Comparison of three methodologies for efficient seed extraction in studies of soil weed seedbanks

M B MESGARAN*, H R MASHHADI*, E ZAND‡ & H M ALIZADEH*

*Department of Agronomy, University of Tehran, Karadj, Iran, and ‡Department of Weed Research, Plant and Pest Disease Research Institute, Tehran, Iran

Received 8 March 2007
Revised version accepted 24 July 2007

Summary
Determination of weed seeds in the soil is tedious and time consuming. To evaluate the different seed extraction methods to improve seedbank estimations, three extraction methods (sieving, cloth bag and flotation) were compared, based on their accuracy and time needed for separation processes and enumeration. Seeds of Datura stramonium, Amaranthus retroflexus, Portulaca oleracea and Plantago major were used to artificially infest soil samples of four textures, namely clay, clay loam, loam and sandy loam containing 19%, 27%, 38% and 65.5% sand respectively. Soil textures had a significant effect on counting time in all extraction methods. In flotation, four submethods involving different solutions and centrifuge rotation speed were examined, but showed no differences, so data were pooled. Counting time in flotation, cloth bag and sieving methods was 9, 16 and 30 min respectively. However, when the time needed for other processes was taken into consideration, flotation and bag methods did not differ significantly. Species-wise seed recovery was not affected by soil texture in the bag method, suggesting an advantage for this approach as its accuracy is not soil texture-dependent. Total seed recovery for flotation, sieving and bag methods was 61%, 67% and 75%, respectively, and was not significantly different. Considering the efficiency of methods, the cloth bag technique could be recommended, because it was as time consuming as the flotation method, but required the same minimum equipments and costs as the sieving method.

Keywords: seed extraction, cloth bags, sieving, flotation, seedbank, sampling efficiency.


Introduction
A knowledge of weed seedbanks is necessary, for example, to carry out studies of population dynamics or to establish weed management programmes (Ambrosio et al., 2004). Bioeconomic models require information about the size and composition of the weed seedbank or seedling population (Wiles & Schweizer, 1999). However, such information is lacking for many weed species and cropping systems (Buhler & Maxwell, 1993), because determining the density of weed seeds in the soil is a tedious and time consuming process (Buhler & Maxwell, 1993; Cardina & Sparrow, 1996), costly (Wiles & Schweizer, 1999) or detrimental to seed viability (Tsuyuzaki, 1993).

The methods that have been proposed for weed seedbank analysis can be ascribed to two main categories: (1) direct seed extraction methods including various sieving techniques (e.g. Smutny & Kren, 2002), elutriation (e.g. Kovach et al., 1988; Wiles et al., 1996) or flotation (e.g. Malone, 1967; Buhler & Maxwell, 1993; Tsuyuzaki, 1994; Goto & Tsuyuzaki, 2004) and (2) germination methods by which the seedbank is assessed via identification and enumeration of seedlings emerged from the soils under controlled conditions (e.g. Cardina & Sparrow, 1996; Barberi et al., 1998; Swanton et al., 2000).

The germination method is simple (Buhler & Maxwell, 1993), but must be carried out over a time period that is too long to make the prediction of actual seedling
populations useful (Ball & Miller, 1989). Some under-
estimations for this method have been also reported. For
example, in the study of Goto and Tsuyuzaki (2004),
mean seed density obtained by flotation method was
about four times higher than that of a germination
method. This underestimation may arise from the
presence of dormant seeds in the soil seedbank. Sieving
methods are appropriate techniques particularly for
detecting large-seeded weeds. Using a water-spray sys-
tem, Kovach et al. (1988) recovered 100% of Avena
fatua L. and Rumex crispus L. seeds (and some other
species). Flotation methods may give a high percentage
recovery of all species (Tsuyuzaki, 1994) and work best
for samples from sandy soils (Wiles et al., 1996).

Soil texture has considerable effects on the efficiency
of these methods. Wiles and Schweizer (1999) found that
the average time to determine presence or absence of
seed in a soil core is 3.78 and 15.25 min for a core from a
field with 37% and 88% sand respectively. Counting and
identifying seeds took almost 2.5 times longer for sand
samples processed by elutriation, rather than by flota-
tion (Wiles et al., 1996). Similar results were reported by

As discussed above there are many advantages and
disadvantages for each seedbank estimation method,
thus, necessitating the evaluation of these methods based
on their accuracy, time required, costs and equipment
requirements. The objective of this study was to com-
pare the efficiency of six different direct seed extraction
methods using soils with different textures and weeds
with different seed sizes.

Materials and methods

Experimental procedures

Laboratory experiments were carried out to compare
three major weed seed separation methods including
flotation (with four sub-methods), sieving and cloth bag
methods. Seeds of Datura stramonium L. [3.3 × 2.5 mm,
thousand seed weight (TSW) of 5.7 g], Amaranthus
retroflexus L. (1.1 × 0.9 mm, TSW of 0.52 g), Portulaca
oleracea L. (1.0 × 0.9 mm, TSW of 0.39 g) and Plantago
major L. (1.0 × 0.6 mm, TSW of 0.15 g), respectively,
were used to artificially infest seed free soil samples of
four textures: clay, clay loam, loam and sandy loam
(Table 1). All seeds were obtained from Weed Research
Institute, Tehran, Iran, with seed measurements (seed
size and TSW) characterised by this institute. Soils were
collected at the University of Tehran Research Farms in
2006. Soil samples were taken from the depth of 50 cm
assuming that the soil is seed-free, as field history
showed tillage depth has never exceeded 25 cm deep.
Some subsamples of these soil textures (prior to the
artificially addition of weed seeds) were subjected to a
sieving method to evaluate whether soils contain weed
seeds. This investigation confirmed our assumption that
soils were seed-free. The soils of each texture type were
taken to the laboratory and sieved (five-mesh: number of
openings per inch length of mesh) to separate stones and
large particles, before subjecting them to the extraction
experiments. For all extraction experiments, soil samples
of 150 g (dried and without stones) were used, which is
nearly equal to a single 3–3.5 cm diameter core (10 cm
depth) with soil bulk density of 1.3 g cm⁻³. Although,
the amount of soil used in this experiment is less than the
normal quantities taken for seedbank determinations,
this insufficiency is a common attribute subscribed to all
extraction methods, making the comparison of methods
reasonable. There are also some examples using cores
narrower than 3.5 cm (e.g. Benoit et al., 1989; Schreiber,
1992; Mulugeta & Stoltenberg, 1997a). In all extraction
methods, 20 seeds of each weed species were added to
seed-free soil samples. Thereafter, seed/soil samples
were subjected to different extraction methods as
described below.

Sievng was performed by placing the samples onto
the sieves (60-mesh) and washing the fine soil particles
through the mesh with running water. The residues were
placed in the plastic dishes and kept at room temper-
ature to be dried for subsequent enumeration. In the
cloth bag method, 10 × 15 cm fine net cloth bags (mesh
opening of 0.25 mm) were used. Samples were poured
into the bags and manually washed under running tap
water. Other processes were the same as sieving method.
The flotation method consisted of four sub-methods
including:

1 The samples were placed in 400 mL centrifugal plastic
tubes and 100 mL of flotation solution was added.
The flotation solution was prepared by adding 250 g
of MgSO₄ (4.1 m) and 25 g of a 2:1 by weight mixture
of sodium hexametaphosphate and sodium carbonate
to 500 mL of distilled water (specific gravity 1.2 g
mL⁻¹). The mixture (soil and solution) was subjected
to centrifugation at 2408 g for 10 min. Prior to
centrifugation, the plastic tubes were manually
shaken for 1–2 min to obtain a homogenous aliquot.

<table>
<thead>
<tr>
<th>Table 1 Properties of four soil types used in seed extraction experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil texture</td>
</tr>
<tr>
<td>Clay</td>
</tr>
<tr>
<td>Clay loam</td>
</tr>
<tr>
<td>Loam</td>
</tr>
<tr>
<td>Sandy loam</td>
</tr>
</tbody>
</table>
2 All components and processes were the same as above, except that MgSO\textsubscript{4} was substituted by K\textsubscript{2}CO\textsubscript{3} to increase the specific gravity (1.4 g mL\textsuperscript{-1}).

3 As with the second sub-method, except that only a 100 mL of 5.5 M K\textsubscript{2}CO\textsubscript{3} was used as flotation solution (specific gravity 1.54 g mL\textsuperscript{-1}). Other processes were the same as above.

4 Like the third sub-method, except that the centrifugation was run at 9632 g.

In all flotation sub-methods, supernatants were filtered through net bags used in the cloth bag method. Seeds from each method were counted and identified using a binocular stereomicroscope with variable magnification. Recovery percentage, time needed for seed identification and enumeration and also total time periods spent to accomplish different tasks (e.g. soil washing, preparing solutions, centrifugation, emptying tubes and bags, etc.) were recorded for each separation method. None of separation methods damaged seeds, so this attribute was not analysed.

**Statistical analysis**

For each experiment, a separate analysis of variance (ANOVA) was performed on the data of recovery percentages, enumeration time and total time spent for a particular extraction method using the PROC ANOVA procedure of SAS (SAS Institute, 2002). In addition to the separate analyses, the whole experiment was subjected to the ANOVA in a factorial arrangement (i.e. extraction methods x soil textures) based on a completely randomised design with four replications. Recovery per cent data were not arcsine square root-transformed before analysis, as data showed homogenous variance distribution and satisfied tests of normality. Differences among treatment mean values were compared using a LSD test at the 0.05 significance level.

**Results**

**Flotation methods**

Neither seed recovery (total and species-wise seed recovery), nor counting time varied significantly among flotation sub-methods (data not shown). As a result, the data for four sub-methods were pooled into the single flotation method. Soil texture only influenced seed recovery of *P. major* (Fig. 1). For this species a higher seed recovery percentage in sandy loam was partly due to the weak adhesion of seeds to soil particles. The *P. major* seeds could be flocculated by soil particles as its seeds become sticky after water imbibition. This attribute not only makes the identification and enumeration of *P. major* seeds difficult (the main reason for poor seed recovery of this species in all methods), but also intensifies the settlement of seeds in heavy soils and prevent them of being brought up to the top of supernatant in flotation method.

Total seed recovery did not differ significantly between soil textures, ranging from 56% in clay up to 63% in a sandy loam soil (Fig. 2). Counting time increased slowly with soil sand content (Fig. 3). Only the sandy loam soil required significantly longer time (14.2 min) for enumeration (Fig. 4). It also took 8 min to complete additional tasks by this method (Fig. 4).

**Sieving method**

Soil texture had significant effects on seed recovery of all species except that of *D. stramonium*, as its large seeds eases seed detection and enumeration (Fig. 1). Sandy loam soil had significantly lower total seed recovery (42%) among the soil textures (about 73% for others) (Fig. 2). Sand particles larger than the sieve mesh size would not pass through the sieve, so the higher the sand content, the more material remained from which to remove weed seeds. The final volume of samples (after being washed) increased exponentially as the sand content of soil increased (Fig. 3).

In sandy loam soil, because of high sand content (65.5%), the fatigue from counting the seeds from a large amount of sand would lead to errors (underestimation) and also a considerable increment in counting time (Fig. 3). Counting time was highly affected by soil texture (Fig. 4). The average time spent for seed extraction was 17 and 56 min for samples with clay and sandy loam textures respectively. An additional 5 min time period was also spent to accomplish other processes of sieving method (for example washing soils, transferring, etc.) (Fig. 4).

**Cloth bag method**

Soils were washed efficiently with cloth net bags in < 2 (± 0.5) min for a 150 g soil sample, irrespective of soil textures (data not shown). Seed recovery for a particular species (Fig. 1) or total weeds (Fig. 2) was not affected by soil textures, providing an advantage for bag method, as its accuracy is not soil texture-dependent.

In contrast to seed recovery, counting time was significantly different among soil textures (Fig. 4). Similar to sieving method, counting time increased (exponentially) with soil sand content. However, the rate of increase was lower than that of the sieving method (Fig. 3). Minimum (7.5 min) and maximum (33 min) counting times were recorded for clay and sandy loam soils respectively (Figs 3 and 4).
Discussion

The cloth bag method was more efficient at recovering seeds of *A. retroflexus* (92%) and *P. oleracea* (78%); however, total seed recovery did not differ among the extraction methods (Table 2), i.e. all methods have nearly the same accuracy. Seed recoveries obtained in our experiment by different methods (Table 2) for large
to small-seeded weeds (D. stramonium, A. retroflexus and P. oleracea), were similar to those reported elsewhere (Kovach et al., 1988; Gross & Renner, 1989; Buhler & Maxwell, 1993; Wiles et al., 1996). However, lower recoveries were found for very small-seeded P. major for the reason discussed previously (very low seed recovery of this species also reduced average total seed recoveries in all methods).

In spite of seed recovery, the time required for enumeration varied greatly between methods (Fig. 5). The average counting time for sieving, cloth bag and flotation methods were 36, 16 and 9 min respectively. Flotation method required less time for enumeration, as lesser amount of materials are retained after processing. Our results resembled those reported by Wiles et al. (1996). They also found no differences between flotation

...
Table 2 Recovery of weed seeds from soil in different seed extraction methods (averaged over soil textures). Twenty seeds of each species were added to a soil sample of 150 g

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Datura stramonium</th>
<th>Amaranthus retroflexus</th>
<th>Portulaca oleracea</th>
<th>Plantago major</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sieving</td>
<td>98a</td>
<td>78b</td>
<td>64b</td>
<td>31a</td>
<td>67a</td>
</tr>
<tr>
<td>Cloth bag</td>
<td>98a</td>
<td>92a</td>
<td>78a</td>
<td>33a</td>
<td>75a</td>
</tr>
<tr>
<td>Flotation</td>
<td>99a</td>
<td>72b</td>
<td>62b</td>
<td>11b</td>
<td>61a</td>
</tr>
<tr>
<td>SED</td>
<td>2.12</td>
<td>5.34</td>
<td>4.76</td>
<td>3.39</td>
<td>6.95</td>
</tr>
</tbody>
</table>

*36 df.
Mean values in the same column not sharing the superscript letter are significantly different at \( P < 0.05 \) (LSD test).

The time required for seed enumeration or total time spent for all tasks of each extraction method. Twenty seeds of each species were added to a soil sample of 150 g. Vertical bars show Fisher LSD values \( (P < 0.05) \).

Methods of soil seed extraction in terms of accuracy, but counting and identifying seeds took almost 2.5 times longer for elutriation than the flotation method. Although the flotation method demanded less time for enumeration, it is restricted by additional efforts, like preparing flotation solutions, shaking the aliquot prior to centrifugation and placing the soil samples into the tubes and then emptying them, etc. When these extra time periods were also taken into consideration, the total processing time for flotation method was 16 min and did not differ significantly from the cloth bag method (Fig. 5). Using the flotation method, Tsuyuzaki (1994), also recovered seeds from soils within 15 min. The time required to count and identify seeds in a core ranged from 44 s to 115.32 min with an average time of 9.56 min in the study of Wiles and Schweizer (1999) for naturally infested soil samples. As shown in Fig. 4, for clay, clay loam and loam textured soil, total time did not differ between cloth bag and flotation methods. Only in sandy loam soil did the bag method require significantly longer time to complete the extraction process, compared with the flotation method. Two other criteria in selecting a proper extraction method are costs and equipment requirements. As there were no significant differences between the four flotation sub-methods, it is advisable to use the third sub-method, because it only requires \( \text{K}_2\text{CO}_3 \) as the flotation solution and a centrifugation speed of 2408 g.

Clearly the cloth bag technique has some advantages over the two other methods tested, which are itemised below: (1) it requires less time for both separation and seed counting processes (Figs 1 and 4); (2) with the exception of \( D. \text{stramonium} \), for which all methods gave a similar percentage of seed recoveries, the recoveries of \( A. \text{retroflexus} \) and \( P. \text{oleracea} \) were significantly higher than those of flotation or sieving methods. When compared with flotation method, it exhibited a similar recovery percentage for \( P. \text{major} \) (Table 2); (3) species-wise and total seed recovery were not affected by soil textures in bag method (Figs 1 and 2), i.e. it was a soil texture-independent method; (4) the cloth bag method requires minimal equipment and costs (no need of a centrifuge set and chemicals). It also occupies such a very small area that space limitation would not be a problem for this method; (5) working with cloth bags is not restricted to laboratories or glasshouse facilities; it could be accomplished wherever there is running water to wash soil-containing bags; (6) it is also easy to work with cloth bags, demanding no prior expertise and (7) a no less important attribute is that cloth bags could be directly used as sampling pockets in soil seedbank studies. Thus, there is no need for preparing additional sampling pockets and the task of replacing soil samples to a secondary device for the subsequent processes of an extraction method is superfluous.

However, the cloth bag method also has some disadvantages, albeit, not solely attributable to this technique. Similar to a flotation method, it would not provide the separation of organic matter and crop debris as well large stones (and large organic particles); the latter is a problem in common with the sieving method. The cloth bag is restricted to seeds larger than the bag mesh size and would not retain small-seeded species. That is also a limitation in the sieving method. In such a case (separation of small-seeded species), the germination method may estimate the seedbank size more
precisely (Goto & Tsuyuzaki, 2004). However, the germination method has also its specific limitations, one being the long-time seedlings need to germinate and emerge. For example, Roberts (1981) suggested continuing the process for 2 years. In the flotation method, a high capacity centrifugation is necessary and limited number and volume of centrifugal vials, as well as the needs for chemical solutions, are among other constrains for the flotation method. Accordingly, the bag method could be recommended as it was as time consuming as the flotation method, but requiring the same minimum equipment and costs of the sieving method.

References