# Peck's penstemon (*Penstemon peckii*) off-site seed storage, and soil seed bank longevity study over a period of 15 years.

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Germinants from seeds stored in soil for 15 years

by

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# Peck’s penstemon (*Penstemon peckii*) off-site seed storage, and soil seed bank longevity study over a period of 15 years.

Abstract:

Given the ecological and legal contexts in which Peck’s penstemon (*Penstemon peckii*) occurs, the potential for this species to form a viable soil seed bank in nature is of interest to USFS land management. In order to assess the potential for seeds of Peck’s penstemon to survive in nature in the soil, an empirical experiment was established in 1992. At that time 120 dacron pouches, each containing 100 seeds were placed in the soil, in a protected place within the range of the species, and samples were removed for testing after 6, 12, 18, 48 and 180 months in the soil. Other seeds from the original bulked collection were simultaneously placed in ex situ frozen storage at the Berry Botanic Garden Seed Bank for Rare and Endangered Plants of the Pacific Northwest and subjected to germination testing.

Results showed clearly that *Penstemon peckii* seeds can survive and germinate for at least 180 months (15 years) in the soil and in the ex situ seed bank. Seed germination was consistently greater in those samples stored ex situ, but the difference between those stored ex situ as compared with those stored in situ diminished over time. The proportion of seeds that remained hard, and thus potentially viable in the soil, diminished to slightly over half following the first winter in the soil, and that proportion remained remarkably constant over the 15 year course of the study. An unexpected phenomenon was discovered during the course of this study in which it has been shown that some seeds can remain dormant through one or more germination trials, only to germinate during a later trial. While this complicated analysis, it is a feature that would appear to reduce the chances of catastrophic loss if all viable seeds were to germinate at the first opportunity.

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## Introduction:

Despite the fact that *Penstemon peckii* is not extremely rare (global rank, or G3), it has a small total range that is almost entirely within the Sisters District of the Deschutes National Forest. Among the threats facing this species is the pressure of ecological succession in the form of encroachment by woody species of its more open optimal habitat.

For management, the degree of threat by woody encroachment would differ depending on whether *P. peckii* is, or is not, capable of forming a long term soil seed bank. The primary question addressed by this empirical study, which began in 1992, concerns the survivorship of *P. peckii* seeds in the soil for 'long' periods of time (Guerrant, 1993). A secondary question addressed is focused around the relative survivorship of seeds in the soil and seeds stored in the Berry Botanic Garden Seed Bank for Rare and Endangered Plants of the Pacific Northwest. An incidental, or peripheral question that arose during the study concerns the ability of viable seeds to remain dormant after being exposed to one or more cycles of otherwise suitable conditions for germination, only to germinate during a later trial.

## Materials and Methods

In July and August of 1992, we, along with the assistance of USFS personnel and volunteers, collected approximately 62,250 seeds from between 3 and 28 individuals at 11 different populations across the species' range (for fuller discussion of Materials and Methods, see Guerrant, 1993).

To summarize, of the *ca*. 62,500 seeds collected, *ca*. 45, 000 were placed into the Berry Botanic Garden Seed Bank for long term storage, and have been maintained separately by maternal lines. A small number of seeds were provided to a researcher at Reed College, Ms. E King, for use in her Senior Thesis research (King, 1993) Approximately a quarter of the seeds from each accession (i.e. individual, or maternal line) having more than 100 seeds were removed, and pooled to make a single statistical sample for use in this study (*ca*. 17,000 seeds). In the fall of 1992, 120 dacron pouches (three layers) were placed just under the soil surface (in stainless steel cages, and covered with a layer of dark colored weed cloth under another lighter colored layer to better mimic the soil with respect to heat absorption). Guerrant, 1993) Each pouch had *ca*. 100 seeds, with 40 pouches placed in each of 3 locations in the forest within its range. The remaining seeds from the bulked sample (*ca*. 5,500 seeds) were returned to long term storage in the Berry Botanic Garden Seed Bank.

Since they were placed out, seeds have been withdrawn (mostly by USFS personnel) and sent (typically by overnight courier) to the Garden for testing on five occasions, approximately 6, 12, 18, 48 and 180 months after being placed in the soil during the late fall, 1992. On each of these occasions, five packets of seeds have been removed from the soil from each of the three field locations, comprising a sample of (5 packets per site * 100 seeds per packet * 3 sites) 1,500 seeds from the field per trial. Each trial also included 500 seeds from the frozen sample retained at the BBG Seed Bank.

Upon receipt at the Garden, tap water was run over and through each packet to remove soil and other debris, after which packets were individually opened and the contents evaluated under a dissecting microscope. Individual objects in the packets were then placed into the appropriate category: hard seeds (and thus potentially viable), empty seed coats, or the remains of germinated seedlings (these were seen only in the initial two trials 6 and 12 months after being placed in the soil.) Seeds were placed in germination dishes on three layers of germination paper, moistened and placed in a controlled environment chamber that alternates between 10ºC and 20ºC. Dishes were examined approximately weekly, though initially more frequently. At the end of each trial, remaining seeds were surveyed, and data obtained on the number that were soft and therefore presumably inviable, and those that were hard and therefore potentially still viable. The tests were both visual, and mechanical – each seed being gently squeezed with forceps. After the initial three-month test, and post-test status tests, we tried something new. We dried down the remaining seeds in the chambers (put lids ajar), and remoistened them after approximately one month to see if any additional seeds would germinate (some did), and then dried them again, and subjected the remaining seeds to a third round of germination testing. Multiple tests were run on some but not all samples. Statistical analyses of the resulting data were conducted using SYSTAT 11.0

## Results

### Initial germination results from ‘fresh’ and ‘dried + frozen’ seeds

Shortly after the seeds were placed in the field, an initial set of data on germination rates for two groups of seeds, a ‘control’ group of ‘fresh’ seeds that had not been dried and frozen, and a group of seeds that had been dried and frozen for a short period of time (to gather baseline data on whether the seeds could, indeed, survive such treatment.) The initial trials lasted approximately 3 months, with dried and frozen seeds germinating at a slightly higher rate than the control or fresh seed (Figure 1; Table 1.) Figure 1 illustrates the time course of germination of the initial series of trials of seeds that had not been stored in the field.

Table 1 provides a summary overview of all the germination trials to date that have been conducted with respect to this project. The table includes incomplete results of the third, and currently ongoing, germination trial on the seeds that were removed after 15 years storage (indicated by asterisks in the table.) Data provided include initial sample sizes, the total number of seeds recovered from the seeds stored in situ, in the soil, the number of hard seeds recovered, as well as the number and proportion of seeds that germinated after the first, second and third trials on particular sets of seeds. Note that not all seed samples have been subjected to multiple tests.

Summary results of the initial trials, only, on all seed samples that had been stored either in situ in the soil, or ex situ in the Berry Botanic Garden Seed Bank are shown in Figure 2. Note that for some reason that we cannot recall, no seeds were removed for testing from the ex situ seed bank after 18 months storage, to compare with those in the soil for that length of time. Note, too, that the seeds stored ex situ germinated at greater rates than those stored in the soil for the first 4 years, but not after 15 years.

Figure 3, however, shows the cumulative total germination of various samples after being subjected to conditions favorable for germination more than once. Only the original samples, and those stored for 15 years were subjected three times to conditions favorable for germination. Only those stored for 6 months were subjected just twice. The relative increase, as indicated by slope (steeper slopes mean greater difference between successive trials), was greatest for the original sample (fresh and dried and frozen). For seeds stored for either 6 or 180 months, note that the seeds stored ex situ had a greater relative increase between tests than did those stored in the soil. Indeed, only a single seed stored in the soil for 180 months (15 years) germinated in either the second, or after a month in the third trial (Table 1, Figure 3). In contrast, those stored ex situ had increased to match the total germination of those stored in situ by the end of the second round of trials, and a greater number of seeds has germinated mid way through the third round of trials for those seeds stored for 15 years.

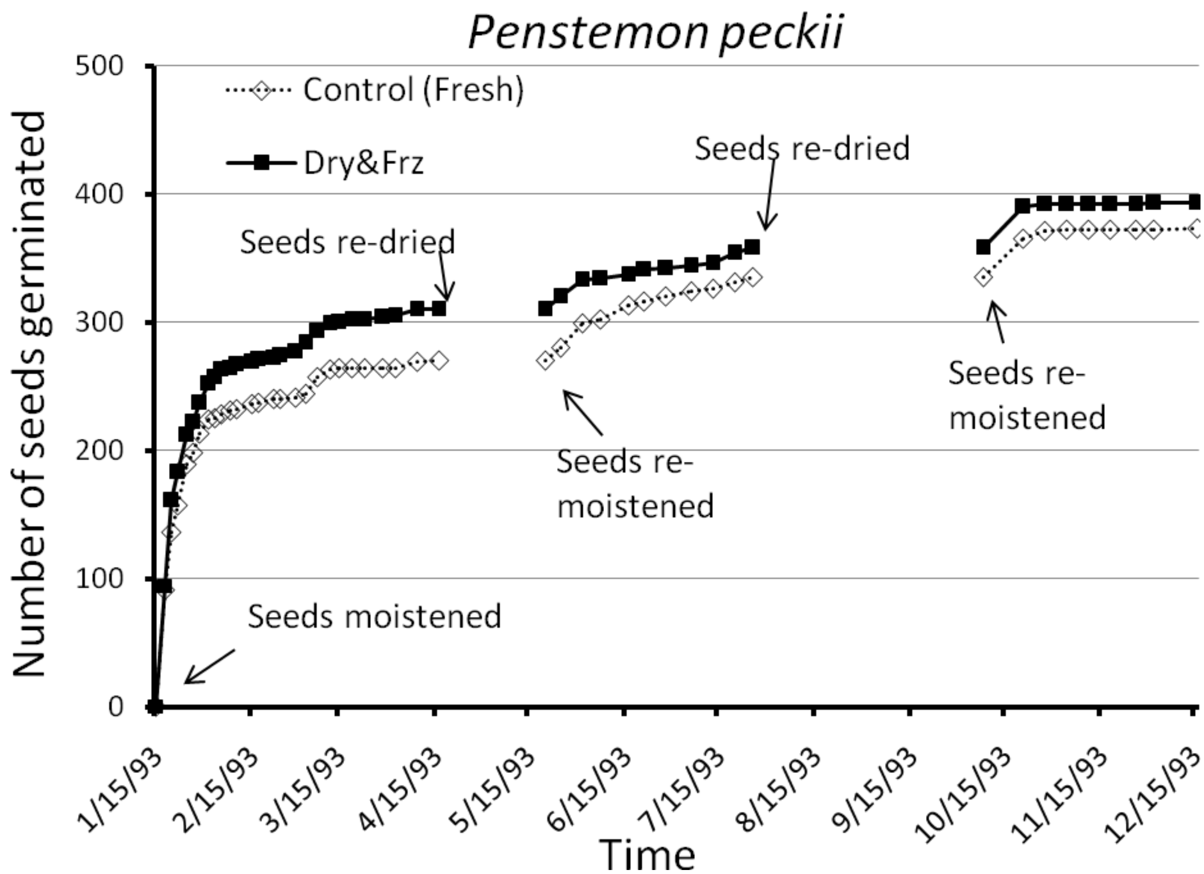


Figure 1. Graphical representation of the time course of germination of the original seed germination trials, both of fresh seed and of seeds that had been dried and frozen. Gaps in time of the data indicate periods of time when seeds were dried between germination trials.

Table 1. Overview figures of initial sample sizes, numbers of seeds recovered from the field, the number of those that were judged to be hard (i.e. potentially viable), as well as the number and proportions of seed to germinate after one or more successive trials of the same seeds.

| | Initial Sample Size | Seeds recovered | Hard Seeds | **Germination, Initial trial** | Germination total after 2nd trial | Germination total after 3nd trial | **Proportion germination, 1st trial** | Proportion germination after 2nd trial | Proportion germination after 3rd trial |
|---|---|---|---|---|---|---|---|---|---|
| Initial tests (Jan 1993) | | | | | | | | | |
| Fresh | 500 | N/A | 500 | **270** | 335 | 373 | **0.54** | 0.67 | 0.75 |
| Dry&Freeze | 500 | N/A | 500 | **310** | 358 | 393 | **0.62** | 0.72 | 0.79 |
| After 1 winter in storage (May 1993) | | | | | | | | | |
| Soil | 1500 | 1484 | 861 | **706** | 738 | N/A | **0.47** | 0.49 | N/A |
| Freezer | 500 | N/A | 500 | **327** | 362 | N/A | **0.65** | 0.72 | N/A |
| After 1 year in soil or freezer (November 1993) | | | | | | | | | |
| Soil | 1500 | 1428 | 853 | **693** | N/A | N/A | **0.46** | N/A | N/A |
| Freezer | 500 | N/A | 500 | **313** | N/A | N/A | **0.63** | N/A | N/A |
| After 18 months in soil or freezer (May 1994) | | | | | | | | | |
| Soil | 1500 | 1408 | 879 | **802** | N/A | N/A | **0.53** | N/A | N/A |
| Freezer | 0 | N/A | N/A | N/A | N/A | N/A | **N/A** | N/A | N/A |
| After 4 years in soil or freezer (November 1996) | | | | | | | | | |
| Soil | 1500 | 1382 | 809 | **741** | N/A | N/A | **0.49** | N/A | N/A |
| Freezer | 500 | N/A | 500 | **291** | N/A | N/A | **0.58** | N/A | N/A |
| After 15 years in soil or freezer (October 2006) | | | | | | | | | |
| Soil | 1500 | 1313 | 865 | **437** | 438 | *438 | **0.29** | 0.29 | *0.29 |
| Freezer | 500 | N/A | 500 | **120** | 146 | *176 | **0.24** | 0.29 | *0.35 |

* indicates 3rd trial in progress as this report being written
N/A indicates either test not conducted, or no number is applicable

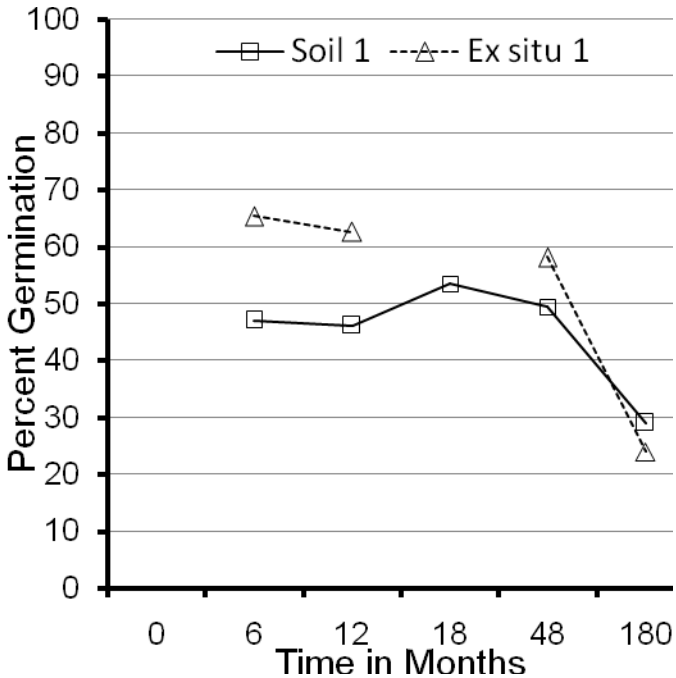


Figure 2. Graphical summary of germination results of initial trials of stored seed.

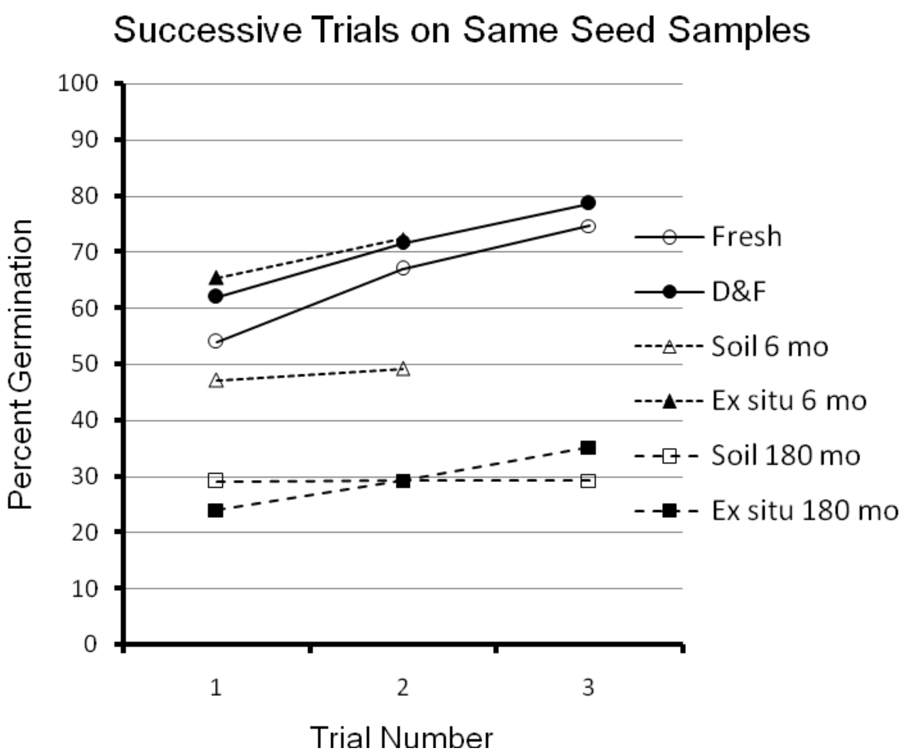


Figure 3. Graphical summary of cumulative germination in those samples that were subjected more than once to favorable conditions for germination.

Seeds stored either in soil or ex situ seed banks

To what degree can seeds remain physically whole in the soil and over what periods of time? Figure 4, and Table 1 show the proportions (of the total number of seed originally placed in the field) of seeds or their identifiable remains that have been recovered after various amounts of time in the soil. The total number of seeds or their remains recovered has diminished over time from a high of *ca* 0.99 (i.e. 99%) after six months in the soil, to a low of 0.88 after fifteen years. The number and proportion of 'hard' seeds, or those that could potentially still be viable, declined rapidly to about 0.57 after six months in the soil, and has remained remarkably constant over the 15 years of this study (varying between ca 0.54 and 0.59). The identifiable remains of germinated seedlings were documented after 6 and 12 months in storage, but not after 18 or more months of storage.

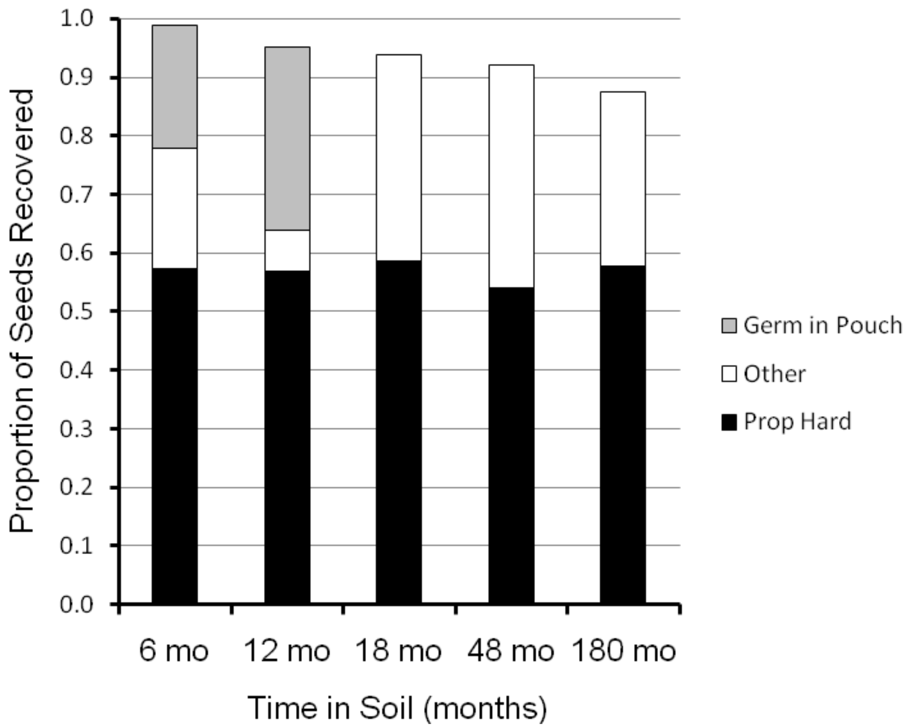


Figure 4. Proportions of the 1500 seeds placed in soil in 1992 that were recovered after various periods of time.

Figure 5 shows the combined percent of seeds (out of 1500 stored in the soil, and out of 500 stored in the ex situ seed bank) that had either germinated or remained hard after the second set of germination trials on seeds stored for 15 years. Note that the cumulative germination percentage of those stored in situ and ex situ were similar, but that a far greater fraction of hard, and potentially still viable seeds remain in those stored ex situ, relative to those stored in the soil.

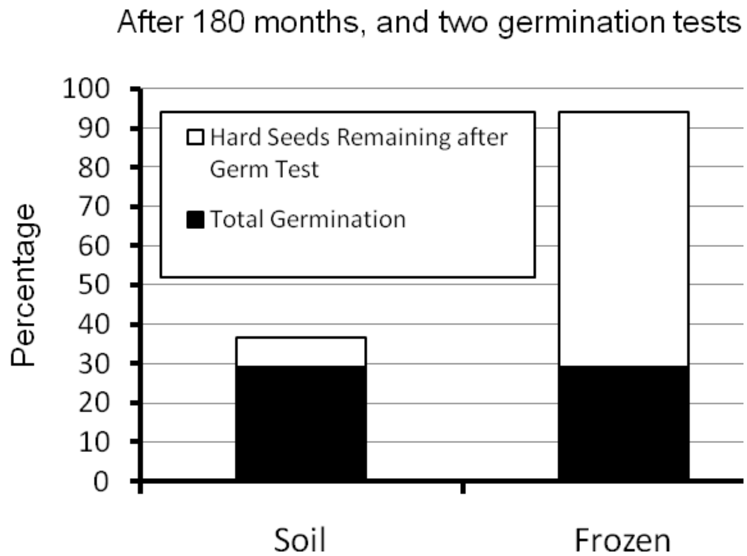


Figure 5. Graphical representation of the combined total germination and remaining hard seeds at the conclusion of the second set of germination trials (the third set of trials are currently underway.)

## Discussion and Conclusions

It is fortunate that the data provide the most confidence in being able to address the primary question in this study (can the species form a soil seed bank). The data provide somewhat less confidence in answering the secondary question (survivorship in ex situ seed bank compared to in situ soil seed bank), in part because of the phenomenon documented in the third, and originally incidental question addressed by this study. That is, the ability of viable seeds to remain dormant through one or more cycles of otherwise suitable conditions for germination, and then germinate during a later trial.

The answer to the central question addressed in this study – Is *Penstemon peckii* capable of forming a viable soil seed bank in nature? – is clear. Seeds of *P. peckii* demonstratively have the ability to survive in the soil and germinate over a span of at least 15 years, and potentially longer.

With respect to the secondary questions, having to do with the relative survivorship of seeds stored in situ and ex situ, the data provide a more nuanced picture. On the one hand, seeds stored ex situ have germinated in higher numbers than those stored in situ after each and every time the two have been compared directly (i.e. after 6, 12, 48 and 180 months of storage.) On the other, the difference in germination between seeds stored ex situ and in situ have progressively decreased the longer they have been stored.

To further complicate the interpretation of results of seeds stored for 15 years, germination of seeds stored ex situ exceeded that of seeds stored in situ, but only during the third trial on those seed samples. That fact draws attention to the final group of questions, concerning the ability of *P. peckii* seeds to remain dormant through one or more cycles of otherwise suitable conditions for germination, and then germinate during a later trial. From an ecological perspective, and as is true within a single season, the ability to stagger germination over more than one season has potential advantages and well as disadvantages. Advantages include the 'bet hedging' aspects of staggering the germination of a particular cohort over more time within a season, or over more than one season. Prolonging germination over a longer period of time, either within or between years, has the effect of reducing the chance of catastrophic failure if, for example, a severe drought, or other environmental disturbance unfavorable for seedling growth occurred in a particular year. Perhaps the most significant disadvantage is that any delay in germination will have the demographic effect of reducing the potential contribution to positive population growth rate of any particular cohort of seeds. On balance, it seems the ability to prolong the time over which a particular seed cohort germinates serves to reduce the chance of catastrophic loss, at the expense of high potential population growth rate.

At more basic, mechanistic, physiological levels, however, these data raise more questions than they answer, and provide no particular insight into what is going on within the seeds. Seeds in which mold or other pathogens consume the embryo, often leaving a soft, gooey mass, are typically interpreted as having either been dead or their health significantly compromised at the outset of a germination trial. In other words, there is reason to think that seeds that can resist rotting, where others of the same sample or species do become moldy and mushy, are potentially viable. If so, then the high proportion of seeds (94%) that had been stored ex situ for 15 years, and subjected to two complete rounds of germination, and which either germinated (29.2%) or remained hard (64.8), suggest that a great many may yet be viable, and capable of germination. Possible explanations include changes in the 'depth' or physiological characteristics of dormancy after long periods of storage. If so, then the progressive reduction in germination rate of seeds stored ex situ is not a measure of viability in storage, but rather a reflection of changing germination requirements. Clearly, more research into this phenomenon is in order.

Acknowledgements

This study could not have been completed without the assistance of many people, not all of whose names we may be aware (i.e. mainly the volunteers who counted out the 120 batches of 100 seeds each that were placed in the packets to be put in the field.). To these we offer our thanks and apologies. Ms. Cindi O'Neil was an early supporter in the USFS of this project, and instrumental in getting it off the ground. Numerous people have assisted in the field, including Dorothy Brantley, Kathleen Cooper, Lizzy King, Ellen Liversidge, Conor O'Neil, Marion Over, and especially over a many year time scale, Maret Pajute. Cindi O'Neil was instrumental in arranging the participation of the Native Plant Society of Oregon, and in organizing volunteers to count and package the large number of seeds. Cal Brantley and Maret Pajute have kindly recovered bags from the field, and sent them to the Berry Botanic Garden. Maret Pajute assisted in the field collection of seeds, and located what have turned out to be superb and secure locations to place the seed packets in the field, and we thank her especially for her long term commitment to this study, and for providing assistance in many different ways. To these and all the other people who have kindly assisted us over the years, we offer our sincere thanks.

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