GERMINABLE SEED/PROPAGULE BANKS

MONITORING AT ITEX SITES

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Objectives and concerns

Knowledge of the availability and abundance of viable seeds in tundra soils is important to an understanding of community processes in a stable or in a changing environment. This includes the actual recruitment from this seed bank into seedlings, juvenile and then adult population.

The study of the seed bank can be laborious if information on the total seed bank over a number of years is to be obtained. For the purpose of ITEX, we need background information on the size and the diversity of seed banks, this would require repeated sampling over a number of years as part of a long-term monitoring program at ITEX sites. Estimates of the **germinable seed (or propagule) bank** would then allow identification of the seedlings to species. This method is suggested for community level studies as it is less tedious than the direct counting of seeds (Simpson et al., 1989) and has given good results in previous arctic and alpine studies (e.g. Freedman et al., 1982; Fox, 1983; Diemer and Pock, 1993; Lévesque and Svoboda, 1995). For the people interested, the **total seed bank** could also be determined after germination by extraction of the seeds and viability tests (Malone, 1967). (For information about the propagule bank for bryophytes, read Lewis-Smith, 1993)

Seed banks are very heterogenous in time and in space (Thompson and Grime, 1979). A standardization of methods between studies has been recommended in the literature (Simpson et al., 1989) and is definitely necessary to allow comparisons among sites and among years in ITEX. Unfortunately, as pointed out by Simpson et al. (1989) it is unreasonable to expect people to use exactly the same method at each site. The range in seed dormancy and other germination requirements between habitats and species makes it impossible. Nevertheless, the method should have a baseline that is comparable between sites and this is what the present protocol is attempting to outline. Here is a list of concerns and background information to consider when planning a seed bank experiment. Please, consider these as well as to the literature cited at the end of this section.

1) There is a lot of variability in seed abundance in soils. A sample collected in the vicinity of a recently flowering individual is likely to have a much higher number of seeds than one collected away from an immediate seed source.

*** REPLICATION is IMPORTANT. Many small samples are better than few larger ones (Benoit et al. 1989)

2) The dormancy properties and longevity of seeds in arctic and alpine soils is relatively little known. Propagules from a large number of species have been able to germinate in the conditions recommended in this manual but it is likely that the requirements for germination of certain species are not met.

*** GERMINABLE seed bank is different from TOTAL seed bank.

3) In relation to (2), the timing of collection of samples and the preservation of samples are important (e.g. if most seeds are shed late in the season a collection early would exclude the current year's crop while a late one would be dominated by it!)

*** Description of time of sampling and consistency (between years) are important

Protocol

In the field:

If the samples are to be collected once in the season, it would be best to do so shortly after snowmelt. The seeds of the previous year would have had a vernalization period and would not have been swamped by the current year's crop. It may be possible to process the samples directly in the field (Diemer and Prock, 1993). If the samples need to be shipped south and processed later, a collection late in the season might be more suitable (to limit time of storage in the field).

The minimum frequency of sampling for a monitoring effort should be once every 3 to 5 years. In the control sites, at least 30 random samples should be collected (this is obviously not suitable in an OTC except if destructive measurements are planned or at the end of the experiment. In this case, following the paired plots design, 2 samples per plot would give 20 samples per treatment).

The samples should be relatively small and kept separate. In soft-substrate sites where it is possible to use soil cores, 7-10cm diameter cores are probably most suitable. In rocky sites it was found convenient to collect the soil of a 10cm x 10cm quadrat with a trowel. In general the top 1cm of soil is recommended for the dry sites, a depth to 3-5cm and up to 10cm was often sampled in alpine and forest tundra (Archibold, 1984; Morin and Payette, 1988; Diemer and Prock, 1993). It is suggested that each layer be kept separate because it would be best if we could compare the uppermost soil seed bank in all sites on the same basis. It is always possible to combine the results later on (suggested divisions, if possible: 1) first centimetre, 2) from 1-3cm, 3) 3-5cm 4) 5-10cm).

Seed banks are usually given in seeds/m^2 while, in fact, the numbers depend on the depth of the samples (i.e. seeds/m^2 per 1cm depth would be different from seeds/m^2 per 3cm depth). In the case of polar desert sites, seeds were found extremely rarely at depths > 1cm (Lévesque unpublished). In an alpine area, Morin and Payette (1988)

determined that 85% of the total seed bank of a 10cm deep core was found in the top 3cm. Thus we recommend sampling the top 1cm in polar desert and a minimum of 3cm for more productive sites (including meadows).

If the samples are not processed in the field, proper storage of soil samples is critical to ensure that seeds do not respire or start to germinate prior to the set up of a germination experiment. In general, drying the samples in the field (in a dark and cool environment) reduces the weight of the samples and minimizes the risk of germination during transport. Samples should then be stored in a dark cool environment as much as possible. A 2-3 month freezing treatment has often been given to samples before germination (Marchand and Roach, 1980; Fox, 1983; Archibold, 1984), and is recommended especially for samples collected late in the season. Certain species may not germinate after a freezing treatment because of their short viability (e.g. *Salix planifolia*; Bliss, 1958) or because of their particular dormancy characteristics. The analysis of the total seed bank, or the germination of a set of samples rapidly after collection might enable these problems to be addressed. Site-specific (and species-specific) problems should be considered before starting the experiment (see Simpson et al, 1989).

In the laboratory:

The following method assumes the availability of a greenhouse or of a growth chamber and has been adapted from the method of Ron Rollo, supervisor of the UBC Botanical Garden's nurseries. We do not recommend a method using a lid (because it is faster and easier for watering and for monitoring seed germination) but some concern should be given to the risk of contamination of the samples, especially if there are potential seed sources in the vicinity of the experiment. Pots with sterilised soil, to serve as controls, should be placed between the pots with samples (Archibold, 1984; Diemer and Prock, 1993). We used 24h light to germinate high arctic samples, slightly different light conditions may be suitable for other sites. In the literature, for alpine and low arctic sites, 16h to 18h seems to have been used with success (Leck, 1980; Archibold, 1984). Temperature near ambient (20-25˚C) is recommended as it has been shown to be near optimum for a number of species. Temperature and light intensity and quality should be monitored.

Each soil sample should be passed through a standard sieve. We used a 2mm mesh sieve, but if some seeds are likely to be larger than that a larger sieve should be used. Fleshy fruits may need to have their seeds separated from the berry (Komulainen et al., 1994). Here the idea is to make the samples more homogenous, to eliminate the rocks and allow the growth conditions to be more similar between samples. It also reduces the volume of the sample and allows more samples to be processed in a given space! This might be impractical for certain soils with high organic matter content, total samples should then be used. For optimal germination, the layer of soil for germination should be thin (<1cm; Fox, 1983) so, depending on the size of the pots and the space available in the greenhouse, the use of subsamples might be necessary. In this case, the total weight of the sample and that of the portion used (subsample) should be taken (0.01g accuracy) to allow conversion of seedlings/subsample to seedlings/sample to seedlings/m^2.

Pots of approximately 10cm x 10cm x 3cm or deeper, with drainage holes should be filled to 2/3 with sterile potting soil. The size of the pots depends mostly of the space available and the size of the sample, deeper pots allow for better rooting zone and are recommended. The sieved sample can be spread on top of this soil (in a thin layer, less than 1cm) covered then by a thin layer of silica aquarium sand (or blasting sand) to keep the surface from drying.

Each pot should be thoroughly watered and put in the greenhouse for approximately 2 months, or until no more new germination is observed over a consecutive 7-10 days period. Germination should be monitored frequently (daily or every 2-3 days, this is especially critical at the beginning), the new seedlings recorded (noting if they are monocotyledons or dicotyledons) and the pots watered if necessary. The samples can be kept in these pots until identification is possible.

Many emerged seedlings will need to be grown until flowering to be correctly identified. To ensure that these plants can grow without too much competition, the previously identified plants should be removed from the densely populated pots. Some voucher specimens should also be preserved.

Calculations:

Once all germinations have been recorded and the seedlings identified, the germinable seed bank value (number of seeds per m^2) at each site, for each species (and in each layer), can be derived from the following relationship

$$G = g \times A$$

and if you used subsamples:

$$G = g_b \times (Wt_a/Wt_b) \times A$$

Where G=germinable seed bank (seeds/m^2); g and g_b=number of germinated seedlings in a sample or in a subsample respectively; Wt_a=weight of the subsample (portion used in germination trial); Wt_b=total weight of sample. The multiplication factor A is used to convert the area of the samples to $1m^2$.

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References

Archibold, O.W. 1984. A comparison of seed reserves in arctic, subarctic, and alpine soils. Canadian Field Naturalist 98: 337-344.

Benoit, D.L., N.C. Kenkel and P.B. Cavers. 1989. Factors influencing the precision of soil seed bank estimates. Canadian Journal of Botany 67: 2833-2940.

Bliss, L.C. 1958. Seed germination in arctic and alpine species. Arctic 11(3): 180-188.

Diemer, M. and S. Pock. 1993. Estimates of alpine seed bank size in two Central European and one Scandinavian subarctic plant communities. Arctic and Alpine Research. 25: 194-200.

Ebersole, J.J. 1989. Role of the seed bank in providing colonizers on a tundra disturbance in Alaska. Canadian Journal of Botany 67: 466-471.

Fox, L.R. 1983. Germinable seed banks of interior Alaskan U.S.A. tundra. Arctic and Alpine Research 15: 405-412.

Freedman, B, N. Hill, J. Svoboda and G. Henry. 1982. Seed banks and seedling occurrence in a high arctic oasis at Alexandra Fjord, Ellesmere Island, Canada. Canadian Journal of Botany 60: 2112-2118.

Gross, K.L. and K.A. Renner, 1989. A new method for estimating seed numbers in soil. Weed Science 37: 836-839.

Lévesque, E. and J. Svoboda, 1995. Germinable seed banks from polar desert stands, Central Ellesmere Island, Canada. *In*: Global Change and Arctic Terrestrial Ecosystems. T.V. Callaghan, U. Molau, M.J. Tyson, J.I. Holten, W.C. Oechel, T. Gilmanov, B. Maxwell and B. Sveinbojorrnsson Editors. Proceedings of papers contributed to the International Conference, 21-26 August 1993, Oppdal, Norway. Ecosystems research report 10, European Commission. Pp. 97-107.

Lewis-Smith, R.I. 1993. The role of bryophyte propagule banks in primary succession : Case-study of an Antarctic fellfield soil. p.55-78 In: Primary Succession on Land. J. Miles and D.W.H. Walton Editors. Blackwell Scientific Publications, Oxford.

Malone, C.R. 1967. A rapid method for enumeration of viable seeds in soil. Weeds 15: 381-382.

Marchand, P.J. and D.A. Roach. 1980. Reproductive strategies of pioneering alpine species: seed production, dispersal, and germination. Arctic and Alpine Research 12: 1370146.

McGraw, J.B. 1980. Seed bank size and distribution of seeds in cottongrass tussock tundra, Eagle Creek, Alaska. Canadian Journal of Botany 58: 1607-1611.

McGraw, J.B. and M.C. Vavrek. 1989. The role of buried viable seeds in arctic and alpine plant communities. p.91-106. In: Ecology of Soil Seed Banks. M.A. Leck, V.T. Parker and R.L. Simpson Editors. Academic Press Inc, San Diego. 462 pp.

Morin, H. and S. Payette. 1988. Buried seed populations in the montane, subalpine, and alpine belts of Mount Jacques-Cartier, Quebec. Canadian Journal of Botany 66: 101-107.

Murdoch, A.J. and R.H. Ellis. 1992. Longevity, viability and dormancy. p.193-229. In Seeds, The Ecology of Regeneration in Plant Communities. M. Fenner, Editor. CAB International, Wallingford, UK. 373 pp.

Simpson, R.L., M.A. Leck and V.T. Parker. 1989. Seed banks: general concepts and methodological issues. p.3-8 In: Ecology of Soil Seed Banks. M.A. Leck, V.T. Parker and R.L. Simpson Editors. Academic Press Inc, San Diego. 462 pp.

Thompson, K. and J.P. Grime. 1979. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. Journal of Ecology 67: 893-921.

Vieno, M., M. Domulainen and S. Neuvonen. 1993. Seed bank composition in a subarctic pine-birch forest in Finnish Lapland: natural variation and the effect of simulated acid rain. Canadian Journal of Botany 71: 379-384.