The other three seed groups were buried in the field. After one winter of burial, the germination of the second group of seeds was tested. The third and fourth group of seeds will be tested similarly after two and five years of burial in the field.

3 The germination rates of fresh seeds ranged from 0.4% in *Linaria alpina* to 98.8% in *Artemisia genipi* (mean = 43.0%)

4 Germination during the winter burial in the field was highly variable. Only three of the species germinated to a significant extent (14–24% of the seeds). The post-burial germination test in the growth chamber showed that in most species, germination rates after one winter of burial were strongly reduced compared to fresh seeds. The total germination rate after one year (sum of germination during and after burial) was positively correlated with seed weight.

5 Scarification was effective in stimulating the germination of hard-coated seeds. Thus, hard-coatedness may provide seeds with the ability to survive in the soil for longer periods.

Keywords: alpine plants, germination, scarification, seed dormancy, seed weight, stratification

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Introduction

Seed germination is one of the most important life history traits of plants, determining population dynamics and the species composition of the vegetation. Persistent seeds in the soil (seed banks) may be regarded as “a reserve of genetic potential” (Simpson *et al.* 1989), and they are important sources for the regeneration of plant communities (Fenner 1992). The persistence of seeds in the soil entails the maintenance of viability (Murdoch & Ellis 1992). The latter can be transient (< 1 year) or persistent (> 1 year), depending on the dormancy type and state of the seeds (Baskin & Baskin 1989). Some seeds germinated after as much as 80–100 years of dormancy in the classical burial experiments of Beal and Duvel (Poschlod 1991; Murdoch & Ellis 1992).

In alpine regions, seed viability and germination have been investigated since the beginning of the 20th century (Körner 1999). An increasing number of seed bank studies appeared during the last two decades (reviewed by Baskin & Baskin 1998; see also Archibold 1984; Hatt 1991; Hilligard 1993; Diemer & Prock 1993; Niederfriniger Schlag & Erschbamer 1995; Urbanska & Fattorini 1998a,b). However, only few of these studies evaluated seed longevity, so that the latter is still poorly known for alpine species (McGraw & Vavreck 1989; Baskin & Baskin 1998). According to a model proposed by Cavieres (1999), alpine species should have seed dormancy mechanisms and form persistent seed banks. On recently deglaciated areas of glacier forelands, a soil seed bank was thought to be absent (Chapin 1993; Stöcklin & Bäumler 1996), but even here Kneringer (unpublished) found several indications of a persistent seed bank on 30 years old moraines.

The present study investigates the germination ability of seeds of nine glacier foreland species (two pioneers, five early successional and two late successional species). It is part of a 5-year burial experiment aimed at testing whether a persistent seed bank is indeed formed on recently deglaciated moraines. First-year results from this long-term experiment were used to examine how germination ability is affected by one winter of burial and to test two additional hypotheses: first, that species with small, low-weight seeds form persistent seed banks whereas heavy seeds are mainly short-lived (Thompson & Grime 1979; Poschlod *et al.* 1991), and second, that independently of size, seeds with an impermeable seed coat have the potential to form persistent seed banks (Baskin & Baskin 1998).

Methods

The burial experiment was carried out on the glacier foreland of the Rotmoos valley (Obergurgl, Ötztal) in the Central Alps (Tyrol, Austria), at 2400 m above sea level. Seeds (= synonym for all sorts of diaspores) of nine species (Table 1) were collected in August and September 2000 on the glacier foreland. Samples of 100 unscathed seeds per species (*Oxyria digyna:* 90 seeds) were sewed into bags (5 cm x 5 cm) of a polyester tissue (SEFAR NITEX, 125 µm mesh width, 37% open areas). Four groups of seeds were prepared: fresh seeds, winter burial, two-year burial and five-year burial. Each group consisted of 45 bags (nine species with five replicates). The protocol of the experiment is reported in Fig. 1.

The first group of seed bags (‘fresh seeds’) was processed immediately. The content of each bag was placed on moist filter paper in Petri dishes, and the soaked seeds were stratified for 10 weeks without light at 4 °C. The germination test in a growth chamber (25/10 °C, 16/8 h light/darkness, 20.000 lux) started on 12 January 2001. Seedlings were counted and removed every third day for a period of 100 days. The germination rate of each species was calculated from the total number of seeds germinated during this period. The ungerminated seeds of *Anthyllis alpestris, Tri-*

Table 1. Species used for the experiment with their role in the colonization of a glacier foreland (P, pioneer species; ES, early successional species; LS, late successional species), type of diaspore, and diaspore mass (mean dry mass per 100 diaspores). Nomenclature according to Adler et al. *(1994),* Anthyllis alpestris = Anthyllis vulneraria *ssp.* alpestris).

Species	Role	Type of diaspore	Diaspore mass (mg)
Saxifraga aizoides	PS	Extremely small seeds	4.9
Saxifraga oppositifolia	PS	Extremely small seeds	8.5
Linaria alpina	ES	Small winged seeds	13.3
Achillea moschata	LS	Small fruits	19.9
Artemisia genipi	ES	Small fruits	21.0
Trifolium pallescens	ES	Round seeds	57.3
Oxyria digyna	ES	Winged fruits	63.2
Geum reptans	ES	Fruits with hairy stylus	64.7
Anthyllis alpestris	LS	Heavy fruits	375.3

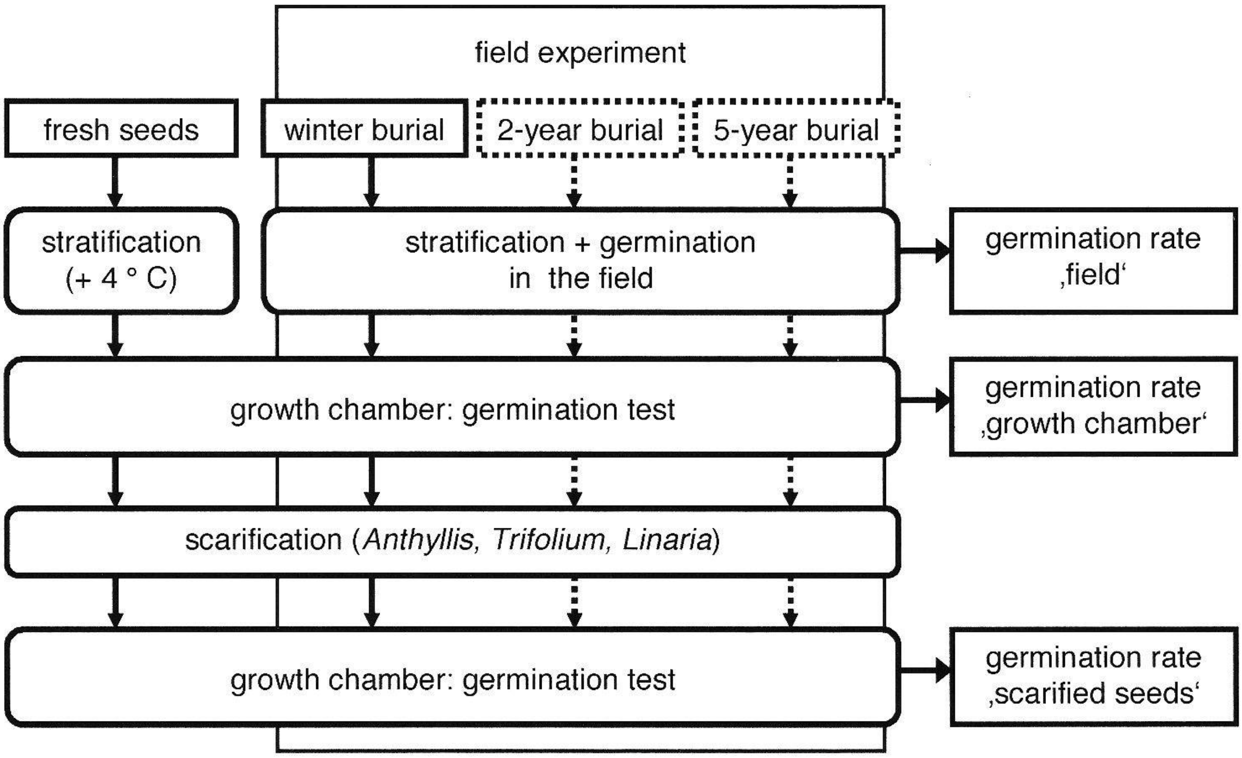

***Fig. 1.** Protocol of the burial experiment and the germination tests. The fresh seeds were used for the germination test in a growth chamber after stratification for 10 weeks. The other three groups of seeds (winter burial, 2-year burial, 5-year burial) were buried in the field in October 2000. The winter burial group was tested in spring 2001, the 2-year and 5-year burial groups will be examined in spring 2002 and 2005, respectively (dashed lines).*

folium pallescens and *Linaria alpina* were dried for one day and then slightly scarified with sandpaper. Afterwards the germination test was resumed for 10 days in the growth chamber under the same conditions, with seeds being checked every second day.

Table 2. Germination rates (mean percentage of seeds germinated ± SD) of fresh seeds incubated during 100 days in a growth chamber, of seeds buried from October 2000 until July 2001 in the field (% germinated during the burial), and total germination of the buried seeds after 100 days of post-burial incubation in a growth chamber (% germinated either in the field or in the growth chamber). The significance of differences in germination rates between fresh seeds and buried seeds (total germination) was assessed with t *tests or Mann-Whitney-*U *tests (***,* P ≤ *0.001; **,* P ≤ *0.01; *,* P ≤ *0.05; ns,* P > *0.05).*

	Fresh seeds	Seeds buried for one winter	
	(%)	Field (%)	Total (%)
Saxifraga aizoides	76.0 ± 14.8	0.0 ± 0.0	0.6 ± 0.6 ***
Saxifraga oppositifolia	2.0 ± 1.2	0.0 ± 0.0	0.0 ± 0.0 **
Linaria alpina	0.4 ± 0.9	0.2 ± 0.5	0.6 ± 0.9 ns
Achillea moschata	68.8 ± 3.8	0.2 ± 0.5	1.0 ± 1.2 ***
Artemisia genipi	98.8 ± 4.0	0.2 ± 0.5	1.6 ± 0.6 ***
Trifolium pallescens	10.8 ± 2.5	14.2 ± 4.2	18.0 ± 5.7 *
Oxyria digyna	69.8 ± 11.5	1.6 ± 2.3	20.7 ± 22.8 **
Geum reptans	43.0 ± 7.8	24.0 ± 12.0	24.6 ± 12.1 *
Anthyllis alpestris	22.0 ± 2.4	20.4 ± 1.3	30.2 ± 3.4 **

The three other groups of seed bags were buried on 20 October 2000 at a depth of 3 cm within a flat bare-ground area which has been ice-free for 30 years and which appeared homogeneous in grain size, soil humidity, inclination, and exposure. Three plots, each of 0.5 m x 0.8 m, were selected and tagged, with a distance of 0.5 m between plots. Each group of seed bags was randomly attributed to one of the three plots, and the seed bags of each group were distributed at random within their plot.

The seed bags of the winter-burial group were dug out on 2 July 2001. The fraction of germinated seeds was determined, and the remaining ungerminated seeds were placed on moist filter paper in Petri dishes. Germination tests (including the test of scarified seeds) were performed under the same conditions as for the fresh seeds. The total germination rate was calculated from the total number of seeds germinated either in the field or during post-burial germination tests in the growth chamber.

Differences in germination rates between fresh seeds and seeds buried for one winter were tested for each species with *t* tests if data were normally distributed and with Mann-Whitney-*U* tests otherwise, using SPSS 10.0

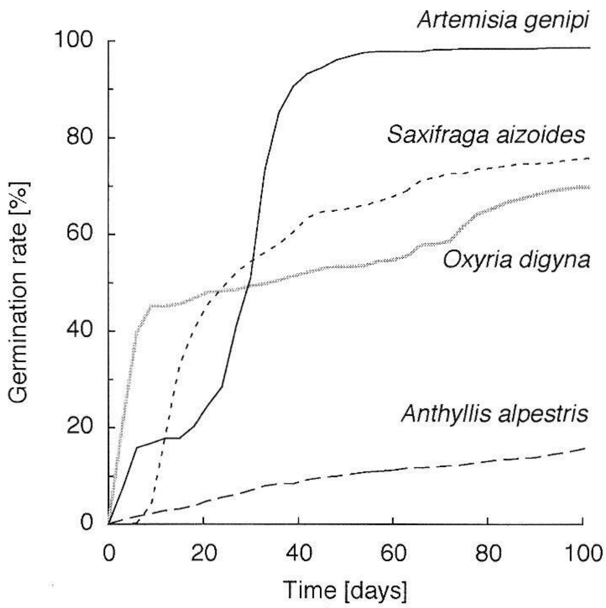

***Fig. 2.** Temporal course of seed germination in a growth chamber for fresh seeds of four alpine species after 10 weeks of stratification at 4 °C.*

Table 3. Total germination rate after scarification (mean percentage of seeds germinated either before or after scarification ± SD) of fresh seeds and of seeds buried during one winter in the field. The significance of differences in germination rate between fresh seeds and buried seeds was assessed with t *tests or Mann-Whitney-*U *tests (*,* P *≤ 0.05; ^{ns},* P *> 0.05).*

Species	Fresh seeds (%)	Buried seeds (%)
Linaria alpina	20.6 ± 12.9	7.8 ± 9.8 ^{ns}
Trifolium pallescens	92.0 ± 1.2	95.8 ± 3.1 *
Anthyllis alpestris	80.0 ± 6.3	79.2 ± 2.3 ^{ns}

for Windows. The total germination rates of winter-buried seeds were used in this test because it was assumed that any seeds germinated in the field did so in spring, i.e. after one winter of burial. The effects of seed weight on total germination rates after winter burial were analysed with linear regression (seed weight log-transformed) using the programme STATISTICA 6.0.

Results

Within the fresh seed group the germination behaviour of the species was quite variable. Germination rates ranged from 0.4% in *Linaria alpina* to 98.8% in *Artemisia genipi* (Table 2). Although most seedlings were observed during the first 30 days, the temporal course of germination also differed considerably among species (Fig. 2). In *Geum reptans, Achillea moschata* and *Oxyria digyna,* germination mainly occurred during the first two weeks, and only a few seedlings emerged later on. *Artemisia genipi* showed two peaks of germination, one in the first week and the other in the fourth–fifth week of the experiment. In *Saxifraga aizoides,* germination started with a delay of one week, and half of the overall germination occurred during the following two weeks. The seeds of *Trifolium pallescens* and *Anthyllis alpestris* germinated continuously during the test period of 100 days (Fig. 2).

After one winter of burial in the field, most seeds had remained ungerminated in six of the species, whereas 14–24% of the seeds of *Anthyllis alpestris, Trifolium pallescens* and *Geum reptans* had germinated (Table 2). Some of the seeds of *Anthyllis alpestris* (4–12%) and of *Geum reptans* (16–30%) were necrotic or attacked by insects or fungi; these seeds were excluded from the germination test in the growth chamber.

The total germination rates of the buried seeds were significantly lower than those of the fresh seeds (Table 2), except for *Linaria alpina, Trifolium pallescens* and *Anthyllis alpestris.* The higher total germination rates of *Trifolium pallescens* and *Anthyllis alpestris* were due to a large number of seeds germinated in the field (Table 2).

Scarification was very effective, especially for the legumes, of which almost all scarified seeds germinated (Table 3). The germination

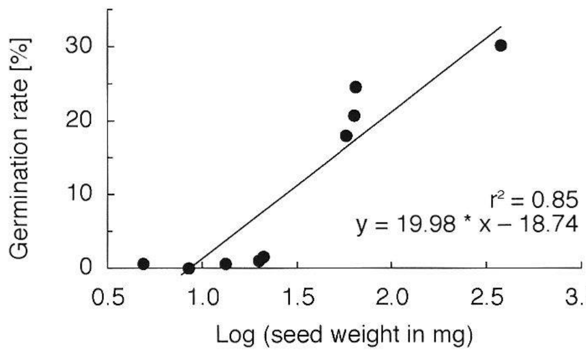

Fig. 3. Linear relationship between log-transformed seed weight and the total germination rate of seeds during and after winter burial in the field.

rate of scarified seeds did not differ between fresh seeds and seeds buried for one winter in *Linaria alpina* and *Anthyllis alpestris,* whereas scarified seeds of *Trifolium pallescens* had a significantly higher germination rate after winter burial (Table 3).

The seed weight of the species determined to a large extent ($r^2 = 0.85$) their total germination rate after one winter of burial in the field (Fig. 3): many of the heavy seeds germinated during or after the burial, whereas seeds with low weight showed little or no germination.

Discussion

INTERSPECIFIC DIFFERENCES IN SEED DORMANCY AND GERMINATION RATES

Strong seed dormancy has been suggested for many alpine species (Fossati 1980; Weilenmann 1981; Zuur-Isler 1982; Urbanska & Schütz 1986, 1988; Flüeler 1992), and stratification has proved to be a successful method to break this dormancy. In this experiment fresh seeds of most species also had a relatively high germination rate after a cold treatment. Although it is difficult to compare the data of different studies, the germination behaviour of the species of the fresh seed group was similar to that found in earlier studies (Mooney & Billings 1961; Fossati 1980; Weilenmann 1981; Schütz 1988; Flüeler 1992; Niederfriniger Schlag 2001). Thus, the ability of seeds to germinate after stratification was demonstrated for all investigated species except *Saxifraga oppositifolia* in this study.

The low germination rate of fresh seeds of *Saxifraga oppositifolia* suggests that this species has strong dormancy mechanisms that cannot be broken by stratification only. Studies of Freedman *et al.* (1982) and Niederfriniger Schlag (2001) support this interpretation, whereas Diemer & Prock (1993) classified *Saxifraga oppositifolia* and *Saxifraga aizoides* as species with transient seed bank. Seeds of *Saxifraga* species are light-weighted – they were the smallest among the nine species included in this study. According to the considerations of Thompson & Grime (1979) and Poschlod (1991), such species would be expected to form a persistent seed bank.

After one winter of burial in the field, *Saxifraga aizoides, Achillea moschata* and *Artemisia genipi* showed surprisingly low germination rates. A high fraction of the seeds probably died during the winter. When their germination was tested in the laboratory after the burial, many seeds seemed necrotic, and fungal attack was more severe compared to the fresh seed group. Schütz (1988) hypothesized that one single cold period might be insufficient to break the dormancy of some alpine species and that higher germination rates could be expected with increasing storage time. *Saxifraga oppositifolia* may have this type of dormancy as well; this would explain why its germination rate was low while the small seed size suggested a persistent seed bank.

Few seeds of *Oxyria digyna* germinated in the field, but the post-burial germination rate in the growth chamber was high. Possibly the temperature in the field was not yet optimal for germination before the buried seed bags were dug out in July. This suggestion is supported by studies of Mooney & Billings (1961) showing that seeds of *Oxyria digyna* had the highest germination rate at 20° C, whereas at 10° C only few seedlings emerged. Given the relatively high post-burial germination rate, we suppose that the seeds of this species are short-lived.

In alpine regions most seedlings appear immediately after snowmelt (Körner 1999; Niederfriniger Schlag & Erschbamer 2000). *Geum reptans* showed a moderate spring germination rate in the field: the seeds probably germinated shortly before they were dug out.

Hardly any of the remaining seeds of this species germinated in the growth chamber. Fossati (1980) observed a similar germination behaviour for *Geum montanum* in seeding experiments: this species germinated only after the first winter, whereas seedlings emerged after the second winter in all other species. According to these observations, it is doubtful that *Geum reptans* has the potential to form persistent seed banks. Relatively high germination rates in the field were also observed for *Trifolium pallescens* and *Anthyllis alpestris* in this study and for other alpine legumes in the burial experiments of Flüeler (1992), suggesting that legumes as well as *Geum reptans* tend to have rather low temperature requirements for germination.

RELATIONSHIPS BETWEEN SEED TRAITS AND GERMINATION BEHAVIOUR

The positive relationship between seed weight and germination rate after one winter of burial could be interpreted in two ways: either the small seeds have lost their viability during the winter or secondary dormancy took place. In the second case, these seeds would still have the potential to germinate after a longer period of burial. These alternative hypotheses can only be validated after germination tests with the seeds buried for two or five years.

Hard-seededness might have caused the low germination rate of *Linaria alpina*. After scarification, the seeds showed a considerably higher germination rate. Schütz (1988) obtained even better results by treating seeds with gibberellin acid. It may be suggested that the embryos lack sufficient growth potential for breaking the seed coat. Almost all seeds of *Trifolium pallescens* and *Anthyllis alpestris* germinated after scarification, both in the fresh-seed group and in the winter-burial group. Scarification is known as an appropriate treatment to prove the germination ability of seeds of alpine legumes (Schütz 1988, Flüeler 1992). The results of this study and those of Schütz (1988) and Flüeler (1992) suggest that the investigated alpine legumes and other species with small, hard-coated seeds might be able to survive in the soil for several years without loosing their germination ability. Thus, hard-coatedness might be one of the seed traits determining the potential of species to form persistent seed banks.

CONCLUSIONS

High variability of germination rates within and among species seems to be a typical feature of alpine plants. Ecological factors as well as genetic components may be the driving forces of this phenomenon. Nevertheless, longevity of seeds should be an appropriate trait that offers glacier foreland plants the chance to survive highly fluctuating conditions in a harsh environment. Which type of dormancy provides seeds with this ability to persist is still unknown. Therefore, one of the major tasks for the future will be the analysis of the dormancy types of these species.

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