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Extractable and Germinable Seedbank Methods Provide Different Quantifications of Weed Communities

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Research Article

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Abstract

Seedbank sampling and quantification methods vary in their ability to describe weed diversity and density, so proper method selection is critical for studying weed communities. The germinable seedbank (GSB) method is commonly preferred over extractable seedbank method (ESB), because the latter is more time-consuming. However, these two methods have only been compared using a few weed species and a relatively small number of samples. A total of 204 weed seedbank samples were used to compare both methods for weed density, richness, evenness, and Shannon-Weiner diversity using a split-sample approach. The two methods yielded dramatically different results. The ESB had 418% higher density and 35% more species per sample but 11% less evenness than the GSB. Diversity was estimated to be only 9% higher using the extractable compared with the germinable method. While the extractable method had higher density and richness overall, this was not true for every species, with only 7 of 14 common species detected in higher amounts by the extractable method. The results indicate the two methods are not strongly correlated, limiting the possibility of generating a conversion factor between methods. Nevertheless, evenness and Shannon-Weiner diversity might allow comparisons between both methods when the emphasis is on characterizing predominant weed species. The GSB is a practical approach to compare treatments or conditions; however, the ESB is more useful to accurately quantify weed species richness, diversity, and density.

Introduction

Characterizing soil seedbanks is important for defining the flora of an area, understanding ecological systems in nature and in agriculture, and forecasting future emergence (Cardina and Sparrow 1996; Forcella 1992; Hoffman et al. 1998; Price et al. 2010). Despite these crucial needs, seedbank collection and characterization are tedious processes, with various methods yielding incomplete results that have been debated, compared, and improved over the years (Ball and Miller 1989; Price et al. 2010). Methods to describe soil seedbanks generally fall into two categories: the germinable seedbank method (GSB) or the extractable seedbank method (ESB). Over time, there have been improvements to both types of methods to increase efficiency in seed recovery and species detection. While the Malone flotation method (Malone 1967) has been widely used, it favors large seeds and misses species during the extraction process (Gross 1990; Mesgaran et al. 2007).

In general, it is considered that the ESB provides a more complete representation of the total seedbank (Abella et al. 2013; Gonzalez and Ghermandi 2012), while the GSB is considered to represent what would emerge in the field (Cardina and Sparrow 1996; Forcella 1992). However, in some cases, the GSB might detect species that were unaccounted for in the ESB, especially species that have very small seeds or camouflage with the soil particles (Abella et al. 2013; Ball and Miller 1989; Gonzalez and Ghermandi 2012; Gross 1990; Marshall and Arnold 1994), even from paired samples (Ball and Miller 1989). Overall, the GSB might underestimate the entirety of the seedbank, and the ESB is still prone to seed losses due to the extraction procedure (e.g., sieving, floating, or repetitions per sample) (Gonzalez and Ghermandi 2012; Mesgaran et al. 2007; Price et al. 2010).

Of the studies that have compared various iterations of these methods in different environments, several have created artificial seedbanks with known numbers of seeds to assess recovery (Buhler and Maxwell 1993; Espeland et al. 2010; Mesgaran et al. 2007; Tsuyuzaki 1994). A few comparisons of natural seedbanks have used paired samples (Abella et al. 2013; Ball and Miller 1989; Forcella 1992; Price et al. 2010), but the majority have randomly assigned samples to each method (Cardina and Sparrow 1996; Gonzalez and Ghermandi 2012;

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Gross 1990; Marshall and Arnold 1994), which reduces the possibility of isolating the variation generated by the seedbank quantification method. In most cases, the sample size used has also been small, ranging from 18 to 80 paired or random samples. An exception was a study conducted by Ball and Miller (1989), who used the flotation extraction method as proposed by Malone (1967) to analyze 144 paired samples. However, the accuracy of seed detection could be negatively affected by the flotation extraction method (Gross 1990; Mesgaran et al. 2007). Furthermore, most studies have examined the similarity of each method for a single or few species independently, and few agricultural seedbanks have been used to compare these methods to characterize weed communities using indexes such as richness, evenness, and diversity. Gonzalez and Ghermandi (2012) reported differences in composition between methods based solely on species richness and species by species inconsistencies. A methods comparison study by Abella et al. (2013) analyzed species composition as single number, but the objective was to identify rare species in natural systems.

Because the literature contains both GSB and ESB methods, it would be useful to describe the relationship between these two methods to facilitate comparisons. This is particularly important to make better use of the increasing number of studies assessing weed diversity and its role in agroecosystem biodiversity. It was hypothesized that the GSB underestimates indexes describing weed seedbank composition compared with the ESB, but these methods provide similar characterization of weed communities. Therefore, the objectives of the present research were (1) to use a large number of paired weed seedbank samples to determine the relationship between the GSB and ESB for parameters that describe weed communities; and (2) to generate a conversion factor to facilitate the comparison of studies using the GSB with others using the ESB, and vice versa, to describe weed diversity in agricultural fields.

Materials and Methods

Seedbank Sampling and Characterization

Seedbank samples were collected from 2012 to 2016 from experimental plots at the North Florida Research and Education Center campuses located in Quincy (42 plots; 84.55°W, 30.6°N) and Marianna (24 plots; 85.10°W, 30.52°N), FL. The soil in Quincy was a Dothan sandy loam (fine-loamy, kaolinitic, thermic Plinthic Kandiudult), and in Marianna the soil was a mosaic of Fuquay coarse sand (loamy, kaolinitic, thermic, Arenic Plinthic Kandiudult) and a Red Bay fine sandy loam (fine-loamy, kaolinitic, thermic Rhodic Kandiudult). Seedbank samples were collected as part of long-term studies assessing peanut (*Arachis hypogaea* L.)–cotton (*Gossypium hirsutum* L.)–bahiagrass (*Paspalum notatum* Flueggé)–bahiagrass rotations (for field and plot details, see Leon et al. [2015] and Leon and Wright [2018]). These seedbank collections generated a total of 204 samples, which provided a good opportunity to compare seedbank methods using more statistical power than previous studies.

In April, before any herbicides were applied, 3.5-cm-diameter soil probes were used to retrieve 20-cm-deep soil cores. Eight cores were collected from each field following a serpentine pattern spacing the samples 1.5 to 3 m apart. The soil cores were pooled and homogenized by manually breaking up soil structure, mixing the soil, and removing plant residue. All seedbank samples were stored in ziplock bags at 4 C in the dark and were analyzed within 4 mo after collection.

To properly assess the impact of the method on seedbank characterization, each seedbank sample was analyzed with both methods. This captured the variability introduced by the method and avoided confounding effects associated with using independent samples for each method, as was commonly done in previous studies (Gonzalez and Ghermandi 2012). Therefore, after homogenization, samples were split into two fractions, a 650 ± 10 g subsample was used to study the GSB and a 200-g sample was used to study the ESB. A smaller sample was necessary to expedite seed extraction in the ESB. For the GSB method, during the third week of May, the sample was spread on a plastic tray forming a 1.5-cm soil layer and maintained in a greenhouse under daily irrigation with micro-sprinklers to maintain field capacity. The greenhouse was maintained at 28 ± 5 C and had natural light (14-h photoperiod). During 5 wk, weed seedlings were identified, counted, and removed once or twice per week depending on emergence rate. This process was repeated after a 2-wk period during which irrigation was withheld and the soil was stirred to favor seedling emergence.

In the case of the ESB, the samples were washed with tap water using very low pressure, and a sieve was used to retain only >150-µm particles and eliminate most of the clay and silt. The washing period was approximately 20 min per sample, and sample stirring was done carefully to avoid any seed damage. This step was important, because our goal was to maximize weed seed counts and avoid losses or underestimations common to mechanical and/or high-pressure elutriation or flotation seed-extraction methods (Gross 1990; Mesgaran et al. 2007). Extractable seedbank samples contained predominantly weed seeds, sand, small plant residues, and invertebrate corpses (exoskeletons) and eggs. Random samples were collected from the material that was not retained in the sieve and analyzed to confirm the absence of weed seeds or seed coat fragments. After being washed, samples were air-dried at 22 C for several days and then transferred to paper bags. Each sample was divided into 10 fractions, and each fraction was spread on a petri dish to form a thin layer that allowed weed seed count and identification under a dissecting microscope. Weed seeds were separated from the rest of the materials with a thin spatula. Weed seeds were identified based on morphology using the weed seedbank collection of the West Florida Research and Education Center in Jay, FL. Only intact seeds that properly represented the morphological traits for identification were counted. Seeds with structural damage, empty seed coats, or partial seed coats were not counted to minimize the bias associated with including nonviable seeds. Seeds that could not be identified based on morphology were germinated, and the resulting plant was grown until it was identifiable. When identification was not possible due to lack of germination, the morphology was noted, and a "dummy" code was assigned and used throughout the study to track the species.

Data Analysis

Weed seed counts from each method were adjusted to 100-g samples and used to estimate the following weed community parameters: total density, richness (S), evenness (J), and Shannon-Weiner diversity (H') (Leon et al. 2015; Sosnoskie et al. 2006). The overall estimates were compared for those parameters using paired t-tests ($\alpha = 0.05$) to provide a rigorous comparison that

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emphasized the differences between methods at the sample level. Also, Pearson's correlation and linear regression analyses were used to quantify the level of similarity between both methods when describing weed community diversity and composition. Also, for each weed species that was detected in both seedbank methods, relative density and relative detection frequency were determined. Relative density was estimated as the proportion of individuals of a given species detected in all samples based on the total number of weed individuals from all species detected in all samples. Similarly, relative detection frequency was estimated as the proportion of samples that contained a given species, regardless of the number of individuals, based on the total number of samples.

Results and Discussion

Paired *t*-tests showed clear differences between the ESB and GSB methods for each characterization parameter (Table 1). The most dramatic difference between methods was for density, which was more than 5 times higher in the ESB compared with the GSB. Although the difference in richness was not as large, the ESB still detected more than 30% more weed species than the GSB. Conversely, the differences between methods for diversity and evenness were smaller. For example, ESB diversity was only 9% higher than GSB diversity, while evenness was 10% higher in the GSB compared with the ESB. Although the variability of these two methods was similar (Table 1), the resulting characterization by all four parameters was different. The GSB is commonly used to compare treatment effects and to correlate soil seedbanks to emerged weed flora the following year (Forcella 1992; Cardina and Sparrow 1996). However, no conversion factor has been established between the GSB and ESB. Studies have found different weed compositions in the same seedbank because detection and overrepresentation of particular species might be favored depending on method (Abella et al. 2013; Gonzalez and Ghermandi 2012; Price et al. 2010).

For all parameters studied, methods were weakly correlated, with coefficients ranging from 0.19 to 0.59 (Table 1). The correlation between ESB and GSB seed density was the only coefficient above 0.5, and thus seed density was the parameter with the largest differences in magnitude and highest variability. Regression analysis confirmed that all parameters had weak positive linear relationships between methods (Figure 1). The best-fit model for density described only 35% of the variation, but all other parameter models explained less than 7%, with the lowest under 4% (Figure 1). Due to the weak relationships, it would be difficult to create a conversion factor that could be reliably used to link both seedbank methods. While the methods have similar precision, these analyses demonstrate there is enough variability introduced by the method on species detection and density quantification that suggest they do not provide similar weed seedbank characterizations.

While ESB density was higher than GSB density overall (Table 1; Figure 1), this trend was not true for every species (Table 2). Only half of the species detected by both methods were found in higher quantity in the ESB (Table 2). Seedbank quantification tends to find different species compositions by different methods, commonly with several species unique to each method in addition to species common to both methods (Ball and Miller 1989; Abella et al. 2013). Paired *t*-tests confirmed that the total number of individuals of each species was different between methods, except for *Ipomoea* spp., *Oxalis* spp., and goosegrass [*Eleusine indica* (L.) Gaertn.] (Table 2).

The density of most species did not correlate between the two methods, except for wild radish (*Raphanus raphanistrum* L.), *Amaranthus* spp., large crabgrass [*Digitaria sanguinalis* (L.) Scop.], and *Cyperus* spp., with r values of 0.23, 0.43, 0.41, and 0.24, respectively. Previous research indicated that the ESB often favored seeds based on size and density (Gross and Renner 1989; Mesgaran et al. 2007; Tsuyuzaki 1994), and the GSB favored seeds based on dormancy relief and germination requirements (Cardina and Sparrow 1996; Gonzalez and Ghermandi 2012; Gross 1990). While most comparison studies introduce a bias to the ESB method by using a floatation method, the present study uniquely exhumed the entire 200-g soil sample, even examining the sieved material for missed seeds. Granting the rigorous exhumation of the ESB, the fact that some species had greater detection in the GSB reinforces the idea that these methods cannot be used interchangeably.

Species that occur more frequently may be predicted to have higher total densities, and this behavior is more likely to occur when the species is evenly distributed across the field or landscape. In the case of weed communities with rare species or species exhibiting highly aggregated distribution, it might be difficult to properly describe the richness and diversity of the community. In the present study, both methods had a positive relationship between the relative density of species and the

Table 1. Comparison of estimation of weed community parameters for extractable (ESB) or germinable (GSB) seedbank methods using paired *t*-tests and Pearson's correlation.

Characterization parameter	ESB		GSB		Paired *t*-test P-value[c]	r	Correlation P-value
	Mean	CV[b] —%—	Mean	CV[b] —%—			
Density[a]	58.0	102	11.2	121	0.0049	0.590	< 0.0001
Richness (*S*)	6.30	35.9	4.0	40.7	< 0.0001	0.254	0.0002
Evenness (*J*)	0.652	23.4	0.726	27.5	< 0.0001	0.190	0.0063
Diversity (*H′*)	1.16	34.0	1.06	37.5	< 0.0001	0.234	0.0008

[a]Total number of individuals in 100 g soil.
[b]CV, coefficient of variation.
[c]Indicates the probability of significant differences between methods.

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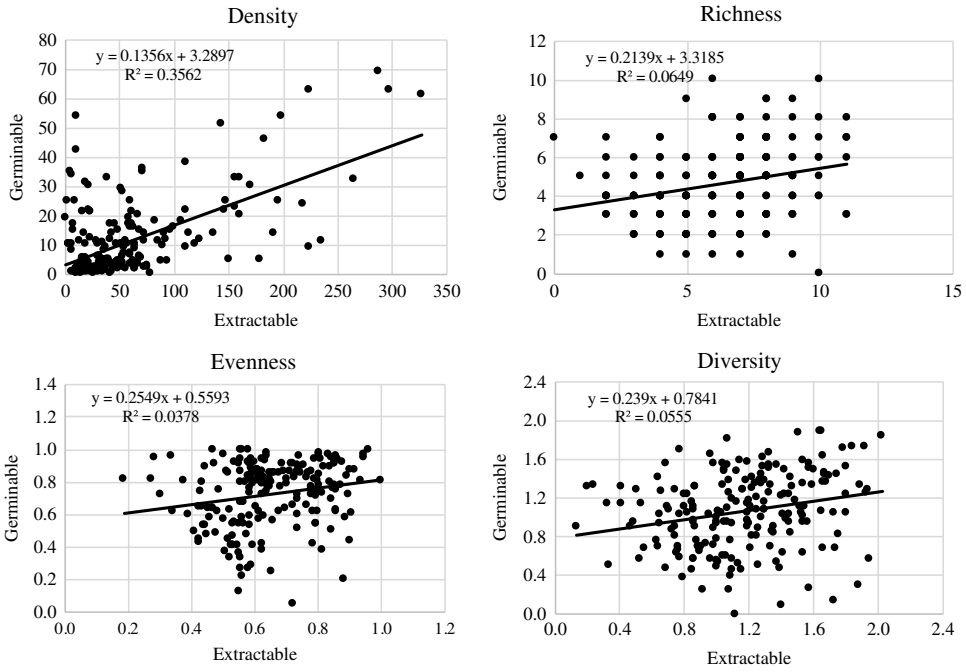


Figure 1. Relationship between extractable (ESB) and germinable (GSB) methods using paired samples for total weed density in 100 g soil; species richness (*S*); species evenness (*J*); Shannon-Weiner diversity (*H′*).

Table 2. Comparison of extractable (ESB) and germinable (GSB) seedbank methods by species composition.

Species	ESB	GSB	ESB	GSB	*t*-test P-value[b]	r[c]
	Frequency[a] —%—		Density —no. of individuals 100 g^{-1} soil—			
Amaranthus spp.	47	13	826	71	0.0001	0.43*
Chamaesyce hyssopifolia (L.) Small	38	31	1,762	576	0.0001	0.09
Cyperus compressus L.	65	65	699	1,090	0.04	0.24*
Digitaria sanguinalis (L.) Scop.	42	34	632	317	0.0143	0.41*
Echinochloa crus-galli (L.) P. Beauv.	6	1	50	4	0.0093	-0.02
Eleusine indica (L.) Gaertn.	61	72	909	1,134	0.2711	-0.06
Gamochaeta purpurea (L.) Cabrera	1	16	6	86	0.0001	-0.03
Ipomoea spp.	4	5	11	27	0.1929	-0.02
Mollugo verticillata L.	97	89	7,619	3,624	0.0001	0.04
Oxalis spp.	30	23	294	318	0.774	0.04
Phyllanthus urinaria L.	2	6	7	40	0.0242	-0.03
Portulaca oleracea L.	54	1	555	7	0.0001	-0.04
Raphanus raphanistrum L.	23	5	221	26	0.0001	0.23*
Sida rhombifolia L.	3	8	11	27	0.0124	-0.03

[a]Relative frequency is the number of samples with at least one individual of the weed species divided by the total number of samples in the study.
[b]Indicates the probability of significant differences between methods for density.
[c]An asterisk (*) indicates that the correlation between methods was significant ($\alpha = 0.05$).

relative frequency at which they occurred (Figure 2). Therefore, when enough samples are collected, both methods can generate data to successfully use techniques such as rarefaction to estimate weed species richness approximating the contribution of rare species (Koellner et al. 2004; Rotches-Ribalta et al. 2015). However, considering that the ESB exhibits higher weed species detection than the GSB, the former might be more useful for rarefaction analysis than the latter.

The results of the present study clearly showed that the ESB and GSB do not provide the same information to describe weed

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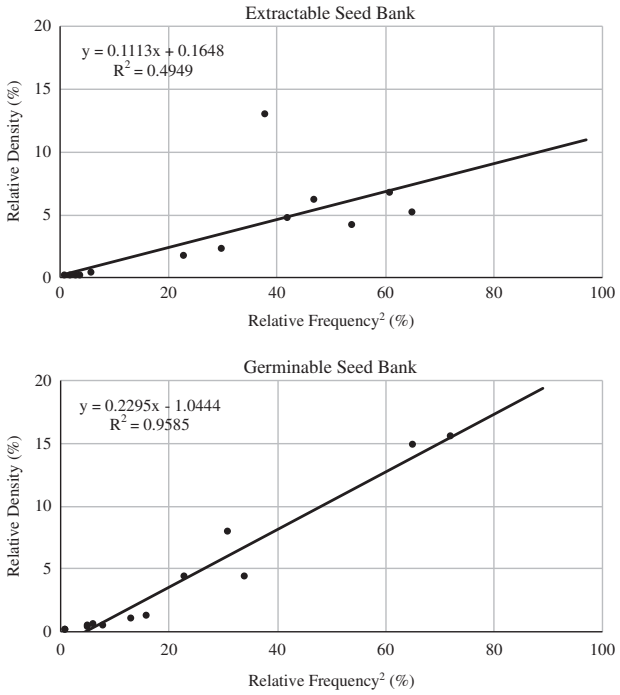


Figure 2. Relationship between relative species frequency and relative species density within extractable (ESB) and germinable (GBS) methods for seedbank characterization. Relative frequency was estimated as the number of samples in which a specific weed species was present based on the total number of samples in the study. Relative density was estimated as the total number of individuals of a species divided by the total individuals for each method.

communities. In addition to having different quantification of certain species as reported by earlier studies, characterization of density, richness, evenness, and diversity differed between methods and lacked a consistent relationship. Nevertheless, it is interesting that although significant, the differences between methods for evenness and diversity were relatively small compared with density and richness. Therefore, estimating diversity and evenness might be a useful way to compare ESB and GSB studies, especially when rare species or species with low abundances within the community represent a minor proportion of the community or are ignored to emphasize predominant weed species. Furthermore, considering that both methods had similar precision (see coefficient of variation columns in Table 1), it is important to determine under what circumstances or for what objectives one might choose one method or the other (Table 3). The GSB is adequate when there is enough greenhouse space available for the number of samples and particularly important for studies that are comparing the effect of different treatments or conditions on the germinable seedbank. Also, this method requires less labor time per sample than the ESB. For example, in the present study, the GSB required an average of 18 min for sample preparation and analysis (including evaluation time), while the ESB required up to 73 min. Key aspects for improving the GSB method include providing opportunities to break dormancy by disturbing soil or cold stratification (Gross 1990), deciding to use undisturbed cores or broken soil in trays (Cardina and Sparrow 1996), reducing space needed in the greenhouse by sieving soil to concentrate samples (Ter Heerdt et al. 1996), or selecting the appropriate watering technique for the soil type (Espeland et al. 2010). However, to characterize the seedbank while maximizing species detection

Table 3. Benefits and limitations of extractable (ESB) and germinable (GSB) seedbank methods for weed community characterization.

Method	Benefits	Limitations
ESB	• Samples have a long shelf life	• Procedure is time-consuming
	• Less space required, so it can be used in studies with large numbers of samples	• Species identification based on seed morphology is challenging and reference collections are limited
	• A more comprehensive description of recent weed community history is provided	• It can overestimate the viable seedbank •A microscope is required
	• Higher seed density and species richness quantification are obtained	
	• Higher detection of rare and less abundant species is provided	
	• Samples can be processed at different times after extraction	
GSB	• Species identification is easy, and individuals can be compared with herbarium samples	• There is a bias toward seeds and species with high germination rates and low dormancy
	• Multiple studies can be found in the literature	• Germination requirements must be known
	• Preparation is not time-consuming	• Large space (greenhouse) requirements limit the number of samples that can be processed
	• Characterization of potential seedling emergence is produced	• The procedure must be conducted shortly after sample collection to avoid viability loss
		• All samples must be germinated at the same time

and quantification, the ESB is still necessary, because it has the best chance of recovering rare species and seeds with long dormancy or specific germination requirements. Multiple efforts have been conducted to expedite seed extraction, including the use of flotation solvents (Buhler and Maxwell 1993; Tsuyuzaki 1994), hydropneumatic elutriator (Gross and Renner 1989), sieving, and cloth bag extraction (Mesgaran et al. 2007). Regardless of the technique used, when the extraction process is done carefully to minimize seed loss, the ESB is useful to process large number of samples, because the samples can be stored in a small area, and seed counts do not have to be done simultaneously for all samples. Researchers should be mindful of these benefits and limitations when considering the best method for study objectives. The present research presents information to help inform the selection of seedbank analysis methods to characterize weed communities for more efficient and reliable research results.

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