Functional Ecology 1996
10, 144–151

TECHNICAL REPORT

An improved method for seed-bank analysis: seedling emergence after removing the soil by sieving

G. N. J. TER HEERDT,*† G. L. VERWEIJ*, R. M. BEKKER* and J. P. BAKKER*

**Laboratory of Plant Ecology, University of Groningen, Biological Centre, PO Box 14, 9750 AA Haren and †Ministry of Transport and Public Works, Directorate IJsselmeergebied, PO Box 600, 8200 AP Lelystad, the Netherlands*

Summary

1. The seedling emergence method for assessing the size of the seed bank is improved by washing soil samples on a fine sieve and spreading the thus concentrated samples in a 3–5 mm thick layer on sterilized potting compost.

2. The method largely increases the number of seedlings that emerge as compared to unconcentrated samples. Hand-sorting afterwards shows that the germination rates vary between 81 and 100% of the viable seeds present.

3. Ninety-five per cent of the seedlings will emerge within 6 weeks using this method.

4. The method greatly reduces the greenhouse space needed and enables examination of large sample volumes.

Key-words: Sample size, seed distribution, seed separation method, seedling emergence method, seedling emergence rates

Functional Ecology (1996) **10,** 144–151

Introduction

In order to perform large-scale studies on the seed content of soils, a method is required that is reliable, fast and not space consuming. Two groups of methods are generally used to estimate the composition of a seed bank, namely seed separation methods and seedling emergence methods (Roberts 1981).

Seed separation methods utilize differences in size or density to separate seeds from the soil. Commonly used techniques are flotation (Malone 1967) and/or sieving (Roberts 1981). The residue still contains soil-material and hand-sorting under a binocular microscope is needed to collect the seeds. The viability of seeds is determinated later (Benz, Koch & Moosmann 1984; Bernhardt & Hurka 1989; Gross 1990). Seed separation methods are very effective in finding large-seeded species (Malone 1967; Fay & Olson 1978; Benz *et al.* 1984). However, they are very time-consuming and ineffective for small-seeded species, especially in large-scale studies. A preliminary study confirmed that this method was too laborious to be used in our research.

In seedling emergence methods the soil samples are spread in trays in a greenhouse and kept under those conditions known to promote the germination of as many species and individuals as possible. The seedlings are identified and counted (Thompson & Grime 1979; Roberts 1981; Bigwood & Inouye 1988; Gross 1990). Ungerminated viable seeds can be detected by hand-sorting afterwards (Moore & Wein 1977). Seedling emergence methods are simple and appropriate for large-scale studies but have some disadvantages. Many authors quote Thompson & Grime (1979) who state that a seedling emergence method is 'not designed to provide a complete assessment of the seed flora present'. According to Major & Pyott (1966) and Galinato & Van Der Valk (1986) species differ greatly in germination requirements, therefore greenhouse conditions are not always suitable for the germination of all species. It is also clear that seeds in a state of 'natural' dormancy will not germinate (Brenchley & Warington 1930; Fenner 1985).

Generally the samples are spread in a layer of 1 cm thickness or more. However, Brenchley & Warington (1930), Bakker (1960), Kropáč (1966), Williams (1969) and Galinato & Van Der Valk (1986) show that only the seeds at the surface of the sample will germinate, especially in clay soils. Seeds deeper in the soil may not germinate because the amount of light that reaches the seeds is too low (Fenner 1985; Grime, Hodgson & Hunt 1988). The deeper a seed is buried, the less affected it will be by daily temperature cycles and the lower the germination rate will be (Grime *et al.* 1981; Thompson & Grime 1983; Fenner 1985). Ideally the method should provide a reliable estimate of both the number of species and the number of individuals. The seedling emergence method, therefore, had to be modified to assure high germination rates.

Another disadvantage of the seedling emergence method is that the soil samples must be kept in the greenhouse for a considerable time. Roberts (1981) suggests that a period of 2 years would be a reasonable compromise but this would be much too long for most purposes.

Moreover, greenhouse space limits the amount of soil which can be studied with a seedling emergence method. According to Brenchley & Warington (1930), Kropáč (1966) and Baralis & Chadoeuf (1980) the bulk can be reduced by sieving. The alternative would be to take fewer or smaller samples.

In this paper we report trials with the seedling emergence method modified by concentrating the soil samples by washing them with water on a fine sieve, and spreading them in a very thin layer. The removal of clay, and other fine material, could stimulate the germination rate by increasing the gaseous exchange and lowering the soil water potential (Kropáč 1966; Major & Pyott 1966; Fenner 1985). Concentration and using thin sample layers ensure that all the seeds are exposed to light and suitable temperatures.

The aim of this study is to test whether these modifications provide a more accurate estimation of the seed-bank composition and distribution, as compared to unconcentrated samples.

Methods

SOIL SAMPLING

Sixteen clay soil samples were taken in the Oostvaardersplassen nature reserve. Two different sub-areas in this marsh were studied. In the lake area (nine samples) there was no established vegetation and the seed densities were low. The mixed area (seven samples) was surrounded by vegetation and the seed densities were high (Ter Heerdt & Drost 1994). Each sample comprised 10 replicates from contiguous quadrats of $15 \times 15\,m^2$ each. Thus 160 quadrats were sampled. This procedure was adopted in order to enable quantification of different methods and to analyse the distribution of seeds in the soil. One litre of soil was taken from each quadrat; a subsample of 925 ml was concentrated, 75 ml was not concentrated.

In a separate experiment one sample was taken from peaty soil and one from sandy soil near the Anloërdiepje (Bakker 1989, p.122). Each sample comprised five replicates from contiguous quadrats of $5 \times 5\,m^2$ each. Two litres of soil were taken from each quadrat; a subsample of 1 l was concentrated, 1 l was not.

Sampling was carried out in early spring, before new seed fall and after natural stratification of the seeds during the winter.

CONCENTRATING

First, the volume of each replica was carefully measured. The replicates were concentrated by washing them with a harsh jet of water through a coarse (4·0 mm mesh width) and a fine sieve (0·212 mm mesh width), removing both coarse and fine soil material, roots and vegetative parts. The mesh was small enough to catch the seeds of *Juncus* spp., the smallest seeds that were expected to be found. If, after concentrating, the volume of a clay replicate was too high to fit in one pot, this replica was subdivided using a sieve with a 0·424 mm mesh width. This procedure also separated smaller and larger seeds, which made it easier to collect the seedlings afterwards.

SEEDLING EMERGENCE

The replicates, both concentrated and unconcentrated, were mixed with water until they became fluid and were then poured into a 3–5mm thick layer on pots or trays filled with steam-sterilized potting soil. A 4 mm thick layer of sterile sand prevented contact between the sample and the potting soil which made it possible to re-collect the samples.

All mud-flat species and helophytes that were expected to be found in the clay samples require a very wet soil, light, high and fluctuating temperatures in order to germinate (Ter Heerdt & Drost 1994). The pots with the samples were placed in tubs filled with water to 4 cm below the soil level. The greenhouse was not shaded and supplementary light was available between 06·00 and 21·00 h. The temperature in the greenhouse was 25 °C between 06·00 and 19·00 h, but during hot summer days the temperature sometimes rose to 38 °C. Between 19·00 and 06·00 h the temperature was 15 °C.

The species that were expected to be found in the peaty and sandy samples appeared to germinate under less wet and hot conditions (Bakker 1989, p.32). Therefore they were only watered from above and the greenhouse was shaded during hot weather.

Seedlings were counted and removed as soon as they could be identified, generally immediately after the cotyledons appeared. Seedlings that were not recognized were transplanted to empty pots and allowed to grow until they could be identified. Nomenclature of species follows Van Der Meijden *et al.* (1990).

Generally germination had stopped after a period of 5–6 weeks. When no further seedling emergence was recorded for more than 1 week, the pots and trays were left drying out for 1 week. When the sample layers had hardened, they were disturbed by crumbling or turning them upside down. They were then provided with water for another 6 week period.

SEED SEPARATION AND HAND-SORTING

After the germination treatment the samples were checked for ungerminated viable seeds. Some of both the concentrated and unconcentrated subsamples were sieved on three sieves (mesh width 4 mm, 2 mm and 0·212 mm) in order to dispose of most of the remain-

ing sand and the mosses which established during the emergence period.

The re-collected material was hand-sorted under a × 96 magnifying binocular. Very small amounts of material were spread in a thin and narrow line, taking care that the particles did not overlap.

A seed was considered to be viable when a cross-section showed that the endosperm was white and appeared healthy.

DATA ANALYSIS

The number of seeds in ith replica was expressed as the number of seedlings per litre (z_i).

The Kolmogorov–Smirnov test on $\log_{10}(z_i + 1)$ transformed data was used to determine whether or not the distribution was lognormal as expected (Sokal & Rohlf 1980).

The number of replicates necessary to assure with 99% probability that a given species will occur at least once in the sample ($q°$) was computed according to Lloyd (1967):

$$q° = \frac{(\frac{m^*}{m} - 1)\ln(100)}{\ln(m^* - m + 1)} \text{ where } m^* = \frac{\sum_{i=1}^{s} z_i (z_i - 1)}{\sum_{i=1}^{s} z_i} \text{ and}$$

$$m = \frac{\sum_{i=1}^{s} z_i}{n}.$$

Results

SEEDLING EMERGENCE

Twenty-eight species were present in sufficient numbers to compare the seedling emergence method with and without concentrating. Species names are not of great importance, to save space they are replaced by numbers. Species names are listed in Appendix 1.

Wilcoxon's signed-ranks test showed that the number of seedlings per litre per species found with concentrating was larger than, or not significantly different from, the number of seedlings per litre found without concentrating (Table 1a,b,c). The number of seedlings per litre of all species together differed significantly between the two methods (Table 1a,b,c).

NUMBER OF SPECIES

With concentrating, many more species emerged in total than without concentrating (Table 2). The number of species per replica found with concentrating was larger than, or not significantly different from, the number found without concentrating. Differences were relatively large (Table 2).

GERMINATION RATES

The clay sample with the highest number of species was checked for ungerminated seeds by hand-sorting.

Table 1a. Clay soil: seedling emergence (median and mean in seedlings per litre), and differences between the two methods tested with Wilcoxon's signed-ranks test. Replicates in which a given species was not present were ignored

	Lake area							Mixed area						
	Median		Mean					Median		Mean				
Sp.	Concentrated	Not concentrated	Concentrated	Not concentrated	n	z_i concentrated > z_i unconcentrated		Concentrated	Not concentrated	Concentrated	Not concentrated	n	z_i concentrated > z_i unconcentrated	
1	2·5	0	2·8	1·6	41	**		13·0	13·3	82·6	41·5	64	****	
2	1·3	0	1·7	0	28	****		1·2	0	1·7	2·1	32	**	
3	1·3	0	1·7	1·1	36	***		1·6	0	1·5	7·1	15	NS	
4	1·1	0	1·1	2·1	13	NS		2·1	0	2·4	1·3	32	**	
5	2·7	0	3·9	2·3	57	***		5·6	0	9·0	5·0	64	**	
6	1·3	0	1·3	2·4	11	NS		3·2	0	5·9	5·7	63	NS	
7	1·5	0	2·9	1·3	40	***		18·4	0	37·4	15·6	64	****	
8	1·7	0	3·8	3·8	42	****		88·5	33·3	291·0	153·5	66	****	
9	7·6	0	20·5	11·7	73	***		171·0	53·3	339·7	130·5	66	****	
10	1·3	0	3·5	4·4	9	NS		10·8	0	53·6	27·7	39	***	
All	10·0	0	25·9	15·9	88	****		675·9	306·7	795·8	369·7	66	****	

NS, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$.

Table 1b. Peaty soil: seedling emergence (median and mean in seedlings per litre) and differences between the two methods tested with Wilcoxon's signed-ranks test. Replicates in which a given species was not present were ignored

	Median		Mean			
Sp.	Concentrated	Not concentrated	Concentrated	Not concentrated	*n*	z_1 concentrated > z_1 unconcentrated
11	19·0	11·0	19·4	14·0	5	NS
12	11·0	6·0	11·6	6·8	5	*
13	3·0	4·0	3·8	9·0	5	NS
14	11·0	5·0	10·4	5·0	5	NS
15	66·5	27·0	63·4	35·6	5	*
16	150·1	72·0	145·7	76·8	5	*
17	840·0	211·0	810·4	266·8	5	*
18	12·0	6·0	11·6	5·6	5	*
19	24·0	12·0	23·0	11·0	5	*
20	4·0	4·0	4·0	6·2	5	NS
21	1·0	0	1·2	0·8	5	NS
22	187·0	138·0	197·8	142·4	5	*
23	4·0	2·0	3·8	2·0	5	NS
24	1·0	2·0	1·4	2·2	5	NS
25	12·0	6·0	17·2	7·8	5	NS
28	2·0	0	3·0	0·2	5	*
All	1357·6	635·0	1342·3	596·6	5	*

NS, not significant; *, $P<0·05$; **, $P<0·01$; ***, $P<0·001$; ****, $P< 0·0001$.

Table 1 c. Sandy soil: seedling emergence (median and mean in seedlings per litre) and differences between the two methods tested with Wilcoxon's signed-ranks test. Replicates in which a given species was not present were ignored

	Median		Mean			
Sp.	Concentrated	Not concentrated	Concentrated	Not concentrated	*n*	z_1 concentrated > z_1 unconcentrated
11	45·0	28·0	48·0	27·4	5	*
14	6·0	5·0	5·2	4·2	5	NS
15	5·3	6·0	6·9	6·2	5	NS
16	11·6	11·0	15·6	9·0	5	*
17	42·4	30·0	55·4	31·0	5	*
18	8·0	13·0	15·0	16·8	5	NS
22	11·0	10·0	10·4	9·4	5	NS
25	109·0	107·0	110·6	95·6	5	NS
26	2·0	3·0	2·0	2·8	5	NS
27	2·0	3·0	4·2	3·8	5	NS
All	315·9	235·0	281·2	211·4	5	*

NS, not significant; *, $P<0·05$; **, $P<0·01$; ***, $P<0·001$; ****, $P< 0·0001$.

Table 2. Number of species (totals, medians and means) and differences between the two methods tested with Wilcoxon's signed-ranks test

	Total		Median		Mean			
Sp.	Concentrated	Not concentrated	Concentrated	Not concentrated	Concentrated	Not concentrated	*n*	z_1 concentrated > z_1 unconcentrated
Clay soil: lake area	14	10	4·0	0	3·9	0·7	88	****
Clay soil: mixed area	20	10	8·0	4·0	7·8	3·7	66	****
Peaty soil	33	26	22·0	16·8	22·4	16·0	5	*
Sandy soil	24	19	14·0	13·0	14·4	12·6	5	NS

NS, not significant; *, $P<0·05$; **, $P<0·01$; ***, $P<0·001$; ****, $P< 0·0001$.

The total number of seedlings per litre found by seedling emergence plus hand-sorting was considered to be 100%. In this clay sample only seeds of the three most abundant species were found and no new species appeared (Table 3a). With concentrating hardly any seeds were present after the germination treatment; the germination rates varied between 81 and 100% but was 100% for the majority of species. Without concentrating many more seeds remained ungerminated in the soil, therefore the germination rates were much lower (33–88%). The data for *Phragmites australis*, *Juncus bufonius* and *Epilobium hirsutum* were unreliable because these species were very scarce.

No ungerminated seeds were found in the remainder of the concentrated samples of both peaty and sandy soil; the germination rates were therefore 100% (Table 3b,c). The only exception was one rare and unknown species. But in unconcentrated samples a considerable number of seeds was still present in the soil; the germination rates were far below 100%.

Without concentrating, in clay, peaty and sandy soil, the number of seeds per litre found by seedling emergence plus hand-sorting was far lower than the total number found after concentrating. This indicated that not all the remaining seeds were found by hand-sorting. Up to 30% may have been missed.

DISTRIBUTION AND SAMPLE SIZE

With concentrating there were fewer zero values in the data of clay samples than without concentrating, causing the data to be less skewed. As a result the distribution of the data with concentrating was more

Table 3a. Number of seeds per litre found by seedling emergence and hand-sorting, with and without concentrating in clay soil

	Concentrated five-core sampling		Unconcentrated 75 ml-subsampling	
	Found by seedling emergence	Found by hand-sorting	Found by seedling emergence	Found by hand-sorting
S. congestus	1164 (95%)	61 (5%)	658 (54%)	308 (25%)
T. latifolia	113 (81%)	27 (19%)	46 (33%)	58 (41%)
C. rubrum	22 (100%)	0 (0%)	11 (50%)	0 (0%)
R. maritimus	125 (95%)	6 (5%)	46 (35%)	40 (31%)
R. sceleratus	11 (100%)	0 (0%)	5 (45%)	0 (0%)
R. palustris	16 (100%)	0 (0%)	14 (88%)	0 (0%)
V. scutellata	71 (100%)	0 (0%)	51 (72%)	0 (0%)
P. australis	2	0	0	0
J. bufonius	1	0	0	0
E. hirsutum	2	0	3	0

Table 3b. Mean number of seeds per litre found by seedling emergence and hand-sorting, with and without concentrating in peaty soil

	Concentrated		Unconcentrated	
	Found by seedling emergence	Found by hand-sorting	Found by seedling emergence	Found by hand-sorting
Gramineae	75 (100%)	0 (0%)	32 (43%)	30 (40%)
Juncus sp.	1197 (100%)	0 (0%)	669 (56%)	396 (33%)
Other sp.	174 (100%)	0 (0%)	85 (45%)	0 (0%)
Unknown	0	3	0	0

Table 3c. Mean number of seeds per litre found by seedling emergence and hand-sorting, with and without concentrating in sandy soil

	Concentrated		Unconcentrated	
	Found by seedling emergence	Found by hand-sorting	Found by seedling emergence	Found by hand-sorting
R. obtusifolius	105 (100%)	0 (0%)	63 (60%)	24 (23%)
Gramineae	84 (100%)	0 (0%)	79 (93%)	0 (0%)
Juncus sp.	51 (100%)	0 (0%)	35 (69%)	0 (0%)
Other spp.	75 (100%)	0 (0%)	59 (79%)	0 (0%)
Unknown	0	3	0	20

often not significantly different from lognormal than without concentrating: lake area 48% against 2%; mixed area 81% against 41%. Owing to insufficient data, peaty and sandy soils were not tested.

With concentrating a sample size of 10 replicates was more often enough to assure with 99% probability that a given species will occur at least once in the sample than without concentrating (Table 4).

Table 4. Percentage species–sample combinations where the number of replicates necessary to assure with 99% probability that a given species will occur at least once in the sample (q°) ≤ 10

	Concentrated	Not concentrated
Clay soil: lake area (n=74)	53	18
Clay soil: mixed area (n=70)	80	58
Peaty soil (n= 28)	96	64
Sandy soil (n= 20)	80	70

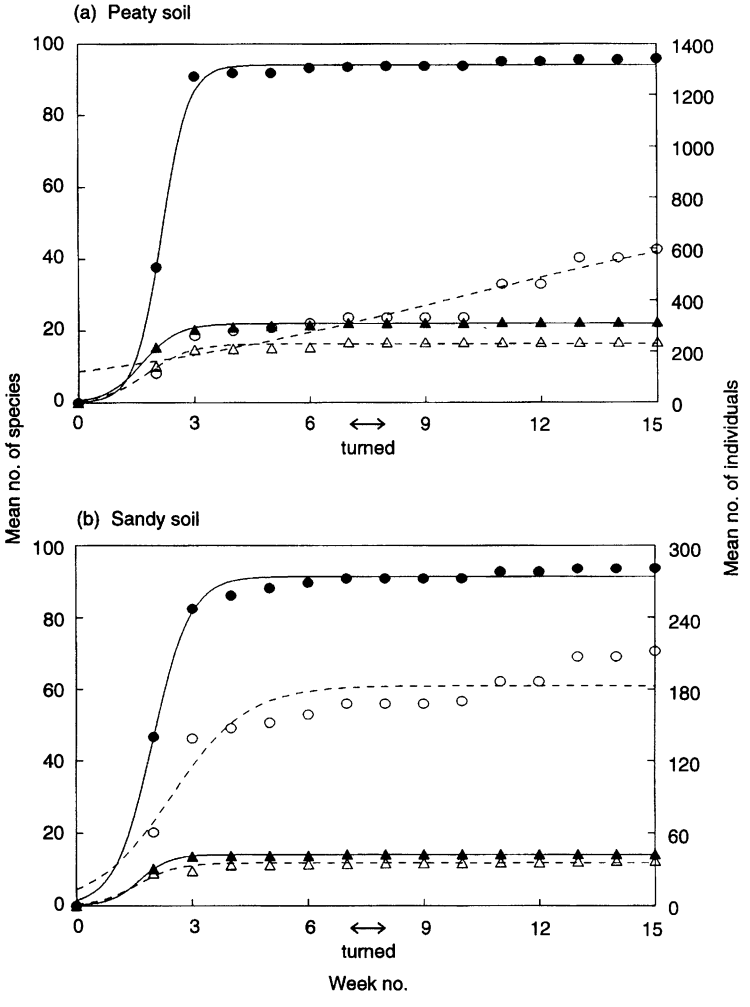

Figure 1. Mean number of species (triangles) and individuals (circles), with (black) and without (open) concentrating, during the 15-week experiment. A: peaty soil, B: sandy soil. The soil layer was turned at week 7 in each case. The lines are fitted to the model:

$$y=M\ \frac{1}{1+e^{a+bx}}\ \frac{1}{1+e^{c}}$$ (Huisman, Olff & Fresco 1993).

TIME

It took about 10–30 min to concentrate a volume of one litre of soil. The time needed increased with the amount of coarse organic matter, litter and roots.

Seedling emergence from peaty and sandy soil samples was counted weekly (Fig. 1). Both the emergence of species and individuals fitted well to an S-shaped curve (R^2>0·5, n=75). Ninety-five per cent of the maximum number of species was reached within 3 to 4 weeks.

Ninety-five per cent of the maximum number of individuals was reached within 4 weeks after concentrating. Without concentrating the number of individuals was still increasing at the end of this study. Extrapolating the S-curve led to the conclusion that it would take 27 (peat) to 16 (sand) weeks to reach 95% of the maximum number of individuals without concentrating. In the clay soil samples 73–100% of the maximum number of seedlings germinated within the first 6 week period, both with and without concentrating. (As shown in Table 1a,b,c, the maximum number of individuals found after concentrating was much larger than without concentrating).

Turning the sample layer upside down and waiting for another 6 weeks did not result in the emergence of many new seedlings in the concentrated samples (Fig. 1).

Hand-sorting was extremely time consuming. Recollecting the remainder of the material of one replica and sieving it again, took 15–60 min. Hand-sorting took 4–14 h of intensive work, depending on the amount of organic matter.

BULK REDUCTION

The reduction of the bulk depended on the type of soil. The volume of clay soil was reduced by approximately 85%, peaty soil by 70% and sandy soil by 55%. The amount of greenhouse space was reduced likewise.

Discussion

The modified seedling emergence method significantly increases the number of individuals and species found in soil samples. The germination rates are more than 80% and it can be assumed that we have developed a complete and reliable assessment of the seed-bank flora in clay, peaty and sandy soils.

Concentrating the samples, and spreading them in very thin layers, takes time, in this study 1 week. However, this is fully compensated for by the high germination rates and the possibility of being able to handle large amounts of soil in a small greenhouse. Using small unconcentrated samples saves time and some greenhouse space, but strongly underestimates the mean density and the number of species. The distribution of the seedlings in small unconcentrated samples is often not lognormal, which limits statistical

analysis. Moreover the number of replicates is often too small to assure that a given species is found. The negative effect of taking small samples is greatest in areas with low seed densities and for relatively rare species.

Some authors have found many more seeds with seed separation methods than with seedling emergence methods (Hopkins & Parker 1984; Poiani & Johnson 1988; Brown 1992). As is shown in this study, this might be caused by the fact that they did not concentrate their samples and used 1–4 cm thick layers. Only Poiani & Johnson (1988) dissect their seeds to see if they were viable. The other authors might have counted dead seeds.

A seed separation method can only be practical if the sample volumes are small and the seeds are relatively large. Both Poiani & Johnson (1988) and Brown (1992) hand-sorted much smaller volumes than they used in their emergence methods. Because the volumes were small, Brown (1992) found fewer species per sample with a seed separation method. Owing to the time-consuming method they used, Bernhardt & Hurka (1989) were forced to sample their sites with only two replicates, losing the ability to analyse their data statistically. Bernhardt (1992) had to pool his 60 cores per sample and only examined two small subsamples, losing all information about distribution and patchiness. Fay & Olson (1978) only recommend their seed separation method for species with large propagules. Finlayson, Cowie & Bailey (1990) were not able to sort the many small seeds from the organic matter that remained after sieving. Gross (1990) found it difficult to distinguish the seeds of closely related species. Malone (1967) developed a seed separation method which he claimed to be 100% efficient but Gross (1990) abandoned this method when trials showed that the salt concentration needed to separate seeds from soil differed among species and that frequent washing resulted in a considerable loss of the sample material. Our study showed that hand-sorting is very time-consuming and that not all seeds that should be present in the sample were found.

Hand-sorting a small proportion of the samples following a germination treatment, though laborious, is always useful for getting an impression of the germination rates. Seeds of woody species often germinate poorly in a greenhouse but they can easily be found with hand-sorting because they are relatively large (Moore & Wein 1977; Schneider & Sharitz 1986; Brown 1992).

A germination treatment should not take more than three months. In this study most individuals and species germinated within 3–6 weeks. Brenchley & Warington (1930) recorded no further germination after 6 weeks, except when the soil was stirred up. Thompson & Grime (1979) found that the germination beyond 5 weeks is negligible. Roberts (1981) recommended a period of 2 years but showed that only a small proportion of the seeds will germinate in the second year.

Removing the seedlings immediately after they appeared is necessary using our concentrating-emergence method because the density of the seedlings can be very high. If the seedlings are not removed soon after their emergence, the germination might be reduced because the emerged plants are shading the soil. Van Der Valk & Davis (1978) recorded that seedlings died after some weeks. This implies that one should identify the seedlings on sight when the cotyledons appear. A preliminary study is needed to identify the seedlings quickly. Seedlings which can not be identified immediately should be transplanted and allowed to grow. Transplantation of seedlings in wet soils such as ours did not lead to any losses.

According to the results discussed here, a reliable method for the estimation of the soil seed bank can be presented:

1. Use a preliminary study of the vegetation and the soil seed bank to get an impression of the species which can be expected and their appearance. This study can also be used to get some information about the distribution and the sample size needed. Favourable germination conditions of many species can be derived from the literature. The time needed for most seedlings to emerge can also be derived from this study.

2. Wash the soil samples with water on a coarse sieve to remove roots, pebbles, etc., and on a fine sieve to remove all clay and silt. The meshes of the fine sieve should hold the smallest seeds one wants to collect; 0·2 mm will do for most species.

3. Spread the concentrated samples in as thin a layer as possible, certainly not thicker than 5 mm.

4. Remove the seedlings as soon as possible. When no further germination is recorded one might disturb the soil layer to enable seeds from deeper in the soil to come to the surface.

5. The presence of remaining seeds should be checked with a seed separation method followed by hand-sorting.

Acknowledgements

We wish to thank Y. de Vries for his help with collecting the soil samples, A. Schutter for her help with the concentrating and counting of the seedlings, S. Nijdam and J. Hogendorf for their assistance in the greenhouse and an unknown referee for advising on statistics. The study of peaty and sandy soils was financed by the European Community Programme Agriculture AIR3-CT 920079 and the Beijerinck Popping Fonds.

References

Bakker, D. (1960) *Senecio congestus* (R.Br.) DC in the lake IJsselmeerpolders. *Acta Botanica Neerlandica* **9**, 235–259.

Bakker, J.P. (1989) *Nature Management by Grazing and Cutting*. Kluwer Academic Publishers, Dordrecht.

Baralis, G. & Chadoeuf, R. (1980) Etude de la dynamique d'une communaute adventive: I. Evolution de la flore adventive au cours du cycle vegetative d'une culture. *Weed Research* **20**, 231–237.

Benz, W., Koch, W. & Moosmann, A. (1984) Ein Extraktionsverfahren zur Bestimmung des Unkrautsamenpotentials in Böden. *Zeitschrift fur Pflantzen Krankheit und Pflantzenschutz* **10**, 106–114.

Bernhardt, K.G. (1992) Besiedlungsstrategien an sandigen Extremstandorten im Tidebereich. *Flora* **187**, 271–281.

Bernhardt, K.G. & Hurka, H. (1989) Dynamik des Samenspeichers in einigen mediterranen Kulturböden. *Weed Research* **29**, 247–254.

Bigwood, D.W. & Inouye, D.W. (1988) Spatial pattern analysis of seed banks: an improved method and optimizing sampling. *Ecology* **69**, 497–507.

Brenchley, W.E. & Warington, K. (1930) The weed seed population of arable soil. I. Numerical estimation of viable seed. *Journal of Ecology* **18**, 235–272.

Brown, D. (1992) Estimating the composition of a forest seed bank: a comparison of the seed extraction and seedling emergence methods. *Canadian Journal of Botany* **70**, 1603–1612.

Fay, P.K. & Olson, W.A. (1978) Technique for separating weed seeds from soil. *Weed Science* **26**, 530–533.

Fenner, M. (1985) *Seed Ecology*. Chapman & Hall, London.

Finlayson, C.M., Cowie, I.D. & Bailey, B.J. (1990) Sediment seed banks in grassland on the Magela Creek floodplain, northern Australia. *Aquatic Botany* **38** 163–176.

Galinato, M.I. & Van Der Valk, A.G. (1986) Seed germination traits of annuals and emergents recruited during drawdowns in the Delta Marsh, Manitoba, Canada. *Aquatic Botany* **26**, 89–102.

Grime, J.P., Mason, G., Curtis, A.V., Rodman, J., Band, S.R., Mowforth, M.A.G., Neal, A.M. & Shaw, S. (1981) A comparative study of germination characteristics in a local flora. *Journal of Ecology* **69**, 1017–1059.

Grime, J.P., Hodgson, J.G. & Hunt, R. (1988) *Comparative Plant Ecology*. Unwin Hyman, London.

Gross, K.L. (1990) A comparison of methods for estimating seed numbers in the soil. *Journal of Ecology* **78**, 1079–1093.

Hopkins, D.R. & Parker, V.T. (1984) A study of the seed bank of a salt marsh in North San Francisco Bay. *American Journal of Botany* **71**, 348–355.

Huisman, J., Olff, H. & Fresco, L.F.M. (1993) A hierarchical set of models for species response analysis. *Journal of Vegetation Science* **4**, 37–46.

Kropáč, Z. (1966) Estimation of weed seeds in arable soil. *Pedobiologia* **6**, 105–128.

Lloyd, M. (1967) Mean crowding. *Journal of Animal Ecology* **36**, 1–33.

Major, J. & Pyott, W.T. (1966) Buried, viable seeds in two California bunchgrass sites and their bearing on the definition of a flora. *Vegetatio* **13**, 253–282.

Malone, C.R. (1967) A rapid method for enumeration of viable seeds in soil. *Weeds* **15**, 381–382.

Moore, J.M. & Wein, R.W. (1977) Viable seed populations by soil depth and potential site recolonization after disturbance. *Canadian Journal of Botany* **55**, 2408–2412.

Poiani, K.A. & Johnson (1988) Evaluation of the emergence method in estimating seed bank composition of prairie wetlands. *Aquatic Botany* **32**, 91–97.

Roberts, H.A. (1981) Seed banks in soils. *Advances in Applied Biology* **6**, 1–56.

Schneider, R.L. & Sharitz, R.R. (1986) Seed bank dynamics in a southeastern riverine swamp. *American Journal of Botany* **73**, 1022–1030.

Sokal, R.R. & Rohlf, F.J. (1980) *Biometry*. Freeman, New York.

Ter Heerdt, G.N.J. & Drost, H.J. (1994) Potential for the development of marsh vegetation from the seed bank after a drawdown. *Biological Conservation* **67**, 1–11.

Thompson, K. & Grime, J.P. (1979) Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *Journal of Ecology* **67**, 893–921.

Thompson, K. & Grime, J.P. (1983) A comparative study of germination responses to diurnally-fluctuating temperatures. *Journal of Applied Ecology* **20**, 141–156.

Van Der Meijden, R., Weeda, E.J., Holverda, W.J. & Hovenkamp, P.H. (1990) *Flora van Nederland*. Wolters-Noordhoff, Groningen.

Van Der Valk, A.G. & Davis, C.B. (1978) The role of seed banks in the vegetation dynamics of prairie glacial marshes. *Ecology* **59**, 322–335.

Williams, J.T. (1969) Biological Flora of the British Isles. *Chenopodium rubrum*. *Journal of Ecology* **57**, 831–841.

Received 13 March 1995; revised 5 July 1995; accepted 21 July 1995

Appendix Species names

Number	Species
	Helophytes and mud-flat species (clay soil)
1	*Chenopodium rubrum*
2	*Epilobium hirsutum*
3	*Juncus bufonius*
4	*Phragmites australis*
5	*Ranunculus sceleratus*
6	*Rorippa palustris*
7	*Rumex maritimus*
8	*Senecio congestus*
9	*Typha latifolia*
10	*Veronica scutellata*
	Grassland species (peaty and sandy soil)
11	*Agrostis sp.*
12	*Cardamine pratensis*
13	*Cerastium fontanum*
14	*Holcus lanatus*
15	*Juncus acutiflorus*
16	*Juncus bufonius*
17	*Juncus effusus*
18	*Phleum pratense*
19	*Plantago lanceolata*
20	*Poa sp.*
21	*Ranunculus acris*
22	*Ranunculus repens*
23	*Rhinanthus sp.*
24	*Rumex acetosa*
25	*Rumex obtusifolius*
26	*Stellaria uliginosa*
27	*Urtica dioica*
28	*Veronica serpyllifolia*