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A New Method for Estimating Seed Numbers in the Soil[1]

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Abstract. **An elutriation system was developed to estimate weed seed densities in soil. The system provided reliable estimates of seed densities for weed species with a broad range of seed weights (0.06 to 9.80 mg) and had no effect on seed viability of three weed species tested. Elutriation of soil samples (up to 60 g) taken from a cultivated field took approximately 15 min. Separating, classifying, and counting seeds is time consuming (20 to 30 min per sample) but provides an accurate estimate of seed densities for weeds with a diameter of 0.5 mm or more.**
Additional index words. **Weed seed abundances, soil seed bank, elutriation.**

INTRODUCTION

Quantifying the diversity and density of seeds in soil is of interest to agronomists, ecologists and evolutionary biologists (3, 6, 9). A number of methods for estimating the soil seed bank by germination or washing of soil samples have been described (6). Generally these methods are time consuming and effective in estimating the densities of only some of the species in soil (3, 4, 6, 8). The unknown reliability of these methods for estimating seed abundances in soil limits their usefulness for developing effective weed management plans.

Weed seed densities are known to be highly variable in soil (6, 8, 10). Thus, to estimate seed densities in soil accurately requires that a large number of relatively small soil samples be taken (6). Community-level surveys of seed diversity in soil also require that a large number of samples be taken across a field (or plant community) to estimate species diversity accurately.

We have developed a method of washing soil samples using a modified hydropneumatic root elutriator (7) that effectively separates seeds of a wide range of plant species from soil samples. The hydroelute system is very efficient in separating fine roots and organic matter from a wide range of soil types (7). Our modification of this system to estimate weed seed densities in soil was developed as part of an extensive survey of weed seed banks under different cultivation and cropping systems in the Long Term Ecological Research (LTER)[3] program in agricultural ecology at the Kellogg Biological Station (KBS)[3] in southwestern Michigan. Here we describe the elutriation method and its validity as a means of accurately estimating densities of agronomically important weed seeds in soil.

MATERIALS AND METHODS

Field sampling. A preliminary set of soil samples was taken from the central portion of the 40-ha LTER field site at KBS. The soil in an Oshtemo series (Typic Hapludalfs; coarse-loamy, mixed, mesic). The field has been under continuous cultivation with conventional, moldboard plow tillage and a corn (*Zea mays* L.)/soybean [*Glycine max* (L.) Merr.] rotation for more than 50 yr.

In late April 1988, prior to spring plowing, 50 soil samples were taken from randomly determined points along a 100-m transect across the middle of the field. At each location, five cores (2.5-cm diameter) were taken to a depth of 5 cm and combined. Soil samples were air dried, weighed to the nearest milligram, and washed using the elutriation method described below to determine weed seed densities in soil.

Elutriation method. Dried soil samples were washed using a modified hydropneumatic root elutriator developed by Smucker et al. (7) to estimate fine root production in compacted soils. For the seed bank quantification study, the primary sieves used to collect fine roots were replaced with a series of stacked sieves (10 cm long) constructed of 8-cm polyvinyl chloride (PVC)[3] pipe with nylon mesh screens at the bottom. The bottom and top edges of the sieves were sanded smooth to a slight angle to allow them to be easily inserted and removed from the connectors. The sieves were stacked in a graded series from largest to smallest, connected with PVC connectors, and placed on top of a 65-cm section of PVC pipe which stood in the bottom collecting pan of the elutriator (Figure 1). Openings (2.5 × 3 cm) cut in the bottom tube allowed water to flow freely from the sieve system into the collecting pan. Soil washed from the sample collected in the collecting pan; water flowed out an overflow into a floor drain.

Three sieves with mesh sizes of 710, 425, and 243 μm were used. These sieve sizes efficiently separated weed seeds and associated organic matter from the soil samples without clogging filters or impeding water flow. Soil samples were placed in the elutriation chamber (Figure 1) and washed for 10 min with the air and water pressure into the high kinetic washing chamber set at 140 kPa and 450 kPa, respectively. Preliminary trials with washing spiked samples with known numbers of seeds showed that this washing duration and air and water pressure allowed recovery of 95 to 100% of the sample seeds. Elutriation of more finely textured or clay soil may require a longer washing time and the use of a chemical dispersant (e.g., sodium hexametaphosphate) to separate organic matter, including seeds and fine roots, from clay

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[3]Abbreviations: LTER, Long Term Ecological Research; KBS, Kellogg Biological Station; PVC, polyvinyl chloride.

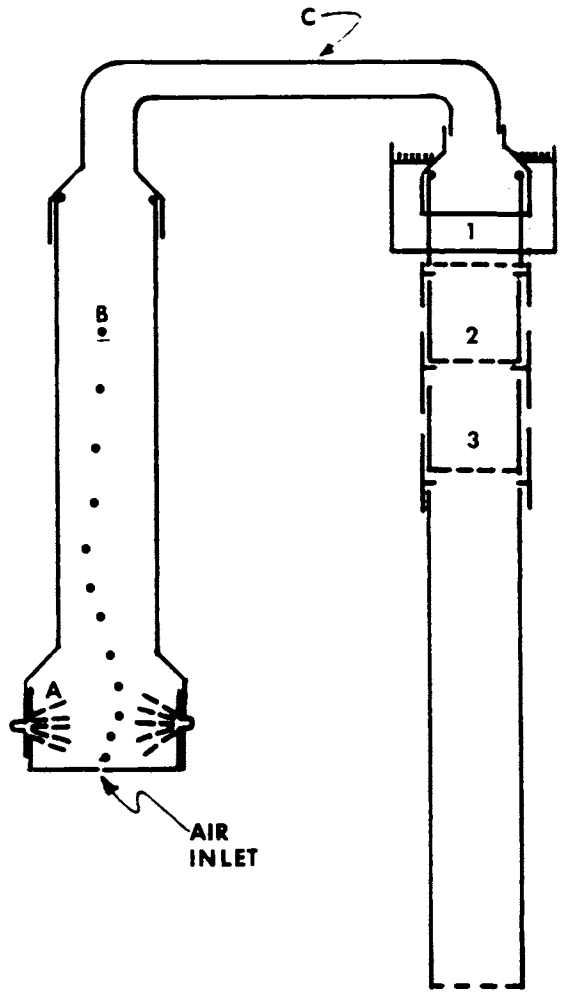

Figure 1. Schematic representation of the elutriation system used to wash soil samples. The soil sample is gently agitated in (A) the high kinetic washing chamber at the base of (B) the elutriation chamber. The removable transfer tube (C) and a size-graded set of collecting sieves (1 = largest mesh size; 3 = smallest) are connected by removable connectors and are mounted on a free-standing tube placed in the bottom collecting pan (not shown) of the elutriation system. Seeds, other organic matter, and coarse mineral fraction from the washed samples are removed from the collecting sieves and washed onto filter paper-lined Buchner funnels (not shown), excess water is removed, and the sample transferred to a petri plate and air dried for 24 h or oven dried at 35 C for 3 to 4 h.

materials (7). In our studies, we have avoided the use of chemical dispersants because the effect on seed viability is unknown.

After washing, sieves were removed from the filtrating system and the collected contents washed into separate Buchner funnels lined with labeled filter paper. The funnels were mounted on a filtration manifold attached to a vacuum/pressure pump (2.7 kPa) which was used to remove excess water from the samples. The filter paper was then placed in a labeled petri plate, allowed to air-dry for 24 h (or oven-dry at 35 C for 3 to 4 h), taped shut, and boxed for storage until seeds were counted and identified.

Each plate was examined under a dissecting microscope (10 to 30×) and all seeds grouped by species and counted. Seeds were keyed to species using weed seed keys (1) and reference weed seed collections from KBS. Counts made from the three sieves were combined to determine the number of species and seeds at each sample point. Having the seed samples separated on different sieve (mesh) sizes facilitated counting and sorting of seeds because large seeds were encountered on the larger mesh and small seeds only on smaller mesh sieves.

Validation of method. To determine what size range of weed seeds would be effectively sampled from soil using this method, the percent recovery of seeds of 13 weed species was determined. Fifty seeds of each species were mixed into the sand fraction of the Oshtemo field soil which had been washed to remove all seeds and elutriated using the method described above. Seeds were run separately and also combined with several species. Two to seven replicates were run for each species.

To determine the accuracy of seed removal and counting from the washed samples, the soil and material remaining in a subset of counted samples was spread over a sterilized soil mix[4] and placed under a mist bench in the greenhouse. The samples were checked daily for germinated seeds and their position on the bench rotated each week. The number and species of seedlings that emerged was determined after 3 weeks.

To ascertain whether the elutriation method had any effect on seed viability, we compared viability of elutriated and unelutriated seeds of three weed species. Seeds for these tests were taken from bulk seed samples collected in the fall of 1987 from local fields. Seeds of common ragweed (*Ambrosia artemisiifolia* L. #[5] AMBEL) and common lambsquarters (*Chenopodium album* L. # CHEAL) were tested for viability using a 0.1% solution of tetrazolium, following the methods of Moore (5). Five replicate plates with 20 seeds per plate were tested. Carpetweed (*Mullugo verticillata* L. # MOLVE) seeds did not stain well with tetrazolium, so viability was estimated by germinating the seeds in an environmental chamber. Five replicate plates (20 seeds/plate) were placed in a chamber set with alternating day/night temperatures (25/15 C) with a 12-h thermoperiod, a light intensity of 55 $\mu E \cdot m^{-2} \cdot s^{-1}$, and a 14 h/10 h (light/dark) photoperiod. Plates were checked every 3 to 4 days and final germination determined after 2 weeks. Differences in mean viability of elutriated and unelutriated seeds were compared by a t-test ($P \leq 0.05$).

[4]Sunshine Seedling Mix #3. Fisons Western Consumer Products, Downers Grove, IL 60515.

[5]Letters following this symbol are a WSSA-approved computer code from the composite List of Weeds, Weed Sci. 32, Suppl. 2. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

Table 1. Estimated seed densities in soil of most abundant weed species determined by elutriation of soil samples.

Species	Frequency[a]	Seed density		Total seeds	
	(%)	(no./sample)[b]	(no./m^2)[c]	(no.)	(%)
Witchgrass	36	0.94 (0,6)	376	30	20
Redroot pigweed	14	0.44 (0,14)	176	22	15
Lambsquarters	16	0.24 (0,4)	96	12	8
Black nightshade	8	0.14 (0,4)	56	7	5
Lifeform:					
Total grasses	44	0.80 (0,6)	320	40	27
Total dicots	84	2.18 (0,15)	870	109	73
Total seeds	. . .	2.98 (0,15)	1190	149	100

[a]Percent of samples (n = 50) in which the species or lifeform (grasses or dicots) was detected.

[b]Mean number of seeds per sample; values in parentheses are observed minimum and maximum values.

[c]Calculated based on an area sampled of 0.0025 m^2 to a depth of 5 cm.

Table 2. Recovery of weed seeds of different sizes by elutriating spiked soil samples.

Species	Seed mass	Diameter[a]	Recovery[b]
	(mg)	(mm)	(%)
Mouseearcress	0.022	<0.5	17 (3)
Carpetweed	0.060	0.5	87 (3)
Witchgrass	0.471	0.5–0.7	87 (3)
Fall panicum	0.655	0.6–0.9	81 (3)
Redroot pigweed	0.204	0.6–1.0	87 (6)
Common eveningprimrose	0.379	0.9–1.3	96 (2)
Common lambsquarters	0.509	1.2–1.6	91 (7)
Buckhorn plantain	0.803	1.0–1.3	99 (2)
Ladysthumb	1.509	1.7–2.0	99 (2)
Barnyardgrass	1.955	1.8–2.4	99 (2)
Yellow foxtail	2.013	1.8–2.1	100 (2)
Common ragweed	5.348	1.8–2.5	98 (6)
Velvetleaf	9.800	2.2–2.6	83 (3)

[a]Data from R. J. Delorit. (1).

[b]Mean percent recovered from 2 to 7 replicates per species (50 seeds/species/replicate); numbers in parentheses are number of replicates.

RESULTS AND DISCUSSION

Field sampling. Elutriation of the field samples revealed considerable variability in seed numbers and species composition among samples (Table 1). A total of 18 weed species were detected in the soil samples, with four species making up approximately 50% of the seeds counted (Table 1). Total weed seed densities estimated ranged from 0 to 6000 seeds/m^2 in each sample ($\bar{x}$ = 1190). This mean density and degree of sample variability are within the range observed in other studies which have used a variety of techniques to estimate weed seed numbers in soil (3, 6, 8, 9, 10). Our estimates are based on sampling only the top 5 cm of soil in the field. However, because this field has been regularly cultivated, the distribution of weed seeds in the soil is likely to be relatively uniform (9).

Elutriation of each soil sample took approximately 15 min: 10 min to wash and 5 min to remove the washed material from the sieves and transfer to petri plates. These time estimates are relatively consistent for soil samples involving up to 60 g of soil from a variety of agricultural and early successional plant communities. Larger soil volumes clog the sieves (particularly with samples from very sandy soils) and should be divided into subsamples for effective elutriation. Soils with a higher clay content may require longer washing times. The water system developed by Kovach et al. (2) for washing soil involves a larger diameter sieve and as a consequence can handle larger soil volumes (up to 1 kg). Our interest was to quantify spatial variability in the soil seed bank (with depth and across the field), which required a large number of samples of relatively small soil volume.

Validation of elutriation method. The elutriation system was very effective in recovering seeds of a broad range of sizes from soil samples. Of the 13 species tested, recovery of seeds was greater than 90% for species with seeds larger than 0.3 mg (Table 2). For species with seed weights between 0.06 and 0.3 mg., recovery was lower, but still greater than 75%. Recovery of mouseearcress [*Arabidopsis thaliana* (L.) Heynh. # ARBTH] seeds (the smallest seeds tested, 0.02 mg) was poor (Table 2). These results are consistent with those of Kovach et al. (2) who also found that recovery of seeds using a water spray system was higher for species with large seeds. Recovery of small seeds might be increased by using smaller mesh sizes in the filtering sieves. However, this would substantially increase the time involved in elutriating samples because small soil volumes would have to be used to prevent sieves from clogging with sand and overflowing.

Overall, visual inspection of the plates was an effective method for counting seeds larger than 425 μm. In the greenhouse, seedlings emerged only from samples collected on the smallest mesh size (243 microns). Only 21% of these samples (6 of 29) had undetected seeds and only a total of 11 seeds emerged from these samples. Of these, five were purslane speedwell (*Veronica peregrina* L. # VERPG) (mass = 0.02 mg) and five were mouseearcress. Seeds of both these species are very light colored which made them difficult to detect in the residue that remained from the washed samples. Carpetweed seeds are also relatively small (Table 2) but have a dark shiny seed coat which makes them easy to detect in the washed samples. Carpetweed did not emerge in the washed soil samples which suggests that most viable seeds were counted.

Elutriation appeared to have no effect on seed viability. Seeds of all three species tested did not differ in viability after being elutriated (Table 3). This result would be expected because elutriation does not expose the seeds to chemicals, and the gentle washing created by the elutriator does not disrupt or damage the seed coat (personal observation).

Counting and classifying seeds to species is the most time-consuming part of this method. Samples counted for these preliminary studies required approximately 5 to 10 min per plate (20 to 30 min per sample) to count. The time required to count a sample is dependent on the number of species and seeds encountered. For example, a single sample from a no-

Table 3. Estimated viability of unelutriated and elutriated seeds.

Species	Viability		Method[a]
	Unelutriated	Elutriated	
	(%)		
Carpetweed	84	85	G
Common lambsquarters	78	79	Tz
Common ragweed	77	80	Tz

[a]Viability determined by tetrazolium (Tz) following methods of Moore (5) or germination (G) in an environmental growth chamber. (See text for details.)

herbicide treatment plot in a separate study had over 500 seeds, primarily common lambsquarters, and took over 3 h to count. Samples taken from an early successional field also had high seed densities (mean of 130 seeds/sample) and up to 18 species per sample[6]. These samples took considerably longer to count, because of the high numbers of seeds and the many species present. Seed numbers in samples from arable fields (with relatively lower species diversity and high seed densities) could be estimated by subsampling randomly selected quadrats of the individual petri plates.

This seed elutriation and identification method provides a means of efficiently and accurately determining seed densities and diversity in soils. The method is particularly effective for estimating seed densities in soil of relatively large-seeded species (both dicots and monocots) that are important agronomic weeds. A large number of soil samples can be washed in a relatively short time and the samples stored for later enumeration. This allows the labor involved to be spread out over several months and does not require the continued commitment of time that more standard germination methods require (6). Counting and sorting seeds to species remains the most time-consuming aspect of the work, and more rapid methods for identifying and counting seeds need to be developed if soil seed bank estimates are to become informative tools in developing weed management systems.

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