COMMUNITY ECOLOGY 13(2): 238-242, 2012
1585-8553/$20.00 © Akadémiai Kiadó, Budapest
DOI: 10.1556/ComEc.13.2012.2.14

Comparison of methods to estimate soil seed banks: the role of seed size and mass

S. L. Gonzalez[1] and L. Ghermandi

Laboratorio Ecotono, Instituto Nacional de Investigaciones en Biodiversidad y Medioambiente, CONICET-Universidad Nacional del Comahue, Quintral 1250 (8400), Bariloche, Argentina.
Tel.: +054-02944-423374. int. 500.
[1] Corresponding author. E-mail: sofia903@yahoo.com.ar; sofiagonzalez@crub.uncoma.edu.ar

Keywords: Grasslands, Method effectiveness, Seedling emergence method, Seed extraction method, Seed mass.

Abstract: There are two main methods for estimating seed density and species composition of soil seed banks: manual seed extraction and seedling emergence. These methods were used to determine and compare seed density and species composition in the soil of a natural grassland in Patagonia. Additionally, known amounts of seeds of different sizes from Patagonian grassland species were mixed with soil to evaluate the efficiency of the seed extraction method, and determine their recovery percentage. Seed density found in the grassland soil with the extraction method was four times higher than that found with the seedling emergence method. Through the use of these two methods, there was very little overlap found in species composition. Small seeds (< 1 mm) were only found with the seedling emergence method, whereas the seeds of species with specific germination requirements were found with the seed extraction method. Seed recovery of grassland species varied from 2.5% for smaller seeds (*Erophila verna*) to 100% for larger seeds (*Rumex acetosella*) with the seed extraction method. This method was more effective in detecting seeds of large-seeded species. Discrepancies in seed detection between both methods may be related to seed dormancy, specific germination requirements, seed size and mass. These two methods are necessary to describe seed density and seed bank composition.

Nomenclature: Correa (1969-1999).

Introduction

There are two commonly used methods to estimate the composition and density of soil seed banks: the seed extraction method and seedling emergence method (Roberts 1981). On one hand, in seed extraction method, the seeds and soil particles are separated considering their different sizes and densities. The most common techniques are seed flotation in oversaturated salt solutions (Malone 1967, Tsuyuzaki 1994) and sample sieving (Roberts 1981); then, extracted seeds are identified under a binocular microscope. On the other hand, the seedling emergence method consists of placing soil samples under suitable conditions for seed germination (i.e., greenhouse or germination chambers); then, emergent seedlings are identified and counted (Thompson and Grime 1979). In general, the seedling emergence method is applied because it is less laborious and useful for a large volume of soil samples (Plue et al. 2012). However, this method requires space in a glasshouse and a long- time seed germination monitoring (usually between 3 and 24 months, Roberts 1981, Leck et al. 1989).

Studies that compared the two methods showed discrepant results in seed density and species composition (Ball and Miller 1989, Gross 1990, Brown 1992, de Villiers et al. 1994, Ishiwaka-Goto and Tsuyusaki 2004, Bernhardt et al. 2008, Price et al. 2010). In general, seed extraction methods detected greater seed densities and richness when compared to the seedling emergence method (Brown 1992, Price et al. 2010). Commonly, these discrepancies are attributed to: 1) the seed extraction method includes apparently healthy but non-viable seeds (Warr et al. 1993) although a viability test can be conducted after extraction, and 2) the seedling emergence method only determines the germinable fraction of the seed bank and fails to detect dormant seeds and those seeds with specific germination environmental requirements (Gross 1990, Manders 1990, Brown 1992). However, few studies have associated these discrepancies with seed characteristics such as seed size and mass, which could limit the ability of a method to detect a species.

Usually, seed extraction methods fail in detecting the smallest-seeded species because seeds could be lost during sample processing (e.g., through sieving) (Gross and Renner 1989, Brown 1992, Mesgaran et al. 2007). According to de Villiers et al. (1994), the seedling emergence method detected a larger proportion of smaller seeded-species, whereas the extraction method also detected tree and shrub species with larger seeds. Therefore, it is necessary to take into account the seed mass and size when evaluating the efficiency of a method for estimating composition and density of soil seed banks.

We compared the two methods, seedling emergence and seed extraction to estimate density and species composition

of the soil seed banks of a grassland in northwestern Patagonia. We expect that the seed extraction method would detect more species with specific germination requirements and larger seeds than the seedling emergence method. Also, we evaluated the efficiency of the seed extraction method (Malone 1967) in separating seeds of different seed mass, using a solution of sodium chloride.

Material and methods

The study area is a fire-prone grassland located in NW Patagonia (San Ramón Ranch, 41°03´19´´S and 71°01´ 50´´W), 30 km east of Bariloche, Argentina. The dominant vegetation is composed of the tussock grasses *Festuca pallescens* and *Pappostipa speciosa* (ex *Stipa speciosa*) and scattered shrubs such as *Acaena splendens*, *Senecio bracteolatus*, and *Mulinum spinosum*. Gaps between tussock grasses and shrubs are colonized by annual grasses and annual and perennial herbs (Ghermandi and Gonzalez 2009). Some of these species require fire-cues to germinate (e.g., *Boopis gracilis)* (Gonzalez et al. 2010).

To compare the two seed bank estimation methods, in April 1999 (after seed dispersal), 120 soil samples of 10 cm in diameter and 3 cm in depth (Ghermandi 1992) were taken randomly from the grassland. Soil cores were stratified at 5 °C for four months, and then were sieved to remove organic debris and stones. For the seedling emergence method, half of the samples (n = 60) were placed on sand in plastic containers (10 × **13 cm Q1 LONG DIameter?)** and kept in a greenhouse. Samples were watered daily and every week identified seedlings were counted and removed. The monitoring started in September and ended ten months later when germination stopped.

The remaining soil samples (n = 60) were used for the seed extraction method and mixed with a saturated sodium chloride solution which separates organic matter and seeds from mineral soil fraction. This solution was prepared by adding 35 g of sodium chloride to 100 ml of distilled water. Then, the mixture (soil and solution) was allowed to settle for 30 min, and the supernatants were filtered through filter paper, and dried in an oven at 35°C. Seeds of each species were separated from the supernatant using a binocular stereomicroscope. Viability of seeds was determined by the pressure test or seed crush test, which consists of visual inspection and application of gentle pressure with forceps to seeds to corroborate the embryo presence (Borza et al. 2007).

In order to evaluate the efficiency of the seed extraction method, seeds of different sizes were mixed with soil in a saturared sodium chloride solution and then extracted. In January-March 2009, seeds from species common to the soil seed banks of the studied grassland were collected (Gonzalez and Ghermandi 2008). The species were *Festuca pallescens*, *Pappostipa speciosa*, *Apera interrupa* (Poaceae), *Rumex acetosella* (Polygonaceae), *Fabiana imbricata* (Solanaceae), *Holosteum umbellatum* (Caryophyllaceae), *Boopis gracilis* (Calyceraceae), and *Erophila verna* (Brassicaceae). Two hundred seeds of each species were selected by using the pressure test (Borza et al. 2007), and 100 seeds selected randomly were weighed and their length was measured under a binocular stereomicroscope. Twenty seeds of each species were mixed with 150 g of soil (n = 10). The extracted soil came from a place near the laboratory without seeds of species present in the grassland community. Soil samples were mixed with a saturated sodium chloride solution as explained above and seeds were identified from soil using a binocular stereomicroscope. The percentage of seed recovery for each species was estimated.

Data analysis

Two sample t-test or non-parametric Mann-Whitney test were used for the comparison of the two seed bank estimation methods. One-way ANOVA with post-hoc Tukey HSD test was used for the comparison of seed recovery among species. We analyzed the relationship of the percentage of seed recovery regarding seed mass and seed length by using the Spearman´s rank correlations. Analyses were performed using STATISTICA v 6.0 software using $\alpha = 0.05$ as a significance level.

Results

The seed density estimated by the seed extraction method was four times higher than that estimated by the seedling emergence method (U = 1104, P = 0.005, Table 1). In addition, more species were found with the seed extraction method (10 vs. 8) and five species with both methods (Table 1).

The most abundant species found with the seedling emergence method were: *Holosteum umbellatum*, *Erophila verna*, *Apera interrupta* and *Rumex acetosella*, representing 44%, 26%, 16%, and 8% of the total seed amount, respectively. On the other hand, the most abundant species detected with the seed extraction method were: *R. acetosella* and *H. umbellatum,* representing 67% and 21%, respectively. Seed density of *H. umbellatum* was similar with both methods (t = 870, P = 0.515), whereas 32 times more seeds of *R. acetosella* were found with the seed extraction method (t = 656, $P < 0.001$). Four-folds the seeds of *A. interrupta* were detected with the seedling emergence method (U = 669, $P < 0.001$). Seeds of the small-seeded species *E. verna* and *Verbascum thapsus* (< 0.20 mg) were only found with the seedling emergence method, whereas seeds of *Acaena splendens, Boopis gracilis*, *Fabiana imbricata, Myosotis discolor*, and *Plagyobothrys verrucosus* were only found with the seed extraction method (Table 1).

Percentage of seed recovery with the seed extraction method varied from 2.5% in *E.verna* to 100% in *R. acetosella* (Table 2). There was a relationship between seed mass and length regarding the percentage of recovery ($\rho = 0.69$, P = 0.04; $\rho = 0.85$, $P = 0.001$, respectively). Species with large heavy seeds had a high percentage of recovery (e.g., *R. acetosella*, *Festuca pallescens,* and *Pappostipa speciosa*) (Table 2). There were lower recovery percentages for the smaller seeds (< 1 mm) (Table 2).

Table 1. Seed density (seeds/m^2) and species composition of the seed bank in a Patagonian grassland detected with the seedling emergence and seed extraction methods. Lower-case letters indicate significant differences between these methods.

Species	Seedling emergence	Seed extraction	Seed mass (mg)
Acaena splendens	-	106	33.3
Apera interrupta	395a	93b	0.30
Boopis gracilis	-	8	0.20
Erodium cicutarium	4	-	0.50
Erophila verna	650	-	0.17
Fabiana imbricata	-	21	0.21
Heliotropium paronychioides	13	42	0.18
Holosteum umbellatum	1091a	2170a	0.30
Myosotis discolor	-	4	0.12
Plagyobothrys verrucosus	-	607	0.60
Rumex acetosella	208a	6705b	0.70
Verbascum thapsus	76	-	0.09
Vulpia australis	55	183	0.50
Total	**2493a**	**9941b**	
Richness	**8**	**10**	

Table 2. Percentage of recovery in grassland species with the seed extraction method using sodium chloride. Seed mass and length are indicated. Different lower-case letters indicate significant differences, Tukey test ($p < 0.05$).

	Recovery (%)	Seed mass (mg)	Length (mm)
Rumex acetosella	100 a	0.68	1.5
Pappostipa speciosa	97.5 ab	7.28	10
Festuca pallescens	94.5 ab	2.30	7.2
Apera interrupta	82 ac	0.30	2
Boopis gracilis	67 bce	0.20	1.4
Fabiana imbricata	28.5 ce	0.21	1
Holosteum umbellatum	25.5 ce	0.30	1
Erophila verna	2.5 e	0.17	0.4

Discussion

We highlighted the contrasting results between the seedling emergence and seed extraction methods in determining the density and species composition of grassland soil seed banks. Discrepancies in seed detection between the methods may be related to seed dormancy and specific germination requirements, as found in other studies (de Villiers et al. 1994, Price et al. 2010). In the present study, we found that the size and mass of seeds play an important role in determining the more effective method to be used.

The seed extraction method using a salt solution was particularly effective in detecting seeds of relatively large-seeded species. Seed recovery was greater than 94% for species with seeds larger than 0.3 mg and longer than 1 mm (e.g., *Rumex acetosella*, *Festuca pallescens,* and *Pappostipa speciosa*). In contrast, small seeded-species (e.g., *Erophila verna*, *Holosteum umbellatum,* and *Fabiana imbricata*) had percentages of recovery lower than 28%. Additionally, when the two methods were compared, seeds of *E. verna* and *Verbascum thapsus* were not detected with the seed extraction method. In other studies, by using this latter method, small seeds of *Plantago major*, *Juncus effusus* var. *decipiens,* and *Erica* spp. were not found (Ferrandis et al. 1999, Ishikawa-Goto and Tsuyusaki 2004, Mesgaran et al. 2007) or only there were a few seeds (e.g., *Erigeron* spp. with seeds < 1 mm^3) (Brown 1992). Mesgaran et al. (2007) also reported that the effectiveness of different seed estimation methods (sieving, cloth bags, and flotation) decreased in species with smaller or equal to 1 mm seeds. However, Gross and Renner (1989) found high seed recovery percentages in species with seeds smaller than those described in the present study (e.g., *Mollugo verticillata* with 0.06 mg). According to Price et al. (2010) seeds smaller than 2 mm were not detected in the seed extraction method because they were lost through sieving. In our study, the seeds of small-seeded species were lost probably during the sample processing. Based on our results and those found in other studies, we recommend using the seed extraction method only if the aim of the study is to estimate the seed banks of larger-seeded species. Using this method, seeds must be larger than 0.3 mg and longer than 1 mm to allow their detection.

Seeds of *Boopis gracilis*, *Plagyobothrys verrucosus,* and *Fabiana imbricata* were only found with the seed extraction method. The seedling emergence method could underestimate the seed number and species composition because it could not detect the species which have specific germination requirements (i.e., temperature, light quality, photoperiod) and dormant seeds (Warr et al. 1993, Baskin and Baskin 1998). These species are all natives and have specific requirements for germination. In the case of *B. gracilis*, this is a fugitive species with long-lived seed banks, which recruits abundantly after fires, stimulated by smoke and favourable post-fire conditions (Gonzalez and Ghermandi 2008, Gonzalez et al. 2010). Therefore, care is needed when applying the seedling emergence method at community level because the history of land use must be considered, in particular in fire-prone systems such as in this present study. In Mediterranean ecosystems where a physically-dormant hard-seeded component needs fire to germinate (e.g., Cistaceae), Ferrandis et al. (1999) suggested the use of both methods simultaneously to estimate seed banks. Based on the results from these methods, we suggest that if a species has very small seeds and specific requirements for germination, they are unlikely to be found by neither of the methods.

In order to analyze the methods efficiency, several aspects must be taken considered. For example in the case of the seed extraction method with salt solution, processing time of samples, and possible loss of seed viability, must be taken into account. The processing time of seed extraction method with sodium chloride used in this study was a lengthy task. Each sample took about 40 minutes (preparation of the solution, time settling, and filtration of supernatant). The time of extraction and identification of the seeds from the supernatant, varied from 30 to 60 minutes depending on the amount of seeds in each sample. Regarding the possible loss of seed viability, some authors have suggested that chemical solutions can alter it (Gross 1984, Tsuyuzaki 1994). In this study seed viability was not tested, and although the seed coat was apparently not damaged, it is possible that sodium chloride affected the viability. Even taking these aspects into account, this study demonstrated that this method is easy to use with simple and inexpensive equipment and effective in detecting large seeds.

The effectiveness of seed extraction and seedling emergence methods for estimating seed density and species composition of seed banks was related to specific germination requirements, seed dormancy and seed size and mass. High discrepancies between these methods restrict the possibility of making generalizations. We suggest that the use of the methods should be determined by the aim of the study. Despite the limitations of the seed extraction method, this may be preferred over the seedling emergence method for studies in which the focus is on determining the seed bank of a subset of large-seeded species or when immediate data are desired. This method should be complemented with some appropriate techniques to determine seed viability. On the other hand, the seedling emergence method is preferred in long-term monitoring experiments and in studies on seasonal changes in seed banks, but care is needed when conducting studies at community level. Seed extraction and seedling emergence methods were complementary to detect the species composition. Therefore, we suggest that both methods are necessary to describe the seed banks and improve the interpretation of the results.

Acknowledgements: This work was supported by Universidad Nacional del Comahue (Project B-109) and Consejo Nacional de Investigaciones Científicas y Técnicas. We thank two anonymous reviewers and the editor for helpful comments. We thank G. Becker, D. Bran, and C. Gittins for their assistance with the field work, and M. Devall, J. Franzese, and H. Murillo for their help in English revision. We also thank San Ramón ranch manager Ing. D. Marty.

References

Ball, D.A. and S.D. Miller. 1989. A comparison of techniques for estimation of arable soil seedbanks and their relationship to weed flora. *Weed Res.* 29:365-373.

Baskin, C.C. and J.M. Baskin.1998. *Seed Ecology, Biogeography and Evolution of Dormancy and Germination*. Academic Press, San Diego.

Bernhardt, K.G., M. Koch, M. Kropf, E. Ulbel and J. Webhofer. 2008. Comparison of two methods characterizing the seed bank of amphibious plants in submerged sediments. *Aquatic Bot.* 88:171-177.

Borza, J.K., P.R. Westerman and M. Liebman. 2007. Comparing estimates of seed viability in three foxtail (*Setaria*) species using the imbibed seed crush test with and without additional tetrazolium testing. *Weed Technology* 21:518-522.

Brown, D. 1992. Estimating the composition of a forest seed bank: a comparison of the seed extraction and seedling emergence methods. *Can. J. Bot.* 70:1063-1612.

Correa, M.N. 1969-1999. *Flora patagónica*. Varios Volúmenes, Colección Cíentfica INTA, Buenos Aires, Argentina.

de Villiers, A. J., M.W. Van Rooyen and G.K. Theran. 1994. Comparison of two methods for estimating the size of the viable seed bank of two plant communities in the Strandveld of the west coast, South Africa. *South Afr. J. Bot.* 60:81-84.

Ferrandis, P., J.M. Herranz and J.J. Martínez-Sánchez. 1999. Fire impact on a maquis soil seed bank in Cabañeros National Park (Central Spain). *Israel J. Plant Sci.* 47:17-26.

Ghermandi, L. 1992. Caracterización del banco de semillas de una estepa en el noroeste de la Patagonia. *Ecol. Austral.* 2:39-46.

Ghermandi, L. and S. Gonzalez. 2009. Diversity and functional groups dynamics affected by drought and fire in Patagonian grasslands. *Ecoscience* 16:408-417.

Gonzalez, S. and L. Ghermandi. 2008. Postfire seed bank dynamics on semiarid grasslands. *Plant Ecol.* 199:175-185.

Gonzalez, S., J. Franzese and L. Ghermandi. 2010. Role of fire on Patagonian grasslands: changes in aboveground vegetation and soil seed bank. In: M. Haider and T. Müller (eds.), *Advances in Environmental Research. Vol. II*. Nova Science Publishers, Hauppauge, NY. pp. 243-264.

Gross, K.L. 1984. Effects of seed size and growth form on seedling establishment of six monocarpic perennial plants. *J. Ecol.* 72:369-387.

Gross, K.L. 1990. A comparison of methods for estimating soil seed banks. *J. Ecol.* 78:1079-1093.

Gross, K.L. and K.A. Renner. 1989. A new method for estimating seed numbers in the soil. *Weed Sci.* 37:836-839.

Ishikawa-Goto, M. and S. Tsuyusaki. 2004. Methods of estimating seed banks with reference to long-term seed burial. *J. Plant Res.* 117:245-248.

Leck, M.A., K.P. Parker and R.L.Simpson. 1989. *The Ecology of Soil Seed Banks*. Academic Press, San Diego, CA.

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

Manders, P.T. 1990. Soil seed banks and post-fire seed deposition across a forest-fynbos ecotone in the Cape Province. *J. Veg. Sci.* 1:491-498.

Mesgaran, M.B., H.R. Mashhadi, E. Zand and H.M. Alizadeh. 2007. Comparison of three methodologies for efficient seed extraction in studies of soil weed seedbanks. *Weed Res.* 47:472-478.

Plue, J., K. Thompson, K. Verheyen and M. Hermy. 2012. Seed banking in ancient forest species: why total sampled area really matters. *Seed Sci. Res.* 22:123-133.

Price, J.N., B.D. Wright, C.L. Gross and W.R.D.B. Whalley. 2010. Comparison of seedling emergence and seed extraction for estimating the composition of soil seed banks. *Methods Ecol. Evol.* 2:151-157.

Roberts, H.A. 1981. Seedbanks in soil. *Adv. Appl. Biol.* 6:1-55.

Thompson, K. and J.P Grime. 1979. Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *J. Ecol.* 67: 893-921.

Tsuyuzaki, S. 1994. Rapid seed extraction from soils by a flotation method. *Weed Res.* 34: 433-436

Warr, S.J., K. Thompson and M. Kent. 1993. Seed banks as a neglected area of biogeographic research: a review of literature and sampling techniques. *Progr. Phys. Geogr.* 17:329-347.

Received March 14, 2012
Revised July 31, August 21 2012
Accepted September 13, 2012