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A COMPARISON OF METHODS FOR ESTIMATING SEED NUMBERS IN THE SOIL

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SUMMARY

(1) Seed-bank species composition and seed density were determined in an annually ploughed field at the Kellogg Biological Station in south-western Michigan, U.S.A. using three different methods: direct germination, germination following cold-stratification, and washing using a modified elutriation system. A fourth method, flotation on a salt-density gradient was abandoned after preliminary trials showed it was inefficient and inaccurate.

(2) Twenty soil cores (2·5 cm in diameter, 15 cm deep) were taken from each of twenty-five sampling points in the field. Cores were divided into three depth intervals (0–2 cm, 2–5 cm and 5–15 cm) and five cores from each quarter of a sampling quadrat were combined and randomly assigned to one of the four methods for estimating the seed bank.

(3) A total of fifty species was detected in the seed bank of this community. Overall, more species were detected with cold-stratification (forty-two species) than with direct germination (thirty-seven) or elutriation (thirty-five). Plots of species–sampling-area curves showed that fifteen to twenty sampling locations were sufficient to determine the number of species present in the seed bank with any of these methods.

(4) Seed-density estimates were significantly higher in the elutriated samples. This was due primarily to the inclusion of inviable seeds in the counts from the elutriated samples.

(5) There were significant differences in seed viability among species recovered in the elutriated samples. Both *Ambrosia artemisiifolia* and *Chenopodium album*, seeds of which were common in the samples, had low viability (25% and 3%, respectively).

(6) Germination methods (particularly when more than one pre-treatment method is used) provide a more complete listing of species present in the soil seed bank of a community than elutriation. However, elutriation methods may be more useful for documenting variation in seed distributions on a large spatial scale or in quantifying the distribution of species with unique, easily identifiable seeds. Elutriation seems particularly well-suited for quantifying the distribution of plants that are considered weeds in agricultural systems, because many of these species have seeds that are easily identified and retain viability when dried.

INTRODUCTION

The existence of a pool of viable seeds in the soil (the seed bank) has been documented for many different types of plant communities (Thompson & Grime 1979; Roberts 1981; Fenner 1985; Leck, Parker & Simpson 1989). Ecologists and evolutionary biologists have become increasingly aware of the role that seed banks can play in maintaining ecological (species) and genetic diversity in populations and communities (Templeton & Levin 1979; Thompson & Grime 1979; Venable 1989). Comparative studies of seed banks in various communities have been limited by the difficulty of accurately determining the numbers of seeds and species present. Extracting seeds from the soil is time-consuming and the results are influenced by the time of year and methods used to take soil samples (cf. Thompson & Grime 1979) and to determine seed numbers (e.g. germination, washing, flotation). Several studies have noted that both sampling time and testing method can influence the species that emerge from the samples (Roberts 1981).

Most attempts to quantify the seed banks of a community have relied on germination methods (Roberts 1981). The limitations of this approach are readily acknowledged (e.g. Thompson & Grime 1979), but it is generally justified as providing an estimate of the readily germinable fraction of the seed bank. However, germination methods are likely to underestimate the seed bank because the specific germination requirements of all the species present in the soil are not likely to be met by a prescribed germination treatment (Roberts 1981; Baskin & Baskin 1989). Germination methods may also require a considerable amount of time and glasshouse space as many species emerge slowly or only after successive periods of wetting and drying (Roberts 1981).

Washing and flotation methods are used less commonly (Roberts 1981) and have the advantage over germination methods for estimating seed numbers because the results are not influenced by differences in germination requirements, but the accuracy of these methods is variable and species with small or cryptically coloured seeds may be missed. In addition, for many species it is not possible to distinguish viable and inviable seeds in the washed samples. Thus seed numbers may be overestimated unless they are adjusted for viability. Flotation methods have the additional difficulty that the specific gravity at which viable seeds can be removed varies among species and they are often ineffective in organic soil (Roberts 1981; K. L. Gross, unpublished). These methods generally require repeated washings, which increase the risk of losing seeds.

Few studies have compared the results of various methods of estimating the soil seed bank in a single community (Roberts 1981). Such comparisons are necessary, however, to determine the relative accuracy of different methods for estimating specific characteristics of the soil seed bank (e.g. total species present, relative abundance at different depths in the soil). Also, such comparisons are needed to interpret the results of studies that have used different methods to estimate the soil seed bank. Here the results are reported of a study in which the soil seed bank of an annually ploughed field was assessed using four different methods. Comparisons are made of the usefulness of these methods in determining specific characteristics of the soil seed bank of the community.

METHODS

Field sampling

Soil samples were taken on 21–22 June 1988 from an annually ploughed research field (Bailey field) at the W. K. Kellogg Biological Station (KBS), Michigan State University, Kalamazoo County, in south-western Michigan, USA. The field has been used for studying competitive interactions in annual weed communities for several years (Miller 1987; Miller & Werner 1987; Goldberg & Miller 1990) and as a consequence has been treated with herbicides and ploughed annually in the spring, and occasionally in the autumn, since 1970.

A 30-m × 50-m grid with twenty-five sampling locations was established at the north end of the field. Points on the grid were located in five rows 6 m apart at 10-m intervals in each row. At each sampling point, twenty soil cores (2·5 cm in diameter × 15 cm deep) were taken from a 0·25-m^2 area. Five cores were taken from each quarter of the sample quadrat. Each of the five cores was divided into three depth classes (0–2 cm, 2–5 cm and 5–15 cm); the five portions from each depth class were then combined and placed in a labelled paper bag indicating the sample location and depth. The four samples (each with five cores) from each sampling location were randomly assigned to one of four treatments or methods for estimating the soil seed bank. Sample bags were allowed to air dry for 24–48 h before processing.

Seed-bank estimation

The four methods for quantifying the soil seed bank to be compared initially were: (i) direct glasshouse germination, (ii) glasshouse germination following cold-stratification, (iii) direct counts following elutriation (washing), and (iv) direct counts following flotation on a density gradient as described by Malone (1967). The flotation treatment was subsequently abandoned when trials with known numbers of seeds of several species showed that the salt concentration needed to separate seeds from soil differed among species. This method also resulted in considerable loss of the sample material because of the frequent washing and transferring of soil that was required.

Glasshouse germination

Soil samples for the direct and stratified glasshouse germination treatments were spread thinly ($<0{\cdot}5$ cm) in separate pots over a base of moistened, sterilized, soil-less seedling mix (Sunshine seedling mix #3) within 48 h after collection. Soil samples from 0–2 cm and 2–5 cm were placed in 10-cm × 10-cm pots filled to a depth of 10 cm with seedling mix. Samples from the 5–15-cm depth were placed in 25-cm × 52-cm trays divided in half and filled with a seedling mix to a depth of 5 cm. Soil from two different samples was placed in each tray. The divider was positioned so that there was no mixing of the soil from the two samples in a tray.

The pots and trays in the direct germination treatment were immediately placed in a temperature-controlled (max. 30 °C; min. 20 °C) glasshouse under natural light and watered once or twice daily so that the soil surface was kept moist. The trays and pots to be stratified were covered with plastic and placed in an unlighted refrigerator at 5 °C. After three weeks of cold-stratification (24 June–15 July), the trays were moved to the glasshouse and watered as above.

Emerging seedlings in both germination treatments were marked with colour-coded plastic tooth-picks and the number of individuals of each species that had emerged from each sample-depth was recorded at regular intervals. Pots were checked frequently (three to four-day intervals) for newly emerged seedlings to assure that no plants emerged and died between censuses. Individuals were identified to species using seedling keys (Kummer 1951; Priwer *et al.* 1980; Stuckey 1981) and reference collections at KBS. Once individuals were identified, they were removed. In September, the remaining unidentified seedlings were removed from the experimental pots and trays, transplanted into separate pots and grown to maturity (=flowering) so that they could be identified. The soil surface in the experimental pots was kept well watered for another four weeks, but few new seedlings emerged during this period. A final census was taken on 4 October and the experiment was terminated.

Elutriation treatment

Elutriation was done with a modified hydropneumatic root elutriator developed by Smucker, McBurney & Srivastave (1982) for estimating fine-root production. This system has been shown to be very effective in separating fine roots and other organic material from a wide range of soil types (Smucker, McBurney & Srivastave 1982). For quantifying the seed bank, the primary sieves used to collect fine roots were replaced by a series of stacked sieves constructed of 7·5-cm polyvinyl chloride (PVC) pipe and nylon mesh screens. The sieves were placed in a graded series, connected with PVC connectors, on top of a section of PVC which stood in the bottom collecting pan of the elutriator. The three sieves, with mesh sizes of 710 μm, 425 μm and 243 μm, made it possible to separate the

seeds (and other associated organic matter) efficiently from the soil without clogging the filters or impeding water flow. Preliminary trials with samples of known numbers of seeds washed through this system demonstrated that this range of mesh sizes effectively captured seeds ranging from 0·06 to 9·8 mg (0·5–2·6 mm in diameter; Gross & Renner 1989).

Samples to be elutriated were stored dry in paper bags in the laboratory until processed. Each depth class of a sample was elutriated separately and washed for 10 min. The contents collected on the sieves were washed into a Buchner funnel lined with a labelled piece of Whatman No. 1 filter paper. Excess water was removed from the samples with a small vacuum pump. The filter paper was then placed in a labelled Petri dish, allowed to air-dry for 24 h, taped shut and boxed for storage until counted.

The elutriated samples were examined under a dissecting microscope (magnification $\times$ 10–30) and all seeds were grouped by species and counted. Only seeds showing no external evidence of damage or disease were counted. Seeds were keyed to species using Delorit (1970) and reference collections of seeds collected at KBS. Counts made from the three sieves were combined to determine the number of species and seeds at a given depth interval for each sample. Separation on different sieve (mesh) sizes facilitated the counting and sorting of seeds, because large seeds were encountered on the larger mesh and small seeds only on smaller mesh sieves.

To determine the viability of seeds collected by the elutriation method, seeds of five species that were common in the elutriated samples were tested. Seeds were removed from elutriated samples and grouped by species and depth. Seeds of four species, *Ambrosia artemisiifolia*, *Chenopodium album*, *Oxalis stricta*, and *Panicum capillare* were tested for viability using tetrazolium following the methods described by Moore (1972). *Mollugo verticillata* seeds did not stain reliably with tetrazolium and so were tested for viability by germination in an environmental growth chamber (14 h light/10 h dark) with alternating temperatures (25/15 °C). Seeds were placed on wet filter paper in 10-cm Petri dishes, sealed with parafilm and placed in a single layer in the growth chamber. Under these conditions, freshly harvested seeds of *Mollugo* germinated rapidly and had an estimated viability of 85%. Germination was monitored at three or four-day intervals and a final count taken after three weeks. Emergence of the cotyledons was the criterion for germination.

Seeds from each depth interval were tested separately for viability. The number of seeds of each species available at each depth interval, and consequently the number of replicates (dishes) and seeds tested per replicate, were quite variable. The available seeds were divided evenly among replicates, with a maximum of twenty (minimum of two) seeds replicate^{-1} and from one to seven replicates.

Statistical analysis

The results of these three methods for quantifying the seed bank were compared by separate one-way ANOVAS on the total number of species, seeds sample^{-1}, and number of seeds at each depth. Seed density and distribution were analysed separately for the seven most common species encountered in the samples. Differences among treatment methods were tested by Tukey's test for a-posteriori comparisons of means. Viability estimates (proportion viable in each replicate) were transformed (angular transformation) for statistical analysis. Untransformed means and standard errors are reported. A portion of one of the elutriated samples was lost during processing, so statistical comparisons are based on a sample size of twenty-four for this method. Results are reported as the mean

TABLE 1. Frequency of species detected in the soil bank of the study field by the three methods for estimating the seed bank. Only species detected in at least 10% of the samples of at least one method are listed. Seed mass determined from a random sample of air-dried seeds from local populations at KBS.

Species	Seed mass (mg)	Frequency by method* Direct	Stratified	Elutriated
Dicots†				
Aster spp.	0·09–0·13	24	8	0
Ambrosia artemisiifolia L.	5·34	32	36	100
Amaranthus retroflexus L.	0·20	0	8	38
Barbarea vulgaris R. Br.	0·44	60	56	79
Berteroa incana (L.) DC.	0·47	20	52	33
Chenopodium album L.	0·51	64	64	96
Daucus carota L.	0·69	40	28	75
Dianthus armeria L.	0·20	8	8	25
Conyza canadensis (L.) Cronq.	0·02	12	4	0
Erigeron strigosus Muhl.	0·03	92	100	0
Hypericum perforatum L.	0·08	4	0	29
Lepidium campestre (L.) R. Br.	2·45	68	56	71
Mollugo verticillata L.	0·06	76	68	79
Oxalis stricta L.	0·22	24	52	92
Plantago lanceolata L.	0·80	32	28	50
Polygonum convolvulus L.	2·25	0	0	17
Portulaca oleracea L.	0·14	36	36	83
Potentilla argentea L.	0·07	16	12	0
P. recta L.	0·27	92	92	67
Rumex acetosella L.	0·51	4	12	8
R. crispus L.	0·87	40	48	75
Silene alba‡ (Miller) E. H. L. Krause	0·67	24	24	17
S. antirrhina L.	0·10	0	0	12
Solanum nigrum L.	0·80	8	12	0
Solidago canadensis L.	0·05	12	40	8
Trifolium repens L.	0·43	16	12	0
Veronica peregrina L.	0·02	40	76	0
Monocots				
Agropyron repens (L.) Beauv.	1·34	0	16	0
Eragrostris cilianensis (All.) Link	1·96	32	40	21
Panicum capillare L.	0·47	32	56	88
Poa compressa L.	0·16	12	20	0

* Frequency is the percentage of samples ($n = 25$; 24 for elutriated) in which at least one seedling was detected from the entire sample.

† Species are listed in alphabetical order, separately for monocots and dicots. Nomenclature follows Gleason & Cronquist (1963) unless otherwise indicated.

‡ See McNeill (1978). Listed as *S. nivea* (Nutt.) Otth. in Gleason & Cronquist (1963).

number of seeds sample^{-1}; to convert to seeds m^{-2} these values should be multiplied by 400.

RESULTS

Estimates of species diversity

Fifty species (forty-three dicots, seven monocots) were identified in the seed bank of this field. Of these, thirty species (twenty-six dicots, four monocots) were detected in at least 10% of the samples using one of three methods (Table 1). Overall, more species were detected in the stratified samples (forty-two species) than by the direct germination

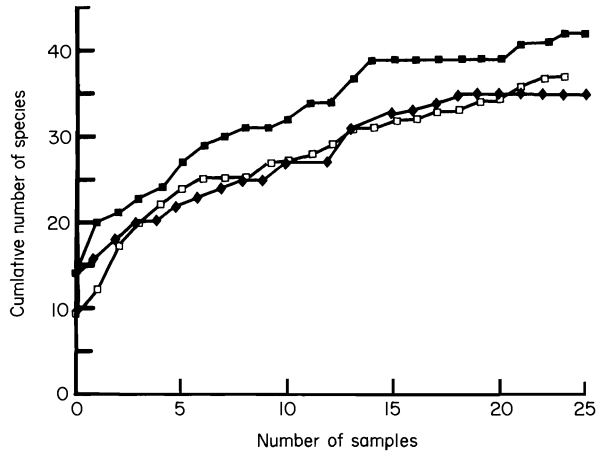

FIG. 1. Cumulative number of species detected in soil seed-bank samples from an annually ploughed field in Michigan by three methods of quantifying seed banks. Estimates determined by direct germination (◆), cold-stratification for 3 weeks prior to germination (■), and elutriation (□).

(thirty-seven species) or elutriation methods (thirty-five species). These differences in number of species are not due to inadequate sampling. Plots of species–area curves for the three methods were similar and all levelled off at approximately fifteen to twenty samples (Fig. 1).

There were also significant differences among the three methods in the number of species detected per sample ($F_{2,69} = 5{\cdot}62$; $P < 0{\cdot}006$). More species were detected per sample by the elutriation method than by either germination method at all depth intervals and for the whole sample (Table 2). These differences were most pronounced in the surface soil samples (0–2 cm and 2–5 cm), where nearly twice as many species were detected per sample by elutriation than by either germination method.

The methods also differed in the frequency of species detected among samples (Table 1). Stratification of the soil samples increased the observed frequency of a number of species (e.g. *Berteroa incana*, *Oxalis stricta*, *Solidago canadensis* and *Veronica peregrina*). Several common 'weedy species' known to have long-lived seeds (e.g. *Ambrosia artemisiifolia*, *Chenopodium album* and *Panicum capillare*) occurred in more than 85% of the elutriated samples, but were much less commonly encountered in the germination methods. However, several species that were relatively common in both germination methods were not observed in the elutriated samples (e.g. *Conyza canadensis* and *Veronica peregrina*). The seeds of both these species are relatively small and light-coloured which may make them difficult to detect in the matrix of sand that is retained on the smallest sieve.

Estimates of seed densities in the soil

Estimates of total seed densities present in soil also differed significantly among the three methods ($F_{2,69} = 10{\cdot}64$; $P < 0{\cdot}0001$). Overall and at all depth intervals, significantly more seeds were detected in the elutriated samples than by either germination treatment. Generally, more seeds were observed in the stratified samples than in the direct germination tests, but differences were not significant (Table 2). With all three methods,

TABLE 2. The number of species and seeds at three depth intervals in the soil seed bank of an annually ploughed field determined by three different methods. Values are the mean ± 1 S.E. ($n = 25$; elutriated, $n = 24$).

		Number sample^{-1}			
Soil depth	Soil volume (cm^3)	Direct	Stratified	Elutriated	$F_{2,72}$†
Species data					
0–2 cm	(8)	$1{\cdot}9 \pm 0{\cdot}3^a$	$1{\cdot}9 \pm 0{\cdot}3^a$	$4{\cdot}4 \pm 0{\cdot}6^b$	14·2***
2–5 cm	(12)	$2{\cdot}4 \pm 0{\cdot}3^a$	$2{\cdot}8 \pm 0{\cdot}4^a$	$5{\cdot}3 \pm 0{\cdot}5^b$	17·7***
5–15 cm	(40)	$8{\cdot}9 \pm 0{\cdot}5^a$	$10{\cdot}3 \pm 0{\cdot}6^{a,b}$	$11{\cdot}9 \pm 0{\cdot}8^b$	5·65**
Total	(60)	$10{\cdot}5 \pm 0{\cdot}5^a$	$12{\cdot}1 \pm 0{\cdot}7^{a,b}$	$13{\cdot}4 \pm 0{\cdot}8^b$	6·2**
Seed data‡					
0–2 cm	(8)	$2{\cdot}2 \pm 0{\cdot}4^a$	$2{\cdot}8 \pm 0{\cdot}6^a$	$12{\cdot}2 \pm 2{\cdot}6^b$	12·97***
2–5 cm	(12)	$3{\cdot}6 \pm 0{\cdot}5^a$	$4{\cdot}3 \pm 0{\cdot}9^a$	$19{\cdot}7 \pm 4{\cdot}3^b$	12·58***
5–15 cm	(40)	$22{\cdot}8 \pm 2{\cdot}1^a$	$33{\cdot}4 \pm 3{\cdot}5^a$	$82{\cdot}3 \pm 16{\cdot}5^b$	10·31***
Total	(60)	$28{\cdot}6 \pm 2{\cdot}5^a$	$40{\cdot}5 \pm 4{\cdot}1^a$	$113{\cdot}6 \pm 23{\cdot}1^b$	11·38***

† F values are comparisons across methods for each variable: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$. Means within a row with the same letter are not significantly different ($P < 0{\cdot}05$) by a Tukey's test for a-posteriori comparison of means.

‡ To convert to number of seeds m^{-2}, multiply each value by 400.

the estimated number of seeds sample^{-1} was highly variable—especially at the shallower depths, where between 10% and 20% of the samples had no seeds (Fig. 2).

For the seven most common species in these samples, seed densities were up to an order of magnitude higher in the elutriated samples than the estimates determined by either germination method (Table 3). Although only whole and undamaged seeds were counted in the elutriated samples, seeds of several species recovered from the elutriated samples had low viability (Table 4). There were significant differences among species tested in viability ($F_{4,32} = 16{\cdot}32$; $P < 0{\cdot}0001$), and a significant species–depth interaction

TABLE 3. Estimated seed densities of the seven most common species determined by different methods. Values are the mean ± 1 S.E. ($n = 25$; $n = 24$, elutriated) number of seeds sample^{-1} (60 cm^3 soil). Values in parentheses are the estimated number of viable seeds in elutriated samples (see text).

	Direct	Stratified	Elutriated	$F_{2,72}$†
Ambrosia artemisiifolia	$0{\cdot}4 \pm 0{\cdot}1^a$	$0{\cdot}6 \pm 0{\cdot}2^a$	$7{\cdot}4 \pm 0{\cdot}8^b$ ($1{\cdot}9 \pm 0{\cdot}2$)	63·2***
Chenopodium album	$1{\cdot}8 \pm 0{\cdot}4^a$	$1{\cdot}2 \pm 0{\cdot}2^a$	$23{\cdot}6 \pm 3{\cdot}2^b$ ($0{\cdot}7 \pm 0{\cdot}1$)	47·5***
Mollugo verticillata	$6{\cdot}2 \pm 1{\cdot}9$	$6{\cdot}7 \pm 1{\cdot}9$	$33{\cdot}2 \pm 15{\cdot}8$ ($5{\cdot}5 \pm 2{\cdot}6$)	N.S.
Oxalis stricta	$0{\cdot}5 \pm 0{\cdot}2^a$	$0{\cdot}9 \pm 0{\cdot}2^a$	$2{\cdot}2 \pm 0{\cdot}3^b$ ($1{\cdot}54 \pm 0{\cdot}2$)	13·2***
Panicum capillare	$0{\cdot}6 \pm 0{\cdot}2^a$	$1{\cdot}2 \pm 0{\cdot}3^a$	$2{\cdot}8 \pm 0{\cdot}7^b$ ($0{\cdot}9 \pm 0{\cdot}2$)	7·2**
Barbarea vulgaris	$2{\cdot}3 \pm 0{\cdot}6$	$2{\cdot}1 \pm 0{\cdot}7$	$3{\cdot}96 \pm 0{\cdot}99$	N.S.
Erigeron strigosus‡	$4{\cdot}7 \pm 0{\cdot}8^a$	$11{\cdot}4 \pm 1{\cdot}6^b$	—	3·75***

† F values are for comparisons of means not adjusted for viability for each species: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$.

‡ t values calculated for comparison of direct and stratified samples only because seeds were not detected in the elutriated samples.

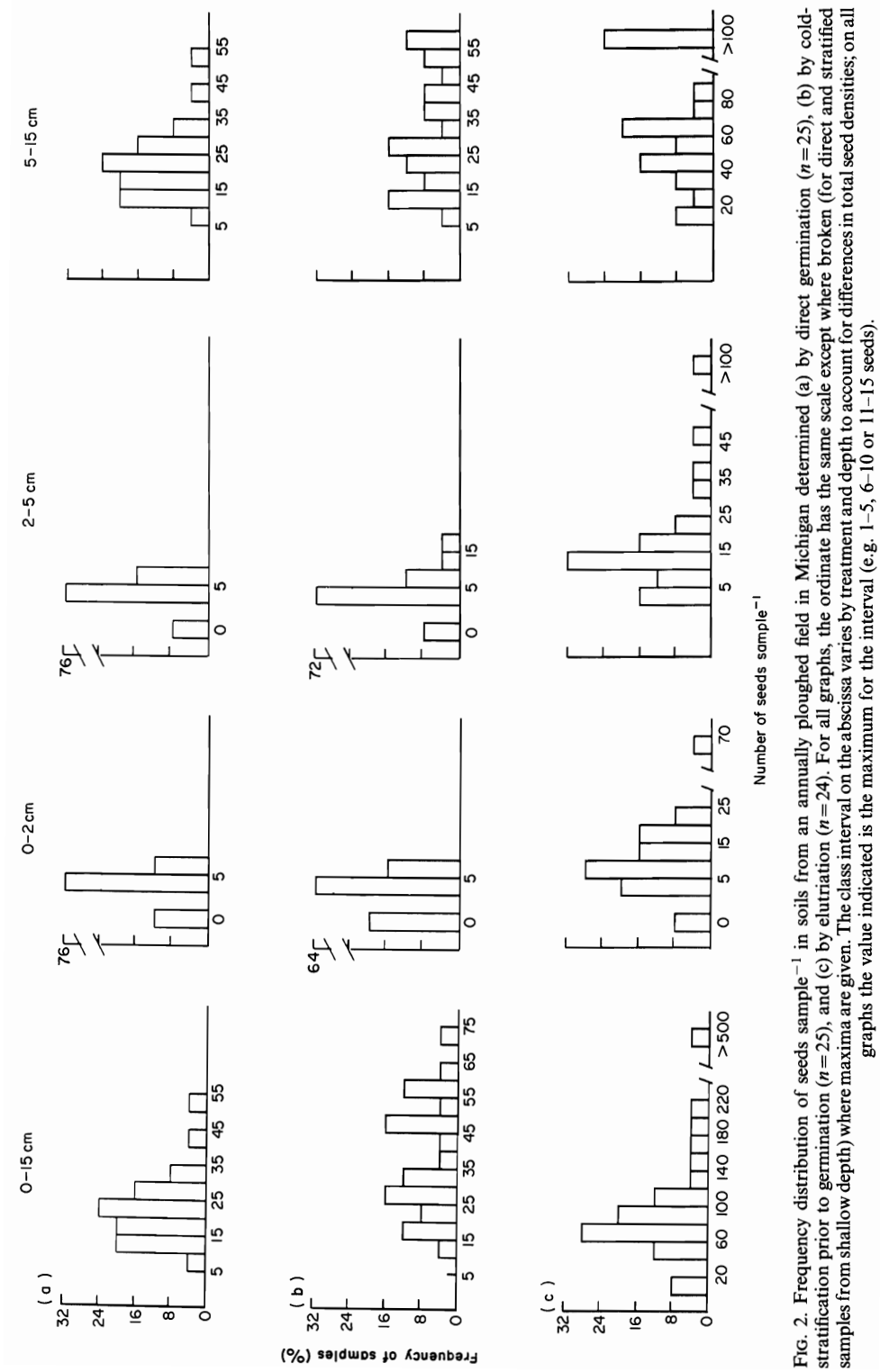

FIG. 2. Frequency distribution of seeds sample^{-1} in soils from an annually ploughed field in Michigan determined (a) by direct germination ($n = 25$), (b) by cold-stratification prior to germination ($n = 25$), and (c) by elutriation ($n = 24$). For all graphs, the ordinate has the same scale except where broken (for direct and stratified samples from shallow depth) where maxima are given. The class interval on the abscissa varies by treatment and depth to account for differences in total seed densities; on all graphs the value indicated is the maximum for the interval (e.g. 1–5, 6–10 or 11–15 seeds).

TABLE 4. Estimated seed viabilities of selected species recovered from elutriated samples. Values are mean (±1 S.E.) percentages of viable seeds at each depth sampled, plus the overall mean. Values in parentheses are the numbers of replicates and total numbers of seeds tested, respectively. Numbers of replicates and seeds replicate^{-1} differed according to availability in samples. All species except *Mollugo* were tested with Tetrazolium; *Mollugo* viability was determined by germination in a growth chamber.

	Percentage viable seeds			
	0–2 cm	2–5 cm	5–15 cm	Mean
Ambrosia artemisifolia	21·4±21·4 (2,14)	16·7±16·7 (2,12)	31·3±8·1 (4,32)	25·2±6·0
Chenopodium album	5·0±5·0 (2,20)	5·0±2·9 (4,40)	1·4±1·4 (7,70)	3·1±1·3
Mollugo verticillata	25·0±15·0 (2,20)	16·0±4·0 (5,100)	13·9±5·1 (5,65)	16·6±3·3
Oxalis stricta	100 (1,2)	50 (1,2)	68±10·2 (5,25)	70·0±9·0
Panicum capillare	0 (1,1)	50 (1,4)	33·3±6·0 (6,42)	31·3±6·6
Mean over all species	25·4±12·3	18·0±4·5	27·6±5·2	

($F_{8,33}=2·43$; $P<0·05$), but no consistent effect of sample depth on viability. This may be due to the small and unequal numbers of seeds tested at each depth. The number of seeds tested for viability was limited by what was recovered in the elutriated samples. If seed densities in the elutriated samples are adjusted for the observed average viability of the five most common species, then there are no significant differences among methods in estimated seed densities (Table 3).

DISCUSSION

Species diversity and composition

The four methods used to determine the soil seed bank differed in effectiveness and gave different results for both the number and abundance of species present in this field. The flotation method that was initially included in the study is probably the least effective of the methods because the density (=salt concentration) at which seeds were separated from the soil varied among species. To separate all the seeds present in soil samples requires repeated washing and rinsing of a sample, which results in considerable loss of the sample. This method is also more costly and time-consuming than the other three.

Of the three methods examined in detail, germination with cold-stratification revealed the greatest number of species. On a per-sample basis, however, elutriated samples had a greater number of species present than either germination method. Differences in the estimated number of species sample^{-1} among germinated and elutriated samples may be due to the failure of some species to emerge in the germination tests because specific germination cues were not met (Baskin & Baskin 1989); however, the elutriation method may overestimate the frequency and density of a species if inviable seeds are present in many samples. This is probably the case in this study as both *Ambrosia artemisiifolia* and *Chenopodium album* occurred in nearly all of the elutriated samples (Table 1) and had low viability (especially *Chenopodium*, see Table 4). The frequent presence of these two species in the elutriated samples could account for most of the observed difference in the number of species sample^{-1} in the germinated and elutriated samples.

The smaller total number of species detected in the elutriated samples as compared to the germination methods is probably due to the difficulty of differentiating between species with similar seed characteristics. This is especially a problem for several congeners that occurred in these samples (e.g. *Potentilla* and *Silene* species) which are distinguished as seeds primarily on the basis of overlapping size characteristics (Delorit 1970). However, seedlings of these species have different leaf shapes and coloration, which makes it relatively easy to distinguish between them. For example, seedlings of *Silene antirrhina*, *S. alba*, *S. noctiflora* L. and *S. peregrina* L. were identified in the direct and stratified samples (*S. noctiflora* and *S. peregrina* were rare). Seeds of *S. antirrhina* are smaller (0·10 mg; 0·5 mm in diameter) and easily distinguished from seeds of the other three species, which are larger (0·6–0·8 mg; 1·0 mm in diameter), but difficult to distinguish from each other (Delorit 1970; K. L. Gross, personal observation). Thus, estimates of *S. alba* frequency in elutriated samples may be inflated if either *S. noctiflora* or *S. peregrina* is common.

Several species that emerged in the germination samples were not observed in the elutriated samples. Validation studies of the elutriation system in which soil samples were spiked with known numbers of seeds of different species have shown that elutriation does not provide a reliable estimate of seed numbers for species with very small ($<0{\cdot}1$ mg) or light-coloured seeds (Gross & Renner 1989). Small seeds may pass through the filters and, especially if the seeds are light-coloured, are difficult to distinguish in the matrix of sand and other material that is retained in the smaller mesh sizes. This may account for the absence of *Erigeron strigosus* and *Veronica peregrina*, both of which were common in the germinated samples (Table 1), in the elutriated samples. However, species with small seeds that are darkly coloured or have shiny seed coats (e.g. *Mollugo verticillata*) are readily detected by elutriation (Table 1). This suggests that seed colour and coat characteristics may be as important as size in influencing detectability by this method.

Several later-successional perennial species, such as *Solidago canadensis*, *Agropyron repens* and *Poa compressa*, which are not known to persist in seed banks, occurred in some of the germination samples but not in the elutriated samples. These seeds probably disperse into the site each year from the surrounding vegetation and represent species that Thompson & Grime (1979) have classified as having a transient (Type II) seed bank. Seeds of these species may only persist in the soil for a short time (one or two years), but they are indicative of the future dominants of the field. Many of these later-successional species have small seeds (Fenner 1985), and would thus be difficult to detect by elutriation. Alternatively, the distribution of these species in a field may be quite patchy, and they will only be detected if a large number of samples is taken (Thompson 1986) or if samples are combined. The manner in which samples were divided among methods in this study prevents a comparison among methods for rare or patchily distributed species; however, germination under conditions typical of spring (or the period of peak emergence in a region) may be the best method for estimating the abundance and distribution of these species in the soil.

Seed densities and viability

The observed differences in estimates of seed densities in the soil appear to be primarily due to counting inviable seeds in the elutriated samples. All of the seeds counted in these

samples appeared outwardly sound, and yet the viability tests reveal that for some species (e.g. *Chenopodium album*) the majority of the seeds counted were inviable. The low viability of the seeds is not an artifact of the elutriation method; previous studies have shown that elutriation does not affect seed viability of freshly collected seeds (Gross & Renner 1989). The extremely low viability (3%) observed for *C. album* seeds in these soil samples is not atypical for this species. Roberts & Ricketts (1979) also observed that the viability of *C. album* seeds removed from soil samples was extremely low. In a separate study at KBS, the viability of *C. album* seeds was examined in soil samples collected from different tillage systems, and samples from conventionally tilled fields had similar low levels of viability (3–6%) (K. L. Gross & D. E. Goldberg, unpublished). The Bailey field, from which these samples were taken, has been ploughed and treated with herbicide annually for more than a decade, and is quite similar to a conventionally tilled agricultural field.

The observed average viability of seeds from these samples (3–30%) is consistent with the levels reported in other studies in which various methods have been used to determine viability (Roberts 1981). The presence of large numbers of inviable seeds in soils makes it difficult to draw conclusions about seed densities from studies using seed counts from elutriated or washed soil samples. Viability testing of some sort is needed to correct these estimates. The causes of the reduced viability of seeds in these soil samples is not clear. Prior site use (particularly fertilization and herbicide levels and tillage practices) may influence the viability of seeds in the soil (Cavers & Benoit 1989; K. L. Gross & D. E. Goldberg, unpublished).

Spatial variability in seed distributions

Accounting for spatial variability is a major challenge to studies which attempt to quantify the density or distribution of species in the soil seed bank (Roberts 1981; Thompson 1986; Bigwood & Inouye 1988; Henderson, Peterson & Redak 1988). Such variability also hampers comparisons among studies because generally it is not clear whether the sampling methods used are comparable or adequate (Roberts 1981; Garwood 1989). The general recommendation for sampling seed banks is that 'a large number of small samples (be taken) rather than a small number of large ones' (p. 4, Roberts 1981). What is meant by a large number of small samples, however, is not clear. Frequently cited studies by Japanese investigators recommend that a minimum soil volume of 400–600 cm^3 is needed to estimate accurately the seed bank of arable fields or grasslands (Numata, Aoki & Hayashi 1964; Hayashi & Numata 1971); however, the number and distribution of samples needed to quantify the soil seed bank of a community will depend on the pattern of spatial variation in species distributions in the soil seed bank (Thompson 1986).

In this study, each sample contained 750 cm^3 of soil and a large number of samples (twenty-five) was taken. The area of the field sampled was relatively homogeneous in species composition and topography. Despite this apparent homogeneity of the sampling area, there were large differences in the distribution and abundance of species in the seed bank among sampling locations. Seed numbers $sample^{-1}$ varied from fourteen to sixty-five and fourteen to ninety-five in the direct and cold-stratified samples, respectively. Even greater variability in seed densities (0–615 seeds $sample^{-1}$) was observed in the elutriated samples, but some of this variation is due to the large number of inviable seeds in these samples. This range of variation in seed numbers among sample points is not atypical for

seed bank studies of arable fields (Thompson & Grime 1979; Roberts 1981; Archbold & Hume 1983; Thompson 1986; Bigwood & Inouye 1988; Cavers & Benoit 1989). Determining the cause of this variation is a major challenge and has important consequences for attempts to use information on seed-bank densities to predict species abundances, particularly in communities where seedling recruitment is highly dependent on seed banks (Wilson, Kerr & Nelson 1985; Wilson 1988).

In this study, some of the among-sample variation can be accounted for by location in the sampling grid. In both the direct and cold-stratified samples, seed number sample^{-1} generally declined from the north to the south end of the sampling grid. There was no apparent east–west trend in the data. Species number was also higher in samples taken from the northern than the southern end of the grid. This pattern may be a result of an 'edge effect'. Samples at the northern end of the grid were located closer to the field edge, and were therefore closer to a late-successional community that surrounded the field and could have served as a seed source. Dispersal from the northern edge into the study area might have been enhanced by ploughing, which was typically done in a north–south direction (J. Bronson, KBS small plots farm manager, personal communication). This is unlikely to be the full explanation, because late-successional species were infrequent in the samples and accounted for a relatively small proportion of the species and seeds counted.

There was also considerable variation in the number of seeds detected by each of the three methods at a single sample location. Although on average there was no significant difference in the number of seeds detected per sample in the two germination methods, at several sample locations there was a two- to threefold difference among these methods in the estimated number of seeds at a single sample point, some of which may be a consequence of the sampling procedure. The soil cores analysed by each method were collected from separate quarters of a 50-cm × 50-cm quadrat at each sampling point and thus were separated by approximately 25 cm. Several recent studies have provided evidence for such small-scale clustering of seeds in the soil (Benoit 1986; Thompson 1986; Bigwood & Inouye 1988), but these studies have not determined if spatial heterogeneity observed in the soil bank at small scales (e.g. < 1 m) from a few (or single) samples (e.g. Thompson 1986; Bigwood & Inouye 1988) can be extrapolated to the whole community. Different factors are likely to determine spatial heterogeneity at small (< 1 m) and large (> 10 m) scales, and the challenge remains to determine the causes and consequences of such heterogeneity at the population and community level.

The time of year at which these samples were taken (20–21 June) may account for some of the observed differences in seed densities with depth (see Table 2). Miller (1987) studied emergence time variation in this same field over four years (1982–85) and consistently observed that the peak periods of seedling emergence in this community occurred in early May and June. Few seedlings emerged after mid-June. Large numbers were observed in the field when these samples were taken, so the seeds recovered in the soil cores would represent the ungerminated fraction or carry over of seeds that remain dormant in the soil. This may account for the higher frequency of samples with no seeds in the 0–2-cm and 2–5-cm depths. Seeds deeper in the soil (5–15 cm) may not have been exposed to the necessary germination cues and thus more seeds remained in the soil.

The results of this study suggest that germination methods (particularly with stratification) may provide a more reliable method for determining the species composition of the seed bank of a plant community than elutriation. The difficulty in distinguishing among species (particularly congeners) with similar seeds, and detecting species with small seeds, results in an underestimate of species composition in elutriated

samples. Moreover, the large number of inviable seeds in the elutriated samples can result in an overestimate of seed densities. For species that are relatively common in the soil seed bank, this bias in seed density estimates can be overcome by determining the viability of seeds in a subset of samples and adjusting density estimates accordingly.

Despite the above-mentioned limitations of the elutriation method, this method may be preferred over germination methods for studies in which the focus is on the dynamics or distribution of a subset of species that occur in the seed bank. For population-level studies, elutriation may be preferred over germination methods because the distribution of a species can be determined across a large number of sample locations (or with depth). If seeds of the species of interest are easily distinguished, then the samples can be counted rapidly. Viability tests can be used to determine the proportion and distribution of viable and inviable seeds in the soil and thus provide information on patterns of seed mortality in the soil. Furthermore, for many evolutionary and genetical studies it is desirable to determine under specific conditions the characteristics (e.g. viability, isozyme frequency, potential growth rate) of the seeds persisting in the soil seed bank. Elutriation has no effect on seed viability, making it possible to save seeds for future testing and analysis.

In addition, for community-level studies in which the goal is to determine the spatial pattern of species abundances across a large area, the elutriation method may have several advantages over germination methods. Most seed-bank studies are criticized because few samples are taken and (in most cases) no attempt is made to determine whether or not sampling was sufficient (Roberts 1981). Surveys to quantify large-scale patterns of species abundance will, of necessity, require that a large number of samples be processed. The glasshouse space required to determine species composition with a large number of samples may be a severe limitation for many researchers. With the elutriation method, large numbers of samples can be processed and stored, allowing the work to be effectively spread out over several months. It is also possible to sample a subset of sample locations to determine the general pattern of spatial variation in the community. Finally, because the samples do not need to be maintained under standard conditions and there is no danger of losing samples to disease, other pests, or to unplanned 'droughts' (non-trivial risks with laboratory or glasshouse germination studies), elutriation may give a more consistently reliable estimate of species abundance and distribution in the soil than germination methods.

The elutriation method is particularly well-suited to agronomic studies in which the goal is to identify large-scale patterns of species abundance and composition so that effective weed-control programmes can be developed (Wilson 1988). Many of the dominant and agronomically most important weed species have large or distinctive seeds which makes them relatively easy to distinguish. Furthermore, the spatial pattern of viable or inviable seed distributions in the soil and any temporal changes that may occur due to changes in tillage or cultivation practices or vegetation development in successional systems can best be detected using the elutriation method to separate all seeds from the soil.

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