Functional Ecology 1999
13, 428–430

TECHNICAL REPORT

The effect of water supply on seed-bank analysis using the seedling-emergence method

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Summary

1. When using the seedling-emergence method to analyse a soil seed bank the greenhouse conditions should match the germination requirements of the species involved. Although the seedling-emergence method is common practice in ecological studies, the germination characteristics of many species are not known, or are only partly known.
2. Before carrying out a large-scale seed-bank study in a wet dune slack, we tested the water requirements of the species in the seed bank, comparing a waterlogged soil with a moist soil. Four species germinated in significantly larger numbers in a waterlogged soil, seven species in a moist soil and 11 species showed no significant difference. When a species was present in low densities, it was often missed using the less appropriate treatment.
3. These results emphasize the need for a preliminary study before carrying out a seed-bank analysis and show the danger of using a 'standard' method to analyse soil samples from different habitats.

Key-words: Germination characteristics, moisture, soil seed bank

Functional Ecology (1999) **13,** 428–430

Introduction

In order to estimate the composition of a soil seed bank, soil samples are often spread in a greenhouse, whereupon the emerged seedlings are identified and counted (Ter Heerdt *et al.* 1996). Essential for this seedling-emergence method is that the conditions in the greenhouse are suitable for the germination of as many species and individuals as possible (Thompson & Grime 1979; Roberts 1981; Gross 1990; Ter Heerdt *et al.* 1996). Yet the germination requirements of many species are not known, or are only partly known. Grime *et al.* (1981) and Grime, Hodgson & Hunt (1988) studied the germination characteristics of several hundreds of species. These species varied largely in their germinability immediately following seed collection, or after dry storage, with or without chilling, with or without scarification and under various conditions of temperature and light flux.

Species may also vary in the amount of water needed for their germination. Some species will germinate under a wide range of conditions of moisture tension, while others are more specific in their requirements (Mayer & Poljakoff-Mayer 1989; Evans & Etherington 1990). According to Van Der Valk, Pederson & Davis (1992) some wetland species germinate in larger numbers in a waterlogged soil than in a dryer soil, while others show the opposite response.

We wanted to study the soil seed bank from a dune slack with a sandy soil, which generally was waterlogged in early spring, but could become much drier later in the season. In order to determine the best germination treatment, we carried out a small scale preliminary study, comparing the emergence of seedlings from a waterlogged soil with a moist soil in the greenhouse.

Materials and methods

Two dune-slack sites harbouring different plant communities were sampled, each with four replicates of one litre of soil each. Each replicate was thoroughly homogenized and split in two equal parts. The parts were sieved and spread in trays filled with potting soil according to Ter Heerdt *et al.* (1996). One part of each replicate was kept waterlogged by putting the trays in larger trays filled with water to 2 cm below soil level. The other part was kept moist by watering it from above every day. This experiment was carried out in June–July, when the weather was quite sunny, causing the moist samples to dry fast, although they did not dry out completely. The seedlings were counted and removed as soon as they could be identified. Nomenclature followed Van Der Meijden *et al*. (1990).

Table 1. Accumulated number of seedlings per species germinating under waterlogged and moist soil conditions. The significance of the difference between the two treatments is tested using the Chi-square test (*$P \leq 0{\cdot}05$; **$0{\cdot}05 < P \leq 0{\cdot}01$; ***$0{\cdot}01 < P \leq 0{\cdot}001$; NS, not significant)

	Both sites			Site A			Site B		
	waterlogged	moist	waterlogged ≠ moist	waterlogged	moist	waterlogged ≠ moist	waterlogged	moist	waterlogged ≠ moist
SPECIES GERMINATING BEST UNDER WATERLOGGED CONDITIONS									
Juncus arcticus ssp. *balticus*	7	0	*	0	0		7	0	*
Juncus articulatus and *J. alpinoarticulatus*	8812	2179	***	5147	1373	***	3665	806	***
Radiola linoides	28	0	***	26	0	***	2	0	NS
Samolus valerandi	3336	2711	***	3283	2665	***	53	46	NS
SPECIES GERMINATING BEST UNDER MOIST CONDITIONS									
Atriplex prostrata	0	6	*	0	0		0	6	*
Chenopodium rubrum	1	10	**	1	0	NS	0	10	**
Hydrocotyle vulgaris	96	127	*	53	71	NS	43	56	NS
Litorella uniflora	35	77	***	34	73	***	1	4	NS
Potentilla anserina	8	30	***	0	0		8	30	***
Ranunculus flammula	29	49	*	29	44	NS	0	5	*
Sagina procumbens	90	137	**	90	133	**	0	4	*
SPECIES SHOWING NO SIGNIFICANT DIFFERENCE BETWEEN TREATMENTS									
Agrostis stolonifera	36	33	NS	18	16	NS	8	17	NS
Calamagrostis epigejos	12	6	NS	3	4	NS	9	2	*
Carex sp.	136	167	NS	48	74	*	88	93	NS
Eleocharis palustris ssp. *palustris*	8	10	NS	1	2	NS	7	8	NS
Galium palustre	33	30	NS	17	9	NS	16	21	NS
Holcus lanatus	4	6	NS	0	0		4	6	NS
Lychnis flos-cuculi	6	7	NS	0	0		6	7	NS
Lythrum salicaria	1598	1666	NS	341	378	NS	1257	1288	NS
Mentha aquatica	48	42	NS	21	14	NS	27	28	NS
Oxycoccus macrocarpos	60	42	NS	6	1	NS	54	41	NS
Potentilla anglica	12	19	NS	4	6	NS	8	13	NS
	21	20		17	15		18	20	
Number of species	22			17			22		

Results and discussion

Twenty-two species were found in this pilot experiment. Four species germinated in significantly larger numbers in a waterlogged soil, seven species in a moist soil and 11 species showed no significant difference (Table 1). When a species was present in low densities, it was often missed using the less appropriate treatment (Table 1). We found 17 species on site A, all detected under waterlogged conditions, two of them were missed under the moist treatment. On site B at least 22 species were present, four of those were missed under waterlogged conditions and two under moist conditions.

If these differences are of any importance or not depends on the accuracy needed. For our actual large-scale study we definitely wanted to detect less common species, such as *Radiola linoides*, *Juncus arcticus* ssp. *balticus*, *Litorella uniflora* and *Ranunculus flammula*, and we needed an accurate estimation of the seed densities. The first species clearly prefers waterlogged conditions, the latter two moist conditions. We therefore chose an intermediate treatment by carrying out the large-scale study in autumn, when the evaporation in the greenhouse was low. The samples were kept very wet, but not waterlogged, by watering them up to two or three times daily. This treatment was successful. In the actual study all species found in this pilot, except *Atriplex prostrata* and *Chenopodium rubrum*, and six other species germinated in rather large numbers (Bekker 1998).

These results show that simply watering the samples daily, practised in many seed bank studies, is not the best treatment when dealing with soil samples from wet dune slacks. The same goes for soil samples from mudflats, whose species prefer waterlogged conditions for their germination (Ter Heerdt & Drost 1994). Using a standard method to analyse soil samples from different habitats easily leads to an underestimation of the number of species and individuals present in the soil. As stated in our previous paper (Ter Heerdt *et al.* 1996), a preliminary study is needed before any time-consuming and expensive seed bank study is started. Without such a pilot, the results might very well be less reliable as wanted.

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Received 27 October 1997; accepted 24 November 1998