EVALUATION OF TWO METHODS FOR ENUMERATING THE SOIL WEED SEEDBANK

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ABSTRACT

Seedbank species composition and density were determined by two methods in two soil types collected from five arable sites in Waikato. The methods were (a) seed extraction by washing and dry sieving, and (b) seedling enumeration under different conditions. The weed seed extraction method was fast and efficient but requires specialised equipment. The seedling emergence method needs more time and specific growing conditions for maximum emergence of some species. Both methods gave similar estimates for grass weeds but the seed extraction method generally found more broadleaf weed seeds. The high ratio of seedlings emerged to seeds extracted demonstrates the potential for using the weed seed content of the soil to predict future weed problems in the field.

Keywords: seedbank, seed extraction, seedling emergence, weed seeds

INTRODUCTION

It has been suggested that estimates of seed bank populations in arable soils could also be used to predict future weed infestations (King *et al.* 1986; Radosevich 1984; Sagar and Mortimer 1976). Such information would have value in planning crop sequences and herbicide usage. It may also be possible to adjust sowing dates of crops to avoid weed emergence peaks and thus minimise yield losses from weed competition.

Determination of the density of viable weed seeds in a soil sample is a tedious and slow process and comparative studies of seed banks have been limited by the difficulty of accurately determining the numbers of seeds and species present. The usual techniques employed by researchers fall into two categories viz., (a) physical extraction of seeds from soil samples by flotation and/or sieving and counting; (b) glasshouse incubation of the soil and enumeration of seedlings resulting from the viable seeds. Both these methods have several advantages and disadvantages (Ball and Miller 1989; Gross 1990; Roberts and Ricketts 1979).

As a first step towards assessing the potential of using the weed seed content in the soil to predict future weed problems, a study was initiated to compare the two main techniques for estimating the weed seed bank of some cropping soils in New Zealand. This paper reports on evaluation of the two methods for determining the seed bank of five different fields representing two major soil types of the Waikato region.

MATERIALS AND METHODS

The two soils used in this study were a Horotiu sandy loam soil (7.3% organic C, 61% sand, 16% clay, pH 5.9) and a Hamilton clay loam soil (2.4% organic C, 34% sand, 29% clay, pH 6.5). Bulk soil samples were collected in September 1994 from three different arable sites for the Horotiu sandy loam soil and from two arable sites for the Hamilton clay loam soil. The individual samples were thoroughly mixed and passed through a 4 mm sieve. From each bulk sample four, 1.85 kg subsamples were used immediately for the weed emergence studies and six other subsamples were air dried after which 500 g soil from each was sent to the MAFQual Official Seed Testing Station (OSTS) at Palmerston North, for weed seed extraction and enumeration.

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Weed seed extraction method

The procedure developed at OSTS for extracting weed seeds from soil samples is as follows. Air-dried soil samples (500 g) were each sub-divided into two 250 g samples. These samples were placed into nylon net bags (screen size 0.25 mm) and soaked in water for 5 minutes to soften soil. After soaking, the bagged soil was placed under running tap water and hand manipulated to remove fine soil particles. After completion of soil washing (5 min), the remaining contents of the bag were oven dried overnight (30°C) on filter paper in 14 cm glass Petri dishes. The dried samples were then passed through a descending series of seven Dodder sieves with screen size ranging from 1.6 mm to 0.5 mm. The material passing through the 0.5 mm sieve was collected in a 9 cm glass Petri dish at the base of the column. After sieving, the contents of each individual sieve were placed into a Leggatte Laboratory Seed Blower to remove a proportion of light empty seed, immature seed, seed coats and fine material from each sieving. Whole seeds from each sieving were extracted, counted and identified using a binocular stereomicroscope with 7x - 10x magnification. Seed viability was determined by "destructive crushing" of seed using forceps during the extraction procedure. Crushed seed containing undecayed (white) endosperm were recorded as potentially viable seed. Crushed empty seed (no endosperm) or decaying seed (discoloured endosperm) were not recorded. Potentially viable seed were well developed and normal in appearance, they had good structural integrity and resisted crushing. In contrast empty and decaying seeds were easily crushed.

Seedling enumeration method

Plastic trays (30 x 40 x 4 cm) were half filled with fine grade vermiculite and thoroughly wetted by sub irrigation. The soil samples (1.85 kg) were placed above the vermiculite and separated by a layer of water permeable weedmat material. Soil moisture was then maintained by top irrigation as required. The trays were maintained in the glasshouse under natural light with day temperatures of 25 - 35°C and night temperatures of 15 - 20°C over the summer of 1994/95. Approximately 1 month after setting up the trays, the emerged weed seedlings were identified, counted and removed. The soil was then allowed to dry before being thoroughly mixed by shaking in a polythene bag, and being set out in the trays again for the next incubation, and the process repeated until seedling numbers emerging in each tray were less than 2.

Weed emergence under different growing conditions

In a separate experiment, different conditions for weed emergence were compared using the soil from one of the Horotiu sandy loam sites. These consisted of three different regimes in addition to the glasshouse conditions described above, viz., (a) maintenance in the glasshouse after an initial period (2 weeks) of chilling at 4°C, (b) a controlled environment with a mean temperature of 17.5°C and a daily amplitude of 7.5°C (ie. from 10 - 25°C), and (c) outside in shadehouse conditions with day temperatures of 20 - 25°C and night temperatures of 7 - 10°C.

Analysis

For comparing the two methods, species were grouped according to growth habit (Table 1) and for each site and each group an analysis of variance of the two methods was carried out. For the 11 most abundant species, the significance levels for each weed group were pooled across sites within a soil type to give an 'overall' significance level for the two methods using Fisher's test (Table 1). For comparision of growing conditions, data were log transformed prior to analysis of variance. From this an approximate least significant ratio (LSR) for use on the untransformed data was obtained (Table 2).

RESULTS AND DISCUSSION

The seed extraction method found a total of 28 species of potentially viable weed seed in quantities ranging from 1 - 1030/kg dry soil while, in a total of seven incubations, the weed emergence method found 36 different species in quantities ranging from 1 - 860/kg dry soil. However many of the species found were in quantities that were too small to analyse. The 11 most common and abundant species are listed in Table 1 in four groups. Group 1 included summer broadleaf weeds and comprised *Amaranthus* spp.,

fathen (*Chenopodium album*), willow weed (*Polygonum persicaria*) and black nightshade (*Solanum nigrum*). Group 2 consisted of winter broadleaf weeds and comprised chickweed (*Stellaria media*), twin cress (*Coronopus didymus*), spurrey (*Spergula arvensis*) and broad-leaved dock (*Rumex obtusifolius*). Group 3 included two annual summer grasses, viz., summer grass (*Digitaria sanguinalis*) and smooth witchgrass (*Panicum dichotomiflorum*). Group 4 comprised the winter grass annual poa (*Poa annua*). These weeds were also found in similar order of abundance in the same two soil types in a field study of the periodicity of weed emergence (Rahman *et al.* 1993).

TABLE 1: Number of seeds/kg of dry soil obtained from two weed seed enumeration methods in a sandy soil (average of three sites) and a clay soil (average of two sites).

Species	Sandy soil			Clay soil		
	No. of seeds[a]	No. of seedlings[b]	Significance of group[c]	No. of seeds[a]	No. of seedlings[b]	Significance of group[c]
Summer broadleaf						
Amaranthus spp.	87	33		39	10	
Fathen	22	27	***	89	65	***
Willow weed	11	6		9	6	
Black nightshade	5	1		24	16	
Winter broadleaf						
Chickweed	1	0		6	6	
Twin cress	15	18	ns	141	104	***
Broad-leaved dock	7	7		0	0	
Spurrey	11	8		171	114	
Summer grasses						
Summer grass	84	33	ns	9	9	**
Smooth witchgrass	347	291		208	167	
Winter grass						
Annual poa	87	64	**	61	74	ns

[a] Determined by soil extraction method
[b] Number of seedlings that emerged in the glasshouse, total from seven incubations
[c] Overall significance of weed group using Fisher's test on the two methods

Of the four groups listed above, the summer broadleaf weeds were found in greater quantities by the seed extraction method compared to the seedling emergence method (Table 1). The winter broadleaf weeds were found in significantly higher numbers by the seed extraction method in the clay soil but not the sandy soil. This is probably an artefact of the low numbers of these species in the sandy soil rather than a true difference due to soil type. Results for grass weeds showed that the differences in the quantities of seed found by the two methods were smaller and less significant than for the broadleaf weeds.

A comparison of the data obtained by the two methods shows a high level of germination of seedlings in the glasshouse in both soil types for the 11 most common and abundant species. For three quarters of the samples, the number of seedlings that emerged in the glasshouse represented 65 - 100% of the weed seedbank estimated by the seed extraction method (Table 1). The remaining samples had 25 - 55% emergence except for black nightshade in the sandy soil where the seed numbers were very low. This is in contrast with the low emergence ratios reported by other workers (Ball and Miller 1989; Hartley and Rahman 1995; Jensen 1969) and suggests that it is possible to achieve high ratios under appropriate growing conditions with some soils. It also demonstrates that the technique used for testing seed viability in the seed extraction method is sensitive and reliable and does not have the shortcomings perceived by Bourdôt *et al.* (1994).

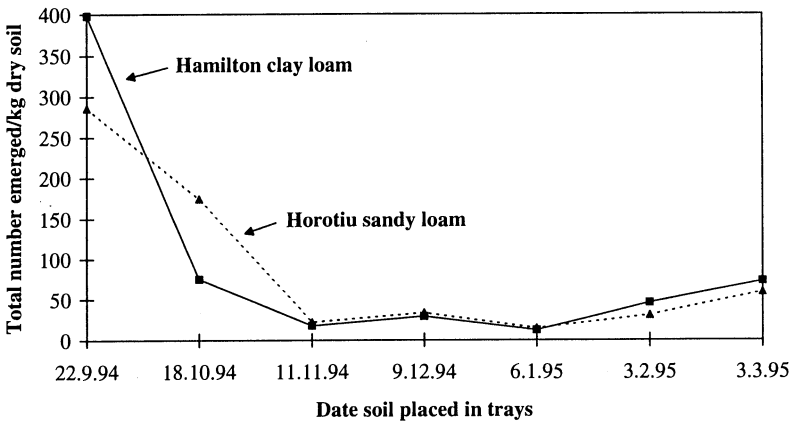

FIGURE 1: Total number of emerged seedlings in the two soil types from each successive incubation (soil mixed between incubations).

Although the seedlings had to be grown in the trays for up to 1 month for positive identification, most had emerged within the first week. Apart from differences in the relative abundance of the species found in the two soil types the overall emergence of the seedlings was very similar with most of the seeds emerging in the first two incubations (Fig. 1). The only species to emerge in significant numbers after two incubations were annual poa and toad rush (*Juncus bufonius*) which are responsible for the upward trend in this graph showing total seedling emergence. This point is highlighted in Fig. 2 through examples of one species from each of the four groups. The seedlings of the summer weeds, *Amaranthus* spp. and summer grass emerged during the first two incubations, while those of the winter weeds viz., twin cress, and especially annual poa, continued to emerge in later incubations.

Some species that were found by the seedling emergence method were not observed by the seed extraction method. For example, annual mouse-ear chickweed (*Cerastium glomeratum*) was present in all sites (average 7 seeds/kg dry soil), and

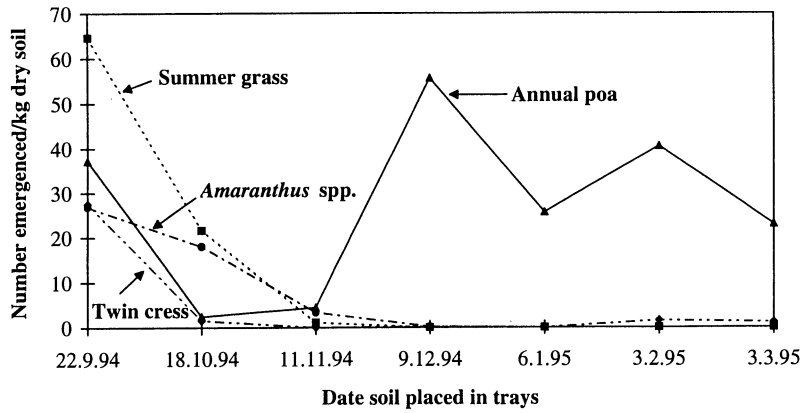

FIGURE 2: Number of emerged seedlings (total of five sites) of four different species from each successive incubation (soil mixed between incubations).

eight other species, including dandelion (*Taraxacum officinale*), willow herbs (*Epilobium* spp.), fleabane (*Pulicaria dysenterica*) and sow thistle (*Sonchus oleraceus*), were recorded in some sites in very small numbers. The reason that the seed emergence method found more species is due to the larger soil samples used for this method (1.85 kg cf. 0.5 kg soil). Another possibility is that very small seeds (less than 0.25 mm) may have passed through the nylon net bags during the initial wash. Two species viz., hawksbeard (*Crepis capillaris*) and *Fumaria* spp. were found through the seed extraction method but did not emerge in the glasshouse trays. A subsample of the soil from each tray is being extracted to see if the seeds that have not emerged can be accounted for in these samples. The remainder will be repeatedly incubated for a period of 2 years.

The weed spectrum in the experiment comparing different growing conditions differed slightly from that in Table 1 in terms of the relative abundance of weed seeds so the weed groupings in Table 2 have been changed. Wild portulaca (*Portulaca oleracea*) replaces black nightshade and fathen in the summer broadleaf group, while hedge mustard (*Sisymbrium officinale*) and *Veronica* spp. replace chickweed in the winter broadleaf group (Table 2).

The four different growing conditions used to determine the soil seedbank gave different results for both the number and abundance of species present. Chilling the soil for 2 weeks before placing it in the glasshouse did not significantly increase germination, possibly because chilling had already occurred in the field. The controlled environment resulted in significantly lower numbers of all species except the winter broadleaf weeds. The outside regime was generally intermediate between the glasshouse and the controlled environment. It is obvious that the conditions for germination need to be selected carefully to suit the particular groups of weed species. For example, if summer growing weeds such as summer grass and thorn apple (*Datura stramonium*) are anticipated in the soil samples then higher temperatures are required for incubation. However, our results suggest that temperature may not be so critical in the case of winter weeds. Other variations in growing conditions such as soil depth, soil amelioration and water method have also been found to affect the emergence of some weed species (Hartley and Rahman 1995).

TABLE 2: Emergence of weeds in 1 kg of Horotiu sandy loam soil (1 site only) under different conditions[a].

Species	No. of seeds[b]	Glasshouse	Glasshouse after chilling	Controlled environment	Outside (shadehouse)
Summer broadleaf					
Amaranthus spp.	95	48	59	50	45
Wild portulacca	6	14	9	0	5
Willow weed	16	10	11	7	5
Winter broadleaf					
Broad-leaved dock	39	32	44	33	21
Hedge mustard	33	55	61	30	58
Spurrey	31	25	24	26	25
Twin cress	39	32	44	33	21
Veronica spp.	17	8	7	19	18
Summer grasses					
Summer grass	239	87	68	18	63
Smooth witchgrass	1031	865	840	36	680
Winter grass					
Annual poa	259	189	238	72	181

[a] Least significant ratio (LSR) (5%) between any two columns is approximately 1.5.
[b] Determined by soil extraction method.

Results show that both methods used in this study provided reliable estimates of the weed seedbank in sites with varying weed seed densities and in soils with different textures. The weed seed extraction method is a fast and efficient technique that provides an immediate result but requires specialised expertise and equipment. While seeds smaller than 0.25 mm could be lost in the initial wash, this method would extract all the larger seeds in the sample and thus provides an accurate estimate of the seedbank for all important species. The seedling emergence method needs more time and specific growing conditions are required for maximum emergence of some species. As most seedlings emerge with the first two incubations, it may be possible to estimate the total seedbank from these numbers. The high ratio of seedling emergence to the total seedbank recorded for the most abundant weed species suggests that a reliable relationship could be established between the weed seedbank and weed emergence. This demonstrates the potential for using the weed seed content of the soil to predict future weed problems but correlations with field emergence will need to be established.

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REFERENCES

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

Bourdôt, G.W., Hurrell, G.A., Rahman, A., Abernethy, R. and Grbavac, N., 1994. Seedling emergence in *Chenopodium album* and the predictive value of soil seed banks. *Proc. 47th N.Z. Plant Protection Conf.*: 1-5.

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

Hartley, M.J. and Rahman, A., 1995. Effect of varying glasshouse and soil conditions on weed seed emergence from soil. *Proc. 48th N.Z. Plant Protection Conf.*: this volume.

Jensen, H.A., 1969. Content of buried seeds in arable soils in Denmark and its relation to the weed population. *Dansk Botanisk Arkiv. 27*: 7-57.

King, R.P., Lybecker, D.W., Schweizer, E.E. and Zimdahl, R.L., 1986. Bioeconomic modelling to simulate weed control strategies for continuous corn (*Zea mays*). *Weed Sci.34*: 972-979.

Radosevich, S.R., 1984. Weed Ecology : Implications for Vegetation Management, Wiley and Sons, New York.

Rahman, A. and James, T.K., 1993. Patterns of weed seedling emergence in two New Zealand soils. *Proc. 8th European Weed Research Soc. Symp., Braunschweig*: 665-672.

Roberts, H.A. and Ricketts, M.E., 1979. Quantitative relationships between the weed flora after cultivation and the seed population in the soil. *Weed Research 9*: 269-275.

Sagar, G.R. and Mortimer, A.M., 1976. An approach to the study of the population dynamics with special reference to weeds. *Appl. Biol. 1*: 1-47.