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Effect of soil depth on seedling emergence in tropical soil seed-bank investigations

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Summary

1. Investigations of tropical soil seed banks have reported large differences in apparent seed densities based on seedling emergence from soil set out under gap conditions. To examine to what extent these differences might reflect different methodologies rather than actual differences in seed density, soil samples of different depths were laid out in germination trays.

2. There was a marked effect of soil depth on total seedling emergence. Germination trays containing soil layers of ≥10 mm markedly underestimated total seed density. Relative abundance of species in the samples did not alter with depth. Almost all seedling emergence occurred within the first 6 weeks for all soil depths.

3. It is recommended that future tropical soil seed-bank investigations should spread soil to ≤5 mm. This should not restrict the sample sizes used in seed-bank investigations as rapid germination from shallow soils allows rapid turnover of samples.

Key-words: Germination, methodology, Panama, seedling emergence

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Introduction

Many investigations have now been made of tropical soil seed banks with a view to determining the relative importance of dormant buried seeds in regeneration. Despite 60 years of these investigations, and more than 40 published studies (Garwood 1989), there is still no consensus as to whether shade intolerant species regenerate primarily from buried seeds or from seeds newly dispersed into pre-existing gaps. This question will never be properly addressed until more thorough investigations have been made that track seed-bank density and composition prior and subsequent to gap formation and which adequately exclude seed rain. In the meantime, little conjecture is possible as enormous variation has been found in seed-bank densities and species compositions, ranging from two to 49 seeds m^{-2}, and two to nine species in an area of 6 m^2 in lower montane forest in Thailand (Cheke, Nanakorn & Yankoses 1979) to over 1000 seeds m^{-2} and 79 species in an area of 3 m^2 in lowland evergreen forest in Queensland in Australia (Hopkins & Graham 1983). To what extent do these differences represent seasonal, annual and geographic variation in seed banks or differences in methodology?

In the majority of soil seed-bank investigations, both temperate and tropical, numbers of viable seeds in the soil are estimated by the seedling emergence technique. This technique provides an estimate of viable seed density in the soil based on the germination of seeds from soil samples maintained under conditions favourable for germination. Although it is recognized that ideal conditions for germination are seldom met, and that therefore the seedling emergence technique may greatly underestimate viable buried seed densities (Chancellor 1986; Simpson, Leck & Parker 1989), critical investigations of the methodology have not been made.

The aim of this study was to examine how methodological considerations might affect measurements of tropical seed-bank densities or composition, by investigating the relationship between the depth at which soil is spread in germination trays, the number of emerging seedlings and the time to seedling emergence.

Materials and methods

Three 10 litre soil samples were collected in July 1992 (mid-wet season) from the surface 3 cm of soil in three locations in old growth forest on Barro Colorado Island, in seasonally moist lowland forest in Panama (for flora and site description see Croat 1978; Leigh, Rand & Windsor 1982). The soil samples were pooled in the laboratory and very thoroughly mixed to ensure that the seeds were homogeneously dis-

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tributed. Volumetric subsamples of this soil were assigned to four soil-depth treatments spread on top of a 10 mm deep layer of washed marine sand in 0·125 m^2 seed trays. In total, five replicates each of 2·5, 5, 10 and 20 mm deep soil were set up. Additionally, two trays of 5 mm deep soil sieved through a 4 mm aperture sieve and two trays with a 5 mm layer of autoclaved soil were also set up.

Seed trays were placed under plastic in a screened shade house (20% full sun) and watered daily. For the first 6 weeks of the experiment emergent seedlings were marked with coloured toothpicks to denote the week of emergence. After week 6 all existing seedlings were removed and the soil was thoroughly mixed. Following this treatment new seedlings were removed weekly until week 13 when the soil was mixed again. There was one final seedling census in week 18. Seedlings were identified by comparison with dried specimens and seedling drawings in the Barro Colorado Island herbarium. Nomenclature follows Croat (1978).

Results and discussion

The expected doubling of total seedling emergence with a doubling of soil depth was not seen except in comparison of the 2·5 and 5 mm depth treatments (Figs 1 and 2). Although total seedling emergence increased with soil depth, in the 10 mm depth sample a total of only 205 seedlings emerged, as against an expected total based on the 2·5 mm sample of 412 seedlings. Likewise in the 20 mm sample 304 seedlings emerged against an expected total of 824. Thorough re-mixing of the soil after 6 weeks and 13 weeks did not substantially increase the number of emerging seedlings in the deeper soil treatments. Sieving of fresh soil to break up larger soil aggregations had no significant effect on either the rate or the total number of seedlings emerging from the 5 mm depth samples. No seedlings emerged from the autoclave-sterilized soil.

The lower seed densities in the deeper soil treatments did not appear to be the result of an under-representation of particular species (Appendix). The proportion of very small seeded *Miconia argentea*, small seeded *Cecropia insignis* and the combined proportion of relatively large seeded species *Trema micrantha*, *Jacaranda copaia*, *Zanthoxylum* spp., *Luehea seemanii*, *Solanum hayesii* and *Ochroma pyramidale* (= *O. lagopus*) was fairly constant between depth treatments. Instead lower seed densities probably indicate that a substantial proportion of seeds were still hidden from germination cues in deep soil even after mixing, or that germination cues were directly affected by soil depth, for example deeper soil layers may buffer diurnal temperature fluctuations, or that seeds germinated and died in the deeper soil treatments without emerging above the soil.

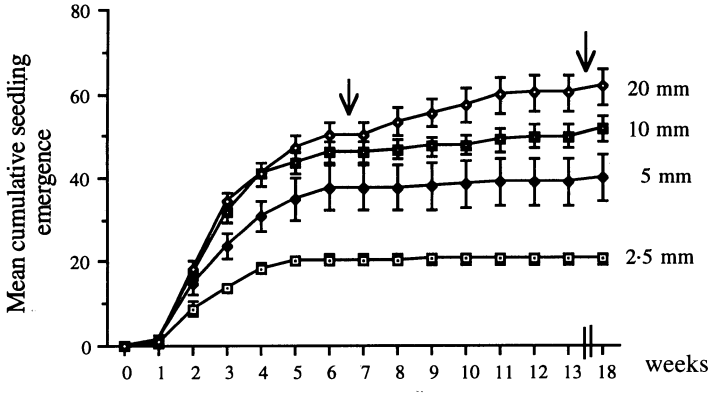

Fig. 1. Mean cumulative seedling emergence (±1 SE). Arrows indicate the time of soil mixing.

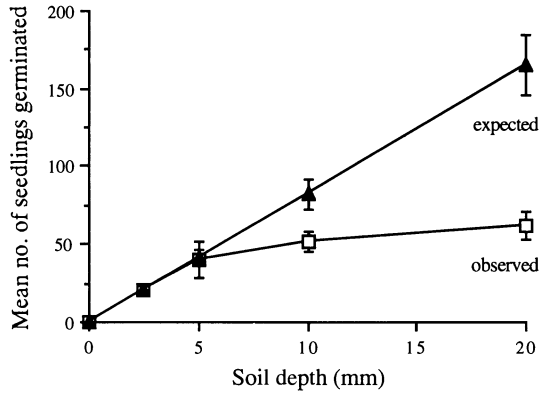

Fig. 2. Observed and expected mean total seedling germination by soil depth (±2 SE). Expected values are based on observed seedling germination from the 2·5 mm soil depth treatment.

Seedling mortality was only recorded in week 6, although as the week of recruitment of seedlings was known, the seedling mortality rate over the first 5 weeks could be estimated at 3·5% per week. Total mortality was not significantly different between depth treatments.

In conclusion these results point to some serious deficiencies in the methodologies of past investigations of tropical soil seed banks. The majority of these investigations have estimated seed-bank densities through the germination of seeds under high light conditions (as opposed to extracting seeds directly) but there have been no previous investigations of the relationship between seed germination and soil depth, and soil depth has frequently not been reported. In a survey of the methods used in 47 tropical soil seed-bank studies, Garwood (1989) found that 41 estimated seed-bank density using the seedling emergence technique but only 23 reported the depth at which soil was spread. Of these 23 studies, in only three cases was soil spread to < 1 cm. While this may not have affected the reported species composition of the seed bank, it has probably led to an underestimation of seed-bank densities.

Similarly, little account has been taken of the need for short census intervals to minimize seedling losses through mortality. Of the same 47 studies only 16

reported using a census interval of 1 week or less. For temperate soil seed banks longer census intervals are usually necessary given the slow rate of seed germination. Chancellor (1986) reports for a cultivated soil in the UK that '... the technique of assessing seed density by counting seedlings arising from 0·2 m depth of cultivated soil will not give a complete total seed count unless continued for many years'. This seems not to be the case for tropical pioneer tree species. Probably because of the brevity of conditions suitable for the establishment of pioneers in tree-fall gaps, germination of these species is rapid, with a median time to germination for pioneer trees in this study of 4 weeks or less and for all germinants of 3 weeks (Appendix). As a consequence, although large amounts of space are needed to process a large volume of soil, turnover of soil samples can be quite rapid, with 6 weeks needed to sample pioneer tree species adequately.

Although this methodology was intended for censusing pioneer species, we expect that non-pioneer species would not form a substantial fraction of the persistent soil seed bank. The median time of germination of 62% of pioneer and non-pioneer species on BCI ($n = 157$) is ≤ 6 weeks (calculated from Garwood 1983). Species with some prolonged period of innate dormancy tend to have large seeds and would be expected to have lower densities in the soil: a different sampling regime would be needed to investigate the long-term behaviour of this group of species (e.g. removal of seeds from soil by sieving and longer observation times).

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Appendix. Numbers of seedlings germinating from soil samples of different thickness, species diaspore mass (without wings) and median time to germination. Data pooled from all replicates at each depth treatment. The unknown category is a multi-taxa group predominantly of very small seeded herbaceous species. Nomenclature follows Croat (1978)

Taxa	Soil depth (mm)				Seed mass (mg)	Median (range) germination time (weeks)
	2·5	5	10	20		
Jacaranda copaia (Bignoniaceae)	0	1	3	9	0·47	3 (3–5)
Trattinnickia aspera (Burseraceae)	0	1	0	0	285	3
Ochroma pyramidale (Bombacaceae)	0	1	2	6	6·6	3 (2–11)
Mascagnia hippocrateoides (Malpighiaceae)	0	0	0	1		3
Miconia argentea (Melastomataceae)	27	59	62	72	0·07	3 (1–18)
Other Melastomataceae	1	0	1	1	0·007–0·35	10 (10–11)
Monocotyledonae	4	3	1	6		4 (2–18)
Cecropia spp. (Moraceae)	12	12	27	43	0·52–0·62	2 (2–6)
Ficus spp. (Moraceae)	0	2	3	4	0·15–2·0	4 (1–18)
Passiflora spp. (Passifloraceae)	0	0	1	1	2·8–3·7	3 (3–3)
Piper spp. (Piperaceae)	3	5	4	4	0·04–3·2	4 (2–11)
Palicourea guinensis (Rubiaceae)	2	0	1	0	14·3	2 (2–5)
Zanthoxylum spp. (Rutaceae)	0	1	1	3	11–36	3 (3–3)
Solanum hayesii (Solanaceae)	1	0	0	2	2·4	2 (1–3)
Turpinia occidentalis (Staphyleaceae)	0	0	1	0		4
Luehea seemanii (Tiliaceae)	2	13	9	11	1·9	2 (1–18)
Trichospermum mexicanum (Tiliaceae)	1	0	0	0	2·5	1
Trema micrantha (Ulmaceae)	2	5	3	8	3·0	2 (2–11)
Unknown	48	87	136	133		4 (1–18)
Total	103	190	255	304		3 (1–18)