Fundy Model Forest

~Partners in Sustainability~

Report Title: Small Scale Variability in a Mixed Temperate Forest Seed Bank

Author: B. MacInnis

Year of project: 1997

Principal contact information: University of New Brunswick, Saint John
100 Tucker Park Road
P.O. Box 5050
Saint John, New Brunswick, Canada
E2L 4L5
Phone: 506-648-5500

File Name:
Biodiversity_1997_MacInnis_Small_Scale_Variability_in_a_Mixed_Temperate_Forest_Seed_Bank

The Fundy Model Forest…
…Partners in Sustainability

"The Fundy Model Forest (FMF) is a partnership of 38 organizations that are promoting sustainable forest management practices in the Acadian Forest region."

Atlantic Society of Fish and Wildlife Biologists
Canadian Institute of Forestry
Canadian Forest Service
City of Moncton
Conservation Council of New Brunswick
Fisheries and Oceans Canada
Indian and Northern Affairs Canada
Eel Ground First Nation
Elgin Eco Association
Elmhurst Outdoors
Environment Canada
Fawcett Lumber Company
Fundy Environmental Action Group
Fundy National Park
Greater Fundy Ecosystem Research Group
INFOR, Inc.
J.D. Irving, Limited
KC Irving Chair for Sustainable Development
Maritime College of Forest Technology
NB Department of the Environment and Local Government
NB Department of Natural Resources
NB Federation of Naturalists
New Brunswick Federation of Woodlot Owners
NB Premier's Round Table on the Environment & Economy
New Brunswick School District 2
New Brunswick School District 6
Nova Forest Alliance
Petitcodiac Sportsman's Club
Red Bank First Nation
Remsoft Inc.
Southern New Brunswick Wood Cooperative Limited
Sussex and District Chamber of Commerce
Sussex Fish and Game Association
Town of Sussex
Université de Moncton
University of NB, Fredericton - Faculty of Forestry
University of NB - Saint John Campus
Village of Petitcodiac
Washademoak Environmentalists

C31

SMALL SCALE VARIABILITY IN A MIXED TEMPERATE FOREST SEED BANK

by

Ben MacInnis

A THESIS PREPARED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
Bachelor of Science with
Honours in Biology

THE UNIVERSITY OF NEW BRUNSWICK
SAINT JOHN
March, 1997

ABSTRACT

A variety of factors cause seeds to exhibit clustered distributions and low abundances in the soil. This has important implications for studies that require an accurate estimate of species richness and species abundance, especially in terms of sampling intensity. The purpose of this study was to determine the fine-scale spatial distribution of seeds in a temperate seed bank following the harvest of the Hayward Brook Watershed and to determine the minimum sampling intensity required for an accurate representation of seed bank species composition. By completely sampling the topsoil of the experimental plot and using the emergence method, eight different species were germinated from the samples. Red Maples showed the highest occurrence and abundance which is likely due to presence of adult Red Maple near the experimental plot prior to harvesting. All germinated species showed clustered distribution which can be attributed to biotic and abiotic factors. It was determined that 190×27.2 cm^3 samples would be needed to accurately estimate the mean abundances of species to a precision of $\pm$ 10%.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

The Biology Department of the University of New Brunswick, Saint John provided facilities and supplies for this project.

I would like to thank, first and foremost, Dr. Kate Frego for all her help, her patience, and her faith in me, without which this project would not have been possible. I would also like to thank Ms. Jennifer Peterson and Angela Hovey for helping with lab work and species identification; Dr. Richard Staniforth (U. of Winnipeg) for his help in the field work and his valuable discussion on seed bank studies; Dr. Paul Cavers (U. of Western Ontario) for his helpful comments on my study; Mr. Greg Adams and the Sussex Tree Nursery for the use of their facilities; Dr. Matthew Litvak for his ecological opinions; and Dr. Jim Kieffer for coordinating the honours program.

I must thank my parents for always encouraging me not to settle for anything less than my best effort, my friends for at least pretending to listen when I complained about my workload, and Jennifer Rae for her constant enthusiasm and support.

INTRODUCTION

The culmination of fertilization in heterosporous plants is the formation of seeds. Seeds are mature ovules formed within the fruits of angiosperms and the cones of gymnosperms. Each seed consists of an embryo surrounded by tough layers of sporophyte tissue called the seed coat or integument; angiosperm seeds also contain a triploid nutritive tissue called endosperm. The embryo develops to a stage of differentiation of embryonic root and shoot before metabolism is suspended. At this point, the seed is signalled to begin dehydration, the extent of which varies between species. Seeds are important adaptively in two respects (Raven and Johnson 1992):

1. their dispersal allows for migration and dispersal of plant genotypes into new habitats, and
2. they are a resistant stage which allows plants to postpone development until conditions are advantageous for growth.

A number of strategies are used by plants for seed dispersal. Species of plants with seeds adapted for water dispersal are said to be hydrochorous (Fenner 1985). These plants are typically found in or near bodies of water or on steep slopes where rainwater can carry seeds downhill. Species which disperse their seeds by animals are zoochorous (Fenner 1985). Such seeds can be attached externally to the fur or feathers, or swallowed with the fruit and later excreted. Plants with seeds adapted for wind dispersal are anemonochorous (Fenner 1985). These seeds can have small wings, hairs, or plumes which can increase the distance they can be transported by increasing their surface/volume ratio. Long distance dispersal of seeds does occur (Cavers et al. 1992), but local seed dispersal dominates (Warr et al. 1993). The success of plant regeneration depends upon the dispersal of seeds into sites with conditions that are conducive to growth. Areas meeting the requirements for germination of seeds are termed "safe sites" (Harper 1977). Success of a species in an area will be dependant on the abundance and location of these regions. If a plant is surrounded by a large number of safe sites, the expected distribution of germination would be close to the parent. There are three basic patterns of seed distribution : random, contagious (also clumped or aggregated), or

regular. In a random distribution, the location of a organism has no bearing on the location of another. In a contagious distribution, the location of one organism means a high probability of finding another member of the same species nearby (Barbour et al. 1987). In a regular distribution, there is a lower probability of finding another than would be expected by the assumption of randomness (Barbour et al. 1987).

Dormancy

Not all seeds germinate immediately following arrival in a "safe site". Seeds have developed characteristics that allow them to survive unfavourable conditions after dispersal from the parent plant. The ability of seeds to halt metabolic growth for prolonged periods is called seed dormancy. Dormancy allows the embryo within the seed to remain viable until germination. Factors such as light, water, oxygen and temperature will affect the duration of seed dormancy. Some seeds of *Lupinus arcticus* (Leguminosae) have been documented to retain their viability even when buried 10, 000 years ago (Baker et al. 1989).

Most seeds found in temperate soils are physiologically and/or physically dormant. Physiological dormancy occurs when an internal mechanism prevents germination of the embryo; this is believed to allow the embryo sufficient time to develop (Northington and Scheidner 1996). Physiological dormancy can be broken by embryo maturation, by the release of a biochemical trigger due to a change in conditions, or by removal of a chemical inhibitor (Harper 1977).

Physical dormancy occurs when the seed coat is lignified so that it is impermeable to water and oxygen, both of which are required for germination. This water-repellant property of the seed coat is due to malpighian and macrosclerid cells which form an solid layer around the embryo. Physical dormancy generally requires the penetration or destruction of some or all this layer in order for germination to occur. Attack by bacteria or fungi, acid scarification by animal digestive juices, or abrasion by rocks or other hard objects are common mechanisms for the breakdown of hard seed coats (Baskin and Baskin 1989). High temperatures, such as the intense heat of forest fires, and/or wide

temperature fluctuations can also cause the deterioration of the seed coat and allow water to enter. Some seeds may require a period of sustained low temperatures, termed stratification (Raven et al. 1992), before germination can begin. This prevents seeds of temperate species from germinating before or during winter.

Many seeds have a weak area in their coat where the breakdown is likely to develop. Once water is absorbed by the seed, germination begins. The uptake of oxygen starts oxidative metabolism and the embryo resumes growth.

Seed banks

The retention of the viability of the seed over time is critical to the formation of the seed bank, a collection of viable but ungerminated propagules on or in the soil or associated litter (Simpson et al. 1989). There are two recognised types of seed banks based on duration of seed dormancy (Thompson and Grime 1979):

1. transient - no seeds remain viable in soil for more than one year, and
2. persistent - some seeds remain viable for more than one year.

A number of studies have shown that there is a decrease in seed density down the soil profile (Kramer and Johnson, 1987). Seeds with long term survival are generally found in all seed layers while short lived seeds are usually found in the upper layers only (Warr et al. 1993). Seeds at or near the soil surface have a greater probability of germinating than those buried deeper because most seeds require light, temperature or moisture cues to trigger germination (Baskin and Baskin 1989). The density of seeds in the upper humus layer has been shown to be extremely variable (Warr et al. 1993). Only the seeds which are capable of replacing the present plants are considered as part of the seed bank, therefore seeds buried at very deep depths are not generally included.

Genetic studies of seeds and seed banks have shown that plants that germinated from seeds with less dormancy will select for seeds with significantly less dormancy (Caver pers. comm.). As a result, continuous destruction of seedling populations over a period of several years will eventually produce a population that has longer dormancy in its seeds (Cavers 1994). Other genetic studies have shown that agriculture selects for

different traits in weed vs. crop species; as a result weed races of crop species have larger seed banks and greater dormancy than crop races of the same species (Toole and Brown 1946 *in* Warr et al. 1993).

Previous studies have shown that dormant propagules vastly outnumber the growing plants of the same habitat (Harper 1977), possibly because the seeds found in seed banks can be from parent plants which vary in both space and time. Studies on seed banks and succession have shown that the majority of seeds present are derived from local plants rather than by immigration (Hill and Stevens, 1981) but that small quantities of non-local seeds could be transported in by wind or water dispersal or by animals and be incorporated into a seed bank (Kellman 1974). A lack of correlation between previous vegetation and the species composition of the seed bank has been found in wetlands (van der Valk and Davis 1976) and in woodlands (Thompson and Grime 1979; Petrov 1977 *in* Warr et al. 1993). This lack of correlation may be explained by the presistence of seeds of species which were present in the past but have been replaced by current species (Warr et al. 1993). As a result, seed banks are often used to reconstruct past community composition. Early successional species may be shade intolerant and have since been lost from the community with only their seeds remaining in the soil (Warr et al. 1993). Studies have shown that species richness as well as seed bank density decreases with successional maturity (between 50-100 years) (Kramer and Johnson 1987; Thompson 1978; Harper 1977). Late successional communities often have low numbers of viable seeds in the seed bank (Whipple 1978).

All major weeds have been shown to produce large seed banks with high seed densities (Jensen 1969 *in* Warr et al. 1993). In contrast, the seed banks of mature temperate woodlands are generally small (Kramer and Johnson 1987). Arctic and subarctic soils also have been shown to contain lower numbers of dormant seeds (Leck 1980). Short-lived seeds of herbaceous species dominate the small seed banks of these areas (Warr et al. 1993).

Importance of seed banks

Seed banks play an important role in the regeneration of plant communities. The dynamics of the seed bank guarantee the ability of a community to maintain itself and to respond to change. The seed bank may contain diverse species, phenotypes, and genotypes that can provide substantial flexibility for community responses (Leck et al. 1989). Seed banks can be found in a variety of habitats including arable lands, grasslands, pastures, landfills, wetlands and forests (Baker et al. 1989). Seed banks can even be found in the beds of lakes that periodically dry up. Darwin (1859) showed that a large number of plants may germinate from a small sample of soil taken from the bottom of a pond. The potential impact of seeds and seed banks on plant community regeneration is based partly on their species composition and probability of germination.

Seedbanks have been recognised for their contribution to the restoration of plant communities after both natural and anthropogenic disturbance (Frego pers. comm.). Plants from soil-stored seeds have been shown to be useful in preventing erosion of bared soil (Skoglund 1992). Also, desirable plants which have been lost on the surface can be restored with the use of seed banks. By removing the sod from a field, the shallow grass seed bank is removed, allowing the deeply buried seeds of the preferred species to germinate (Warr et al. 1993).

Seed banks have specifically been shown to play a major role in regeneration and recolonization in managed woodlands after harvesting (Warr et al. 1993). By killing buried seeds in a experimental plot cleared of vegetation and comparing the results to a control plot, Putz and Appanah (1987) showed that buried seeds play a more important role in revegetation than freshly dispersed seeds. The importance of buried seeds in regeneration has also been shown to depend on the gap size (Warr et al. 1993): regeneration from buried seeds is more significant in larger gaps and becomes less important in smaller gaps.

Seed bank heterogeneity

The heterogeneity of the seed bank is an important factor in determining the impact of the seed bank on the plant community. Survival, germination, and seedling competition can be affected by the densities and spatial distribution of the seeds (Warr et al. 1993). Studies on tropical (Kellman 1978), desert (Reichman 1984), and grassland soils (Schenkeveld and Verkaar, 1984) showed wide spatial variability in the seed bank at the large scale. Thompson (1986) studied small scale heterogeneity within the seed bank by completely analysing the number and distribution of seeds in a small area of soil. He showed significant clustering of the main species in the seed bank. Clustering of seeds at any scale can be the result of environmental and/or biological factors (Thompson 1978). Seeds falling directly down from the plant or coming from a single fruit will result in a clustered pattern. Wind- or water-borne seeds can collect in depressions which will also result in clustering (Harper, 1977), as can the activity of animals interacting with seeds (Frego pers. comm.; Mull and MacMahon 1996).

The spatial distribution of seeds is an important, but frequently overlooked, aspect in designing seed bank sampling methodology.

Study types

Simpson et al. (1989) state that the most critical question to be addressed when initiating seed bank studies is project design. The lack of standard methodology for seed bank studies is apparent when reviewing literature. With the number of possible variables in sampling techniques and lab research, two studies rarely use the same methods.

There have been two major approaches to seed bank studies (Cavers 1994). The autecological approach tests seeds of a particular species for dormancy and viability by burying a known number of seeds in a known area and then removing them after a period of time. One problem with this type of study is the fact that the seeds must be contained in a mesh bag when buried so that they can be removed easily. The success of seeds buried in these bags has been shown to be less than natural seeds (Cavers 1994).

A second approach is synecological: studying seed banks to determine the seed bank species composition. This usually involves the collection of soil cores from a study area, e.g. using a knife to remove blocks of soil (Warr et al. 1993), often taken randomly. The soil cores are usually taken to a greenhouse and spread on a sterilized medium where seedlings can be counted and identified.

When soil cores are used to describe a population of seeds, the number and size of each sample are important considerations. The distribution of seeds in soil has often been found to be clustered and many species occur at low densities (Warr et al. 1993). This uneven distribution of seeds in the soil requires a large number of soil samples if an accurate estimate is desired (Benoit et al. 1992; Bigwood and Inouye 1988). The labour involved in extracting the soil cores, maintaining the soil samples in the greenhouse, and counting and identifying the seedlings is substantial (Cavers 1994). Since seeds are very small, it is possible to use small sized samples without harming the organism (Bigwood and Inouye 1988) but the time and labour involved in processing small soil cores makes very small soil samples impractical. One possible method is to combine all the samples into one large sample, however this technique would not be suitable for spatial heterogeneity studies.

Previous studies have attempted to calculate the total volume of soil needed to determine species densities within certain parameters. Champness (1949 *in* Bigwood and Inouye 1988) determined that 200 samples of 25 cm^2 cores were needed to estimate the species density within 10% of the population mean. However, Bigwood and Inouye (1988) showed that this calculation does not always produce a reliable indication of the required number of samples. Thompson (1986) utilised a formula for the sampling of benthic invertebrates (Elliot 1977 *in* Warr et al. 1993) from which he determined that a minimum of 50 × 25 cm^3 samples was needed in his study of seed bank heterogeneity of grasslands. Major and Pyott (1966) have stated that all investigators have certainly taken too few cores.

The spatial distribution of the soil samples also must be considered. Random sampling is often a requirement for statistical methods, but systematic sampling can also

be used for seed bank studies. This involves the use of transects of contiguous cores (Kramer and Johnson 1987; Kellerman 1974). This method is simple and efficient but can cause problems with chance resonance with natural clustering patterns, and consequent problems of spatial autocorrelation (Warr et al. 1993).

After the procedure for collecting samples is determined, the next step is to determine the seed species and densities in the samples. One method for accomplishing this involves separating the seeds from of the soils by flotation (e.g. Benoit et al. 1989; Henderson et al. 1988; Price and Reichman 1987; Malone 1967). The samples are immersed in water and the floating seeds are skimmed off the surface. This method can give an accurate estimation of the seeds present in a sample of a soil but has several limitations: (1) it gives no indication of whether the seeds are viable or not, (2) seeds are often difficult to identify, therefore species may be over or underestimated, and (3) this process is time consuming and small seeds may be lost in the extraction. The advantage for using this method is that it does not discriminate for germination factors and dormancy (de Villiers 1994). This method may be very effective for large seeds such as *Rubus fruticosus* (Warr et al. 1993). Often, the dominant seeds are tested for viability after counting.

An alternative method to seed flotation is the emergence method (e.g. Kitajima and Tilman 1996; Sem and Enright 1996; Warr et al. 1994). In this case, the soil samples are placed in a greenhouse, watered, and tended until germination. Once the seedlings have emerged and are identified, they can be removed and the soil samples stirred to promote further germination. Great care must be taken to keep the seedlings alive until identification (Warr et al. 1993). When emergence methods are used, the size of the seed bank is likely to be underestimated due to the fact that a single treatment will not meet the specific germination requirements for of all the species present in the soil (de Villiers 1994). Further, seeds of some species will germinate over long periods of time which may require keeping the samples for a year or more (Warr et al. 1993). Emergence methods also generally require large amounts of greenhouse space and there is an inherent delay in obtaining results. The advantages for using the emergence method

are that (1) each seedling represents a viable seed, (2) the effort required is spread over a larger period of time, and (3) seedlings are usually easier to identify than seeds (de Villiers 1994). Gross (1990) suggested that emergence methods provide more information on species composition whereas separation methods are more useful for studying variations in seed distribution, especially with seeds that are easily identifiable (Warr et al. 1993).

OBJECTIVES

This study was undertaken to:

(1) determine the fine-scale spatial distribution of germinable seeds in a temperate forest seed bank, and

(2) apply the information in determining how best to sample this seed bank to accurately describe its species richness and abundance.

METHODS

Study site

The study site was located within the Hayward Brook watershed in Westmorland County near Petitcodiac, New Brunswick, Canada (lat. 45° 53' N long. 65°11' W) (Hovey unpublished). The Hayward Brook watershed is an area of coniferous, deciduous and mixed forest with lower elevations dominated by Balsam Fir (*Abies balsamaea*) and Red (*Picea rubens*), Black (*P. mariana*), and White (*P. glauca*) Spruce and higher elevations dominated by Red Maple (*Acer rubrum*), Sugar Maple (*A. saccharum*), Striped Maple (*A. pennsylvanicum*), White Birch (*Betula papyrifera*), Beech (*Fagus* sp.) and Trembling Aspen (*Populus tremuloides*) (Sims unpublished). This region is classified as Acadian Forest Region (Scoggan 1978) and is located between temperate and boreal zones of eastern Canada.

Two bedrock types underlie the Hayward Brook study area (McLeod et al. 1994 *in* Hovey unpublished). The Boss geomorphical district is composed of grey and carbonaceous mudstone, red sandstone, limestone, quartz sandstone, and quartz pebble conglomerates. The PET 3 geomorphic district forms reddish grey soils and is composed of fine to medium grained sandstone and red mudstone.

Annual precipitation in the Hayward Brook watershed is approximately 130 cm; snow levels often exceed 300 cm, approximately 16% of annual precipitation (Gowan and Brodo 1988 in Sims unpublished).

Sampling procedure

The sample plot (1.92m × 1.28m) selected for this study was located near the area designated #7 on the F transect in ongoing studies by Drs. Mark Roberts and Kate Frego within the Fundy Model Forest. The area was clear-cut by Irving Forestry in August 1995. The sample plot had been cleared of large trees, was free of rocks and deadfall and sloped slightly downward to the southeast.

Soil samples were collected 28 September 1996 by removing the upper layer of the soil in a contiguous grid design of 24 × 16 units. Each unit was removed using a

cylindrical can with diameter of 8cm and depth of 3.4 cm (27.2 cm^3). The inverted can was forced down into the topsoil and a knife was used to cut around the edge. Each soil sample was placed in a plastic ziploc bag labelled with its grid position.

Samples were processed at the University of New Brunswick in Saint John. Stones, large leaves or roots, and mosses were removed; the samples were transferred to labelled paper bags and placed in cold storage at a temperature of -5°C at the Sussex Tree Nursery, headquarters of Fundy Model Forest in Sussex, New Brunswick on 3 October 1996.

After six weeks, the soil samples were removed from cold storage and transferred to 150mm diameter plastic petri dishes and placed in the greenhouse at UNBSJ. The soil samples were thoroughly moistened with a dilute fungicide Nodamp® (10 ml per litre). The petri dishes were then placed on shelves in the greenhouse and moistened regularly with distilled water for a total of 99 days.

Once the seeds had germinated, the seedlings were transferred into sterile soil in larger growing trays. The trays were placed over metal bins containing water and clear plastic was laid over the trays to maintain high atmospheric humidity. Soils were moistened by spraying daily with distilled water.

Representative seedlings of the eight germinated species were transplanted to larger pots and grown under ideal conditions to hasten maturation and identification.

The number and location of species and seedlings in each sample was recorded. The total number of each species was then tabulated and the mean number of seedlings per sample calculated.

The index of dispersion for each species within the sample grid was calculated using the formula:

$$I_D = \frac{\sigma^2(n-1)}{\mu} \quad \text{(Southwood 1978)}$$

where I_D is the index of dispersion, σ^2 is the population variance, μ is the population mean and n = number of samples. The calculated values were compared to the χ^2 table

value (α=0.05, 383df, χ^2_{crit} = 388, 430) to determine whether the spatial distribution of seedlings differed significantly from randomness.

The minimum sampling intensities needed to ensure estimates within 10% and 5% of the population mean were calculated for two species: Red Maple was selected because it had the highest variance (i.e. it was the most spatially heterogeneous species), and Honeysuckle because it had one of the lowest means (i.e. it was a rare species). The precision of sample means was then tested by repeated random sampling at: n = 2, 4, 6, 8, 10, 18, 26, 40, 50, 60, and 104 from the population of Red Maple; n = 2, 4, 6, 8, 10, 12, 18, 26, 51, 104, 255 and 300 from the population of Honeysuckle. Confidence intervals were calculated for the estimates of the mean at each sample size. Means and confidence intervals were plotted and compared to 10% and 5% of the population mean to determine minimum sampling effort .

RESULTS

Eight species of seedlings were present in the samples (Table I). As of 14 March 1997, five of the seedling types were identified at least to the genus level : Red Maple (*Acer rubens*) , Gooseberry (*Ribes* sp.), Aster (*Aster* sp.), Sedge (*Carex* sp.) and Honeysuckle (*Viburnum* sp.) The remaining three will be classified as grasses 1 and 2 and unknown dicot.

Figure 1 shows the frequency distribution of species richness of the 384 samples. Over 75% of samples contained ≤ 2 species. Only 1% of the samples taken contained 5 different species. No sample contained 6 or more of the 8 species.

The overall percent frequency of individual species is shown in Figure 2. Red Maple showed the highest occurrence (44.8% of samples). Seven of the 8 species were present in fewer than 30% of samples. The unknown dicot was found in only 0.5% of samples.

Figure 3 shows the percent abundance of individuals species as a percentage of the total 943 seedlings. Red Maple comprised 41.46% of all seedlings; Sedge comprised 24.92%. The relative abundances of the remaining 6 species were all less than 11%.

The mean seedling density per species found in the 384 samples is shown in Figure 4. Red Maple had a mean of 1.01 seedlings per sample. Sedge had a mean of 0.611 seedlings per sample. The remaining 6 species averaged less than 0.25 seedling per sample.

The calculated indices of dispersion were all larger than the critical upper limit of χ^2, ie. all species showed contagious spatial distribution (Figure 5).

Figure 6 shows the mean and confidence intervals (CI) for increasing number of samples for Honeysuckle. Figure 7 shows the means and confidence intervals for increasing sample sizes for Red Maple. Increasing sample size showed similar trends in mean and confidence intervals for both species. The calculated mean seedling abundances for both species approached the known population mean, and the confidence intervals declined with an inverse logarithmic pattern.

Table I. Comparison of vascular plant species present in quadrat F7 of Hayward Brook Watershed before harvesting, and germinated seedlings. (Hovey unpublished)

Species	Pre-harvest vegetation (1995)	Germinated species (1997)
Bracken fern (*Pteridium aquilinum*)	✓	
Bunchberry (*Cornus canadensis*)	✓	
Gooseberry (*Ribes* sp.)		✓
Balsam Fir (*Abies balsamaea*)	✓	
Black Spruce (*Picea mariana*)	✓	
Red Maple (*Acer rubens*)	✓	✓
Blueberry *(Vaccinium myrilloides)*	✓	
Blueberry (*V.. angustilfolium*)	✓	
Honeysuckle (*Viburnum* sp.)	✓	✓
Aster (*Aster* sp.)		✓
Sedge (*Carex* sp.)		✓
Starflower (*Trientalis borealis*)	✓	

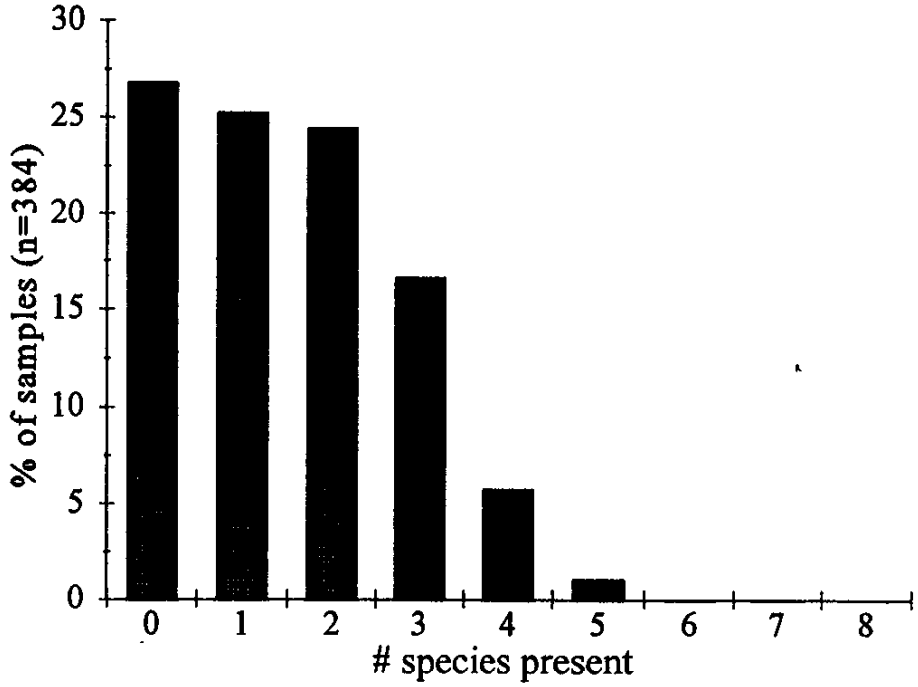

Figure 1. Species richness of germinated seedlings from the Hayward Brook soil samples

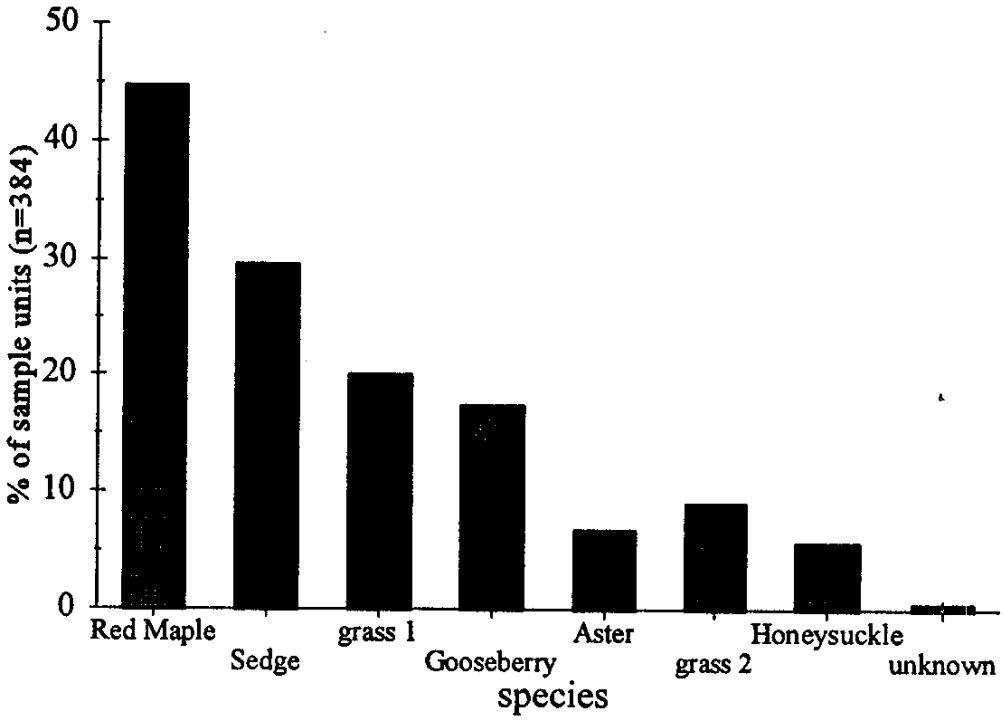

Figure 2. Percentage of sample units containing germinated species from Hayward Brook soil samples.

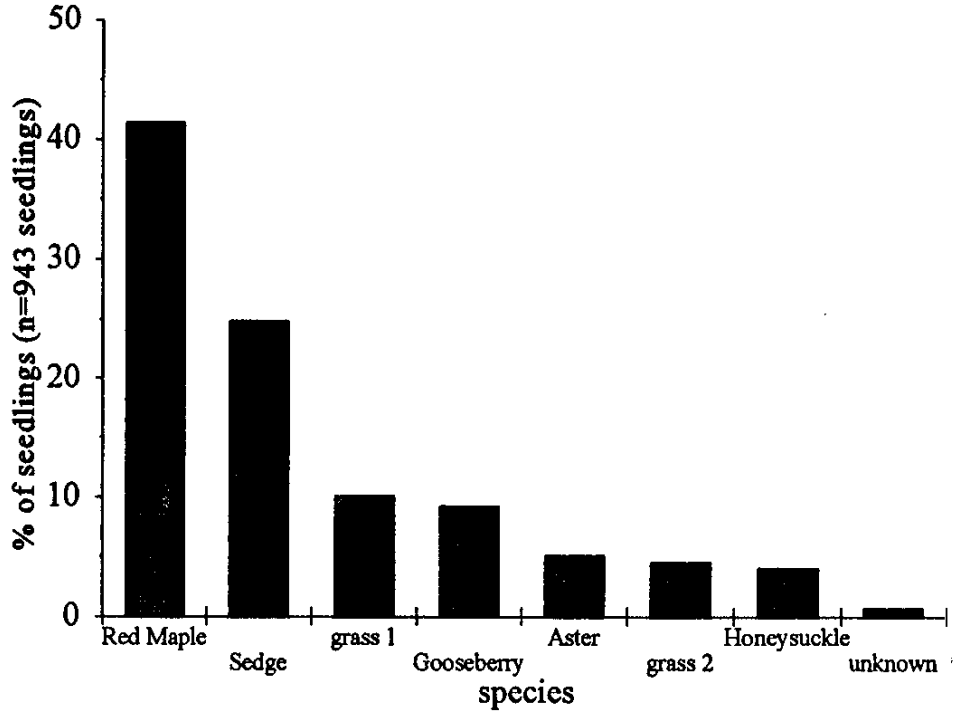

Figure 3. Relative species abundance of germinated seedlings from Hayward Brook soil samples.

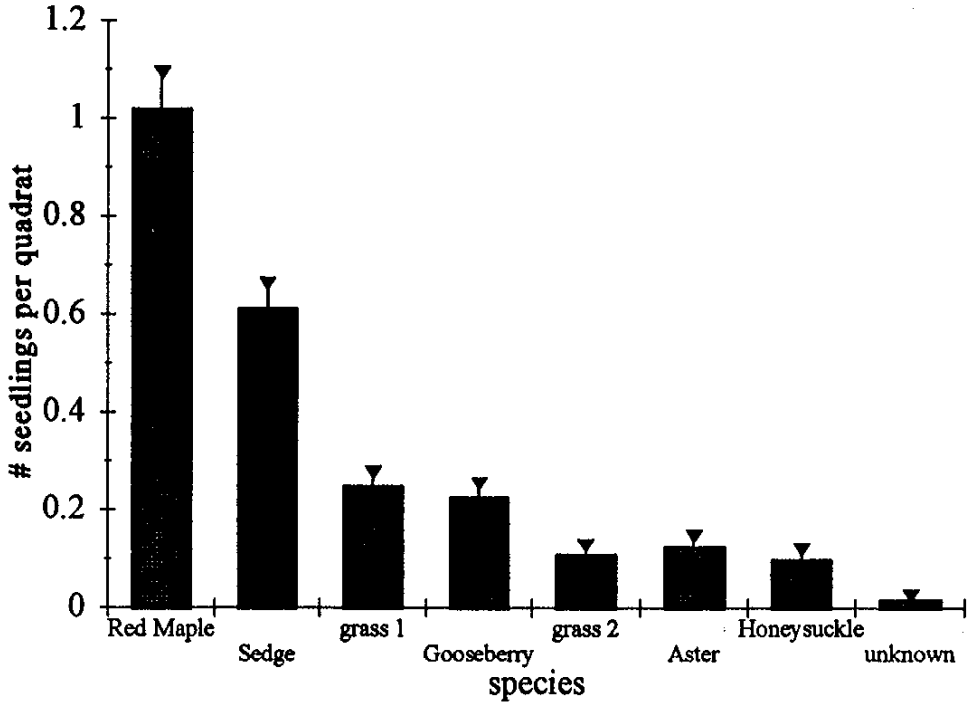

Figure 4. Average seedling density of germinated species from Hayward Brook soil samples. (standard deviations are shown)

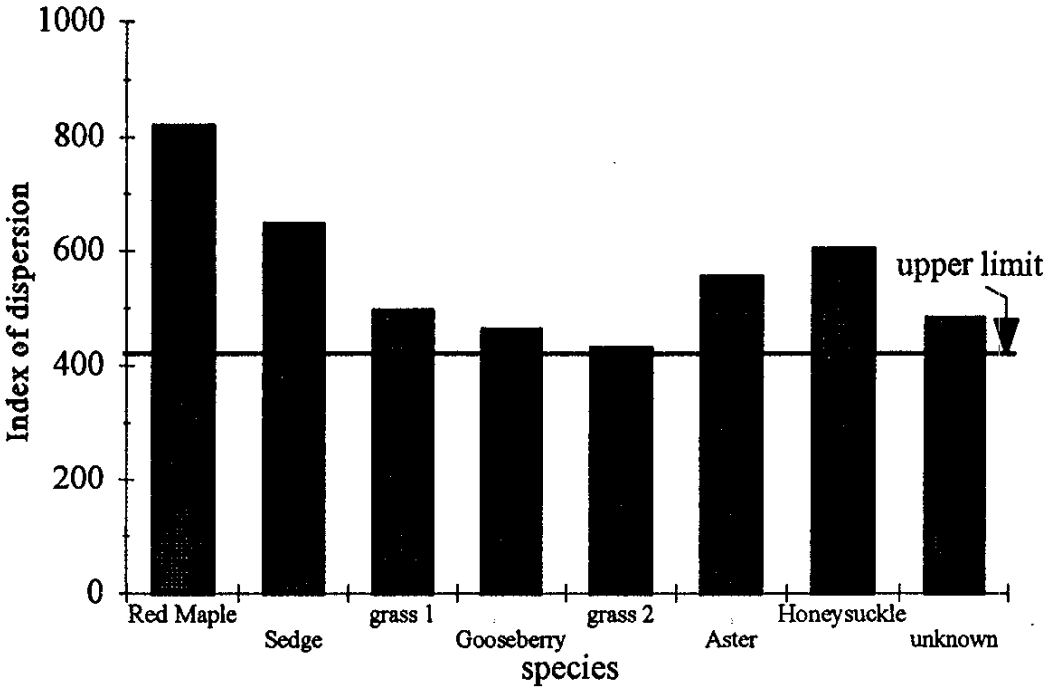

Figure 5. Index of dispersion of germinated seedlings from Hayward Brook soil samples. Upper limit line represents upper critical χ^2 value.

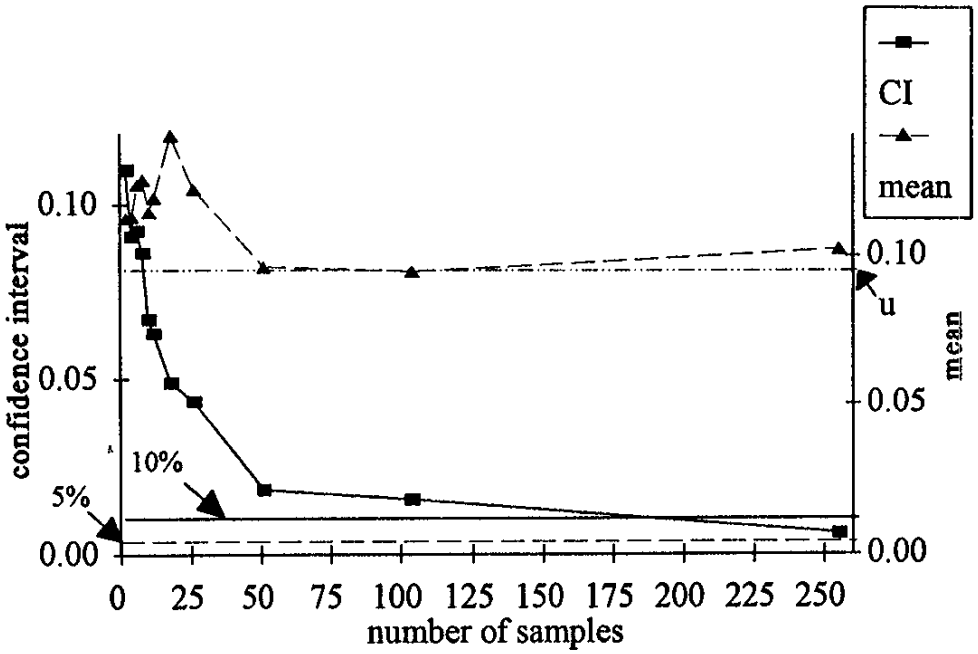

Figure 6. Estimates of mean abundance and the respective confidence limits from random samples of Honeysuckle (*Viburnum* sp.) in Hayward Brook soil samples. CI line designates confidence interval ($\bar{x} \pm t_{\alpha\,(2),\nu}\, s_{\bar{x}}$) of estimates of mean abundance; mean represents estimates of mean abundance; u represents population mean; 10% represents a confidence interval within 10% of the population mean; 5% represents a confidence interval within 5% of the population mean.

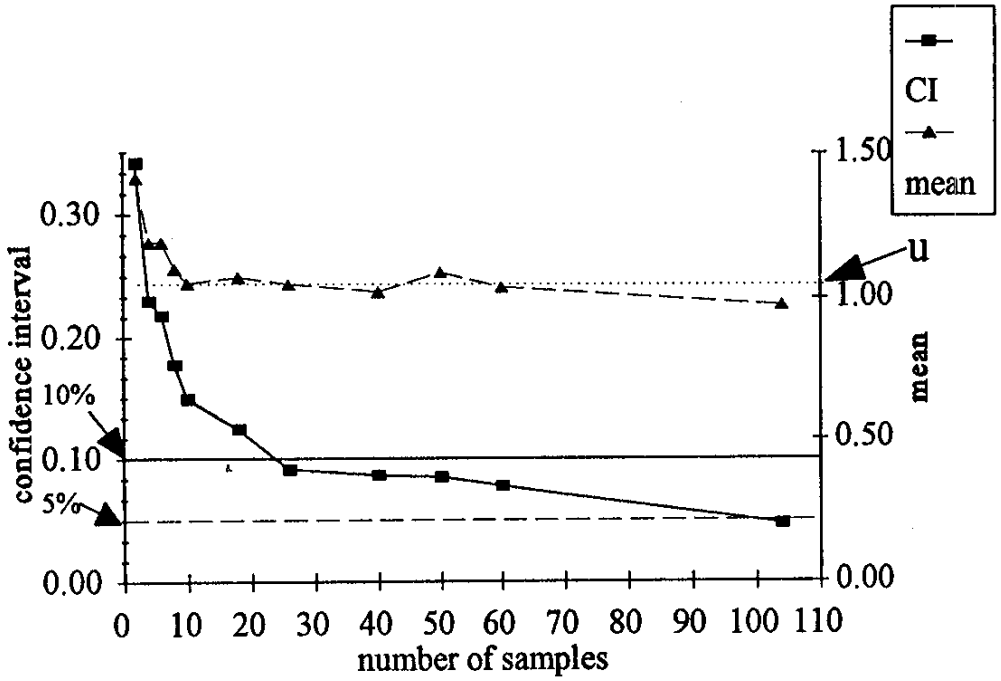

Figure 7. Estimates of mean abundance and the respective confidence limits from random samples of Red Maple (*Acer rubens*) in Hayward Brook soil samples. CI line designates confidence interval ($\bar{x} \pm t_{\alpha(2),\nu} s_{\bar{x}}$) of estimates of mean abundance; mean represents estimates of mean abundance; u represents population mean; 10% represents a confidence interval within 10% of the population mean; 5% represents a confidence interval within 5% of the population mean.

DISCUSSION

Species richness

Species present in the samples included herbs (unknown grasses, sedge and dicot), trees (Red Maple), and shrubs (Honeysuckle). Species richness of the sampled plot's seed bank is low when compared to the known flora present near sampling site before harvesting, which contained at least 9 vascular species: 3 herbs, 3 shrubs and 3 trees (Table I) (Hovey unpublished). Such dissimilarity between seed banks and the associated vegetation has been found in other temperate woodlands (e.g. Warr et al. 1994). There are several reasons for low seed bank species richness. (1) The absence of seedlings of most deciduous tree species may be due to the characteristically short life span of their seeds (Warr et al. 1994). (2) Other mature forests (between 50-100 years) such as this area have shown decreases in species richness as well as seed bank density with successional age (Kramer and Johnson 1987; Thompson 1978; Harper 1977). (3) Timing of sampling can affect species density and richness. Sem and Enright (1996) have shown that the maximum density of seed rain occurs in late autumn for some species of temperate trees. The experimental samples in this study were collected in early fall just before the annual seed rain. (4) Thompson and Grime (1979) stated that some transient seeds germinate in fall while some require period of chilling before germination. It is possible that seeds of the current year, which would normally germinate immediately, were inducted into a dormant state by the cold storage treatment.

Species abundance

Red Maple was the most abundant species in the seed bank in terms of occurrence (44.8% of samples), relative abundance (41.46% of seedlings) and seedling density (mean of 1.01 seedlings per sample). Although the Maple's winged "key" (samara) increases its dispersibility, long distance dispersal is not a likely explanation as the fruit is heavy and is not generally carried further than a few metres from the parent plant. The data on species present before harvesting show that mature Red Maples were present in quadrat F7 (Hovey unpublished). The abundance of local seed source

coupled with experimental conditions that meet the specific germination requirements for Red Maple presumably account for its high abundance.

The presence of Honeysuckle can also be explained by the local seed source, i.e. presence of adults in the preharvest community (Hovey unpublished). The fleshy fruit of this species is also not suited for long distance wind dispersal, but is likely to fall near the parent shrub.

The presence of Aster seeds in the sample plot cannot be explained by the presence of adult plants in the previous vegetation. However, Aster seeds are suited for long distance wind dispersal. It has been shown that sites in early successional stages, as our recently cleared sample plot, tend to have large numbers of windborne seeds (Fenner 1985).

The remaining five species showed much lower occurrences (<30% of samples), abundances (<25% of seedlings) and mean seedling densities (<0.7 seedlings per quadrat). One explanation for the rarity of these five species is experimental: the treatment may not have met their germination requirements. Even with homogeneous treatment of the soil samples, species differ in germination stimuli and the micro-environment surrounding the seed will determine whether it receives the stimuli, conditions and resources required for germination (Harper 1977). Possible ecological reasons for species absence or rarity are: (1) low seed production of that species in the sample plot area, (2) poor dispersal from parent plants, (3) heavy seed predation, (4) or high seed mortality (Fenner 1985). Also, the length of time given for the seeds to germinate may affect species abundances and richness. Warr et al. (1993) and Roberts (1981) state that a minimum of two years of experimental germination is required for an exhaustive estimate of all viable seeds. For reasons of practicality, this length of study is rarely seen.

To successfully determine the abundance of viable seeds, the emergence method is sometimes followed by a separation technique to extract seeds that have not yet germinated (Cavers 1994; de Villiers 1993). A tetrazolium test is then used to determine

their viability (Cavers pers. comm.). However, these procedures are beyond the scope of this study.

Spatial distribution

The seedlings of all eight species showed contagious distribution within the sample plot, which fits the general expectation that seeds have clustered spatial distribution (Bigwood and Inouye 1988). This supports the conclusions of Thompson (1986) who also found significant clustering of the main species in a small plot of a pasture seed bank. The concentration of seeds in a small area can be the result of the dispersal strategies of the species, and/or of biotic or abiotic factors which may involve biological tolerance or chance. For example, the reproduction and dispersal strategies of the species can cause the seed distribution to be aggregated. The fruits of some species, including grasses, Sedge, and Honeysuckle, tend to fall close to the parent, resulting in a dense local distribution of seeds. As well, multi seeded fruits may produce a clump of seedlings in a small area. This could eventually result in a clustered pattern of adult plants and perpetuate the aggregation.

Biotic environmental effects can result in a clustered seed distribution. For example, granivores such as rodents and ants can also affect seed density and composition (Inouye et al. 1980). Mull and McMahon (1994) found that seed densities were high near harvester ant nests but very low close to the foraging trails of these ants. Although animal activity was not documented in this study, the foraging of small animals and insects has been shown to increase following a clear cut (Frego pers. comm.).

Abiotic environmental factors such as wind and water can cause seeds to be concentrated in certain areas. Post dispersal movement can accumulate seeds in areas such as cracks or depressions (Harper 1977). Wind and water redistribute surface-lying seeds and deposit them in depressions or the wind shadow of shrubs (Reichman 1984). Seeds of Aster, grasses and Sedge are small and therefore could easily be dispersed by this movement.

Because species have specific ranges of ecological tolerance, micro-environments which are suitable for one species, either in terms of germination or later growing conditions, will tend to become densely populated with that species (Barbour et al. 1987). For example, depressions in areas that are prone to surface desiccation may contain moist soil which enhances germination (Bigwood and Inouye 1987) which in turn produces an aggregated seed source. The plot used in this study was sloped slightly with no visible depressions or habitat irregularities at the scale of metres. Microsite differences may exist at a minute spatial scale.

Implications of contagious distribution

The clustered distribution of a seed bank results in an inherent variability in samples (Bigwood and Inouye 1988) which has important implications in terms of both the sample number and volume required to estimate its characteristics. A high sampling intensity is needed for highly variable populations and/or when the species being studied are present in low numbers. Both conditions were clearly present in this study. Although the number of samples needed for an accurate representation of soil stored seed banks has been discussed in many publications (e.g. Warr et al. 1993; Bigwood and Inouye 1988; Thompson 1986; Major and Pyott 1966), no consensus has yet been reached. Studies have attempted to provide a reasonable estimate of density in all but the most rare and/or clustered species (Warr et al. 1993) but seed bank researchers commonly reduce sampling to a 'reasonable' number at the expense of precision (Benoit et al. 1992). Ideally a preliminary study should be made to determine local variability on which to base sample size. Even where this is not feasible, it is desirable to obtain sufficient data to calculate whether increasing the sampling intensity would bring about a worthwhile gain in accuracy (Roberts 1981).

This study has attempted to ascertain the minimum sampling intensity needed to estimate the mean seedling abundance of a locally rare species and the most clustered (highest variance) species encountered. For Honeysuckle (rare: mean seedling density 0.09896), it was shown that 190 - 27.2cm^3 soil samples would be needed to obtain an

estimate of the mean abundance with standard error within 10% of the population mean (Figure 6). No significant drop in the precision was found after 300 samples. The high number of samples required for estimating the abundance of Honeysuckle is likely due to its rarity in the sample plot.

For Red Maple (clustered: variance 2.18456; χ^2 value 821.709), 25 - 27.2 cm^3 soil samples would be sufficient to obtain a estimate of the mean within 10% (Figure 7). One hundred samples would be required for an estimate within 5% of the mean. The high number of samples needed for a precise estimate of Red Maple density is likely due to its clustered distribution (high variance).

Figures 6 and 7 clearly demonstrate the generalization that precision increases with an increase in sample numbers, but there is a law of diminishing returns (Green 1979). The standard error of the mean will decrease in proportion to the square root of the sample number. For example, an increase from n=4 to n=9 will reduce the standard error and the width of the confidence limits by one third. To achieve another reduction of one third, the sample number must to be increased to n=21 (Green 1979). There is a limit at which the confidence width of the estimation of the mean will no longer decrease sufficiently to warrant the costs of a further increase in sampling intensity. Based on the data for Honeysuckle, this study found that a sample intensity of more than 190 - 27.2cm^3 soil cores would be needed to estimate the mean of all species present within 10%. To estimate the mean of all species within 5% is not feasible. It has been shown that for the rarest species it is probably impossible to sample with an intensity that will result in a reasonably small standard error (Bigwood and Inouye 1988).

In this study, the minimum sampling size for a rare species of n=190 of 27.2 cm^3 (precision of ± 10% of mean) soil cores shows that previous studies using less than 100 samples (e.g. Benoit et al 1989; Thompson 1986) may not give reliable estimates of seed or seedling densities of all species present.

Conclusions

The fine-scale distribution of seeds in the sampled area was shown to be clustered for all germinated species. This has important implications when considering the necessary sampling intensity. It was determined that 190-27.2 cm^3 soil samples (precision ± 10%) would be needed to accurately estimate the mean abundance of all species but the rarest. To estimate the mean abundance within 5% of common species would require 100 - 27.2 cm^3. It is not reasonable to attempt to estimate the mean abundance of rare species within 5%.

Future work

Future work on this topic could include: (1) continued germination and growing of seedlings so that all species present could be identified to the species level, (2) extraction of non germinated seeds from the soil samples, coupled with a viability test, to determine total seed bank content, and (3) more sophisticated statistical analyses to determine the most effective spatial sampling design.

LITERATURE CITED

Baker, H. 1989. Some Aspects of the Natural History of Seed Banks. In Ecology of soil seed banks. Edited by M. Leck, V. Parker and R. Simpson. Academic Press, Toronto, Ont.

Barbour, M., Burk, J. and Pitts, W. 1987. Terrestrial Plant Ecology (2nd ed.). Benjamin Cummings. Don Mills, Ont.

Baskin, J. and Baskin, C. 1989. Physiology of dormancy and germination in relation to seed bank ecology. In Ecology of soil seed banks. Edited by M. Leck, V. Parker and R. Simpson. Academic Press, Inc., Toronto, Ont.

Benoit, D., Kenkel, N. and Cavers, P. 1989. Factors influencing the precision of soil stored seed bank estimates. Canadian Journal of Botany 67: 2833-2840.

Benoit, D., Derkesen, D. and Panneton, B. 1992. Innovative approaches to seedbank studies. Weed Science 40:660-669.

Cavers, P., Kane, M. and O'Toole, J. 1992. Importance of seedbanks for establishment of newly introduced weeds - a case study of Proso Millet (*Panicum milaceum*). Weed Science 40: 630-635.

Cavers, P. 1994. Seed banks: memory in soil. Canadian Journal of Soil Science 75: 11-13.

Champness, S. 1949. Notes on the technique of sampling soil to determine the content of buried seeds. Journal of the British Grassland Society 4: 115-118. Cited in

Bigwood, D. and Inouye D. 1988. Spatial pattern analysis of seed banks: an improved method and optimized sampling. Ecology 69 (2):497-507.

Darwin, C. 1859. The origin of species by means of natural selection. Random House, Toronto, Ont.

de Villiers, A., Rooyen, M. and Theron, G. 1994. Comparison of two methods for estimating the size of the viable seed bank of two plant communities in the Stranveld of the west coast, South Africa. South African Journal of Botany 60 (1): 81-84.

Elliot, J. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. Ambleside Freshwater Biological Association, 128-131. Cited in Warr, S. J., Thompson, K. and Kent, M. 1993. Seed banks as a neglected area of biogeographic research: a review of literature and sampling techniques. Progress in Physical Geography 17(3): 329-347.

Fenner, M. 1985. Seed ecology. Chapman and Hall. New York, NY.

Gowan, S. and Brodo, I. 1988. The lichens of Fundy National Park, New Brunswick, Canada. Quarterly Journal of the American Bryological and Lichenological Society 91: 255-325. Cited in Sims, M. "Distribution of forest floor bryophytes in a north temperate forest." Honours thesis. University of New Brunswick, 1996.

Green, R. 1979. Sampling design and statistical methods for environmental biologists. Wiley- Interscience, Toronto, Ont.

Gross, K. 1990. A comparison of methods for estimating seed numbers in soil. Journal of Ecology 78, 1079-1093.

Harper, J. 1977. Population Biology of Plants. Academic Press. New York, NY.

Henderson, C., Petersen, K. and Redak, R. 1988. Spatial and temporal patterns in the seed bank and vegetation of a desert grassland community. Journal of Ecology 76: 717-728.

Hill, M. and Stevens, P. 1981. The density of viable seed in soils of forest plantations in upland Britain. Journal of Ecology 69:693-709.

Hovey, A. "Structure of herbaceous community in a mixed forest in the Hayward Brook Watershed, New Brunswick." Honours thesis. University of New Brunswick, 1996 .

Inouye, R., Byers, G. and Brown, J. 1980. Effects of predation and competition on survivorship, fecundity, and community structure of desert annuals. Ecology 61: 1344-1351.

Jensen, H. 1969. Content of buried seed in arable soil in Denmark and its relation to the weed population. Danske Botaniske Arkiv 27: 1-55. Cited in Warr, S. J., Thompson, K. and Kent, M. 1993. Seed banks as a neglected area of biogeographic research: a review of literature and sampling techniques. Progress in Physical Geography 17(3): 329-347.

Kellman, M. 1974. Preliminary seed budgets for two plant communities in coastal British Columbia. Journal of Biogeography 1: 123-133.

———. 1978. Microdistribution of viable weed seed in two tropical soils. Journal of Biogeography 5: 291-300.

Kitajima, K. and Tilman, D. 1996. Seed banks and seedling establishment on an experimental productivity gradient. Oikos 76: 381-391.

Kramer, N. and Johnson, F. 1987. Mature forest seed banks of three habitat types in central Idaho. Canadian Journal of Botany 65: 1961-1967.

Leck, M. 1980. Germination in Barrow, Alaska, tundra soil cores. Arctic and Alpine Research 12: 343-349.

Leck, M., Parker, V. and Simpson, R. 1989. Ecology of soil seed banks. Academic Press, Inc., Toronto, Ont.

Major, J. and Pyott, W. 1966. Buried, viable seeds in two California bunchgrass sites and their bearing on the definition of a flora. Vegetatio 13: 253-282.

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

McLeod, J., Johnson, S. and Ruttenburg, A. 1994. Geological map of southeastern New Brunswick. NBDNRE, Mineral Resources Division. NR-6. Cited in Hovey, A. "Structure of herbaceous community in a mixed forest in the Hayward Brook Watershed, New Brunswick." Honours thesis. University of New Brunswick, 1996 .

Mull, J. and MacMahon, J. 1996. Factors determining the spatial variability of seed densities in a shrub-steppe ecosystem: the role of harvester ants. Journal of Arid Environments 32: 181-192.

Northington, D. and Schneider, E. 1996. The botanical world (2nd ed.). Wm. C. Brown, Toronto, Ont.

Petrov, V. 1977. Reserve of viable plant seeds in the uppermost soil layer beneath the canopies of coniferous and small-leaved forests. Vestnik Moskovskogo Universiteta Biologiya 32: 43-55. Cited in Warr, S. J., Thompson, K. and Kent, M. 1993. Seed banks as a neglected area of biogeographic research: a review of literature and sampling techniques. Progress in Physical Geography 17(3): 329-347.

Price, M. and Reichman, O. 1987. Distribution of seeds in Sonoran desert soils: implications for heteromyid rodent foraging. Ecology 68: 1797-1811.

Putz, F. and Appanah, S. 1987. Buried seeds, newly dispersed seeds, and the dynamics of a lowland forest in Malaysia. Biotopica 19: 326-333.

Raven, P. and Johnson, G. 1992. Biology (3rd edition). Mosby Year Book, Toronto, Ont.

Raven, P., Evert, R. and Eichorn, S., 1992. Biology of plants (5th ed.). Worth Publishers, New York, NY.

Reichman, O. 1984. Spatial and temporal variation of seed distributions in Sonoran Desert soils. Journal of Biogeography 11: 1-11.

Roberts, H.A. 1981. Seed banks in soil. Advances in applied biology. 6: 1-55.

Scoggan, H. 1978. The Flora of Canada: Part 1 - General Survey. National Museum of Natural Sciences. Ottawa, Ont.

Sem, G. and Enright, N. 1996. The relationship between seed rain and the soil stored seed bank in a temperate rainforest stand near Auckland, New Zealand. New Zealand Journal of Botany 34: 215-226.

Southwood, T. 1984. Ecological methods with particular reference to the study of insect populations (2nd ed.). Chapman and Hall, New York, NY.

Simpson, R., Leck, M., Parker, V. 1989. Seed banks: general concepts and methodological issues. In Ecology of soil seed banks. Edited by M. Leck, V. Parker and R. Simpson. Academic Press, Inc., Toronto, Ont.

Sims, M. "Distribution of forest floor bryophytes in a north temperate forest." Honours thesis. University of New Brunswick, 1996.

Schenkeveld, A. and Verkaar, H. 1984. The ecology of short-lived forbs in chalk grasslands: distribution of germinative seeds and its significance for seedling emergence. Journal of Biogeography 11: 251-260.

Skoglund, J. 1992. The role of seed banks in vegetation dynamics and restoration of dry tropical ecosystems. Journal of Vegetation Science 3: 357-360.

Thompson, K. 1978. The occurrence of buried viable seeds in relation to environmental gradients. Journal of Biogeography 5: 424-430.

_____. 1986. Small scale heterogeneity in the soil stored seed bank of an acid grassland. Journal of Ecology 74: 733-738.

Thompson, K. and Grime, J. 1979. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. Journal of Ecology 7: 236-241.

Toole, E. and Brown, E. 1946. Final results of the Duvel buried seed experiment. Journal of Agricultural Research 72: 202-210. Cited in Warr, S. J., Thompson, K. and Kent, M. 1993. Seed banks as a neglected area of biogeographic research: a review of literature and sampling techniques. Progress in Physical Geography 17(3): 329-347.

van der Valk, A. and Davis, C. 1976. The seed banks of prairie glacial marshes. Canadian Journal of Botany 54: 1823-1838.

Warr, S. J., Thompson, K. and Kent, M. 1993. Seed banks as a neglected area of biogeographic research: a review of literature and sampling techniques. Progress in Physical Geography 17(3): 329-347.

Warr, S., Kent, M. and Thompson, K. 1994. Seed bank composition and variability in five woodlands in south-west England. Journal of Biogeography 21:151-168.

Whipple, S. 1978. The relationship of buried, germinating seeds to vegetation in an old-growth Colorado subalpine forest. Canadian Journal of Botany. 56: 1505-1509.