Weed seedbank estimation, spatial distribution, decline and potential for predicting future weed populations

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Summary

Seedbank species composition and density were determined in soils collected from five arable sites in 1994 and from a further six sites in 1995. The methods used were (i) seed extraction by washing and dry sieving, and (ii) seedling enumeration under different growing conditions. Both methods gave similar estimates for grass weeds but the seed extraction method generally gave higher values for broadleaf weed species. There was significant variation between samples within each of the sites, both in terms of the number of species and the number of seeds or seedlings recorded. No obvious differences due to variations in soil type were noted in the weed density or weed seed numbers between the six sites. Soil samples collected from the headlands of maize fields produced seedlings of more weed species, although the number of seeds or seedlings were not significantly different between samples collected from inner and outer areas of the fields. The initial rate of seedbank decline varied between sites from different parts of the country. However, the seedbank of most grass and broadleaf weeds present declined in the absence of seed input over the four year period to between 1 and 2% of the original number, although this still represented a very large seedbank. The high ratio of seedlings emerged to seeds extracted in all samples demonstrates the potential for using the weed seed content of the soil to predict future weed problems in the field.

Introduction

The weed seedbank as a reservoir of weed seeds in the soil or on the soil surface largely determines the species composition and potential densities of weeds that subsequently interfere with crops during the growing season. Some knowledge of the weed seedbank may therefore be appropriate for integrated weed management programmes (Forcella 1993). However, a number of problems are inherent in the estimation of the seedbank size of arable weeds which usually have annual life cycles (Benoit *et al.* 1992, Forcella 1993). Determination of the density of viable weed seeds in a soil sample is a tedious and slow process and comparative studies of seedbanks have been hampered by the difficulty of accurately determining the number of seeds and species present. The usual techniques employed by researchers fall into two categories viz. (i) physical extraction of seeds from soil samples by flotation and/or sieving and counting, (ii) incubation of the soil and enumeration of the seedlings emerging from the viable seeds (Ball and Miller 1989, Gross 1990).

Studies on weed seedbanks have demonstrated large variability in both density and composition between samples (Benoit *et al.* 1992) and multiple soil samples need to be collected from different locations within a field to get a reliable estimate (Dessaint *et al.* 1991, 1996, Forcella *et al.* 1992, Wilson and Aebischer 1995). Our observations also indicate that the edges and corners of arable fields tend to be the areas of greatest botanical diversity.

Weed seedbanks generally decline exponentially over time, and the rate of decline increases with the frequency of cultivation (Roberts and Dawkins 1967). Data from field experiments show that similarly, the number of seedlings emerging annually from regularly disturbed soil declines exponentially for most weed species (Popay *et al.* 1994, Roberts 1964, Wilson and Lawson 1992). Thus in addition to estimating the weed seed populations in the soil, some knowledge about the persistence of weed seedbanks is important for developing long term weed management strategies.

The work described in this paper summarizes a number of field and glasshouse studies conducted over the past five years with the following objectives:

i. to compare the two main techniques for estimating the weed seedbank of some cropping soils in New Zealand,
ii. to examine the distribution of arable weed seeds in different parts of arable fields,
iii. to estimate the rate of seedbank decline for certain weed species, and finally
iv. to assess the potential of using the weed seed content in the soil to predict future weed problems in New Zealand cropping systems.

Materials and methods

Comparison of techniques

The techniques evaluated in this study were the weed seed extraction method and the seedling emergence method. Bulk quantities (50 kg) of soil were collected in September 1994 from three different arable sites on Horotiu sandy loam soil (7.3% organic C, 61% sand, 16% clay, pH 5.9) and two arable sites on Hamilton clay loam soil (2.4% organic C, 34% sand, 29% clay, pH 6.5). The soil from each site was passed through a 4 mm sieve and thoroughly mixed. From each bulk sample four sub-samples were obtained with a riffle sampler and used immediately for the seedling emergence studies. Six further sub-samples were air dried after which 500 g soil from each was sent to the MAFQual Official Seed Testing Station (OSTS) for weed seed extraction and enumeration. In August 1995 bulk soil (50 kg) was collected from a further six maize fields representing a variety of soil types ranging from peaty loam to clay soils and processed in a similar fashion.

For the weed seed extraction method the air dried soil samples were each subdivided into two 250 g samples, placed in nylon net bags (screen size 0.25 mm), soaked in water for five minutes, and then washed under running tap water. The remaining contents of the bag were oven dried overnight in petri dishes and then passed through a descending series of seven Purity Test Sieves (Hoffman Manufacturing Company, Albany, Oregon, USA) with screen sizes ranging from 1.6 to 0.5 mm. Whole seeds from each sieving were extracted, counted and identified. Seed viability was determined by 'destructive crushing', in which seeds containing undecayed endosperm were recorded as potentially viable.

For the seedling emergence method, soil samples (1.7–2.1 kg) were placed in plastic trays (300 × 400 × 40 mm) half filled with vermiculite and separated by water permeable weedmat material. Trays were placed in a glasshouse under natural light with day/night temperatures of 25–30/15–20°C and with soil moisture maintained by top irrigation. After one month the emerged seedlings were counted and removed. The soil was then air dried, mixed and set out in trays for the next incubation. This process was repeated until two or less seedlings emerged in each tray (usually 7–9 repeat incubations). After this, the trays were stored at 4°C for two weeks and the incubation process was repeated again until no more seedlings emerged.

For comparing the methods, species were grouped according to phenology (Table 1) and for each site and for each group an analysis of variance was carried out. For the 11 most abundant species, the

significance levels for each group were pooled across sites within a soil type to give an 'overall' significance level using Fisher's test.

In a separate experiment, different growing conditions were compared for weed seedling emergence using the soil from one site, viz. (i) glasshouse conditions as described above, (ii) maintenance in glasshouse after an initial period (2 weeks) of chilling at 4°C, (iii) controlled environmental conditions with a day/night temperature of 25/10°C, and (iv) outside in a shadehouse with day/night temperatures of 20–25/7–10°C.

Spatial distribution of a weed seedbank

Six fields (5–20 ha) that had been used for growing maize for several years and which had a variety of soil types ranging from peaty loam to clay were selected. Each field was divided into eight areas for sampling according to Figure 1. The outer area was always 20 m wide and generally conformed to the headland planting area for the maize crop. Each area was sampled by taking 60 cores along transects using a 25 mm diameter soil corer set to 100 mm depth. Transect and sampling spacings were designed according to the size and shape of each field to produce an even and regular sampling pattern over the whole area. The samples were collected after harvest of maize crop but before cultivation, in August 1995. The cores were mixed while still field moist and sub-samples taken for enumeration of the weed seedbank using the two methods described above. In the seedling emergence method the soil samples were each incubated seven times. Means and standard deviations were obtained for each species at each site. For spatial distribution, analysis of variance was carried out on the averaged data (inner vs. outer areas of fields) using sites as replicates.

Rate of decline of weed seedbanks

This experiment was conducted to determine the rate of seed loss of certain weed species from the soil seedbank in the absence of seed input, when the soil was cultivated (to a depth of 100 mm) monthly over a period of four years. Each experiment consisted of four 1 × 1 m plots on an arable site in three different parts of News Zealand viz. Ruakura near Hamilton, Lincoln near Christchurch and Invermay near Dunedin. Each plot was divided into four quarters and the monthly re-cultivation (with a hand trowel) was staggered by seven days in each quarter. At the beginning of the experiment in September 1992 and annually thereafter soil samples were collected to 100 mm depth from each plot. After thorough mixing and air drying, a sample of 500 g of soil from each quarter was sent to OSTS for measurement of the seedbank using the weed seed extraction method described above. For Figures 4 and 5, the data were analysed using repeated measures methods.

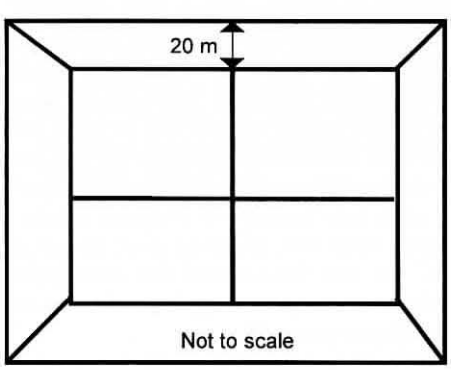

Figure 1. General representation of the division of each field into four outer and four inner sampling areas.

Table 1. Estimates of weed seeds kg^{-1} of dry soil obtained from two 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]	Ratio[C]	No. of seeds[A]	No. of seedlings[B]	Ratio[C]
Summer broadleaf						
Amaranthus spp.	87	33	2.6	39	10	3.9
Chenopodium album L.	22	27	0.8	89	65	1.4
Polygonum persicaria L.	11	6	1.8	9	6	1.5
Portulaca oleracea L.	2	5	0.4	121	45	2.7
Solanum nigrum L.	5	1	5.0	24	16	1.5
Total	**127**	**72**	**1.8*****	**282**	**142**	**2.0*****
Winter broadleaf						
Stellaria media (L.) Vill.	1	0	–	6	6	1.0
Coronopus didymus (L.) Sm.	15	18	0.8	141	104	1.4
Rumex obtusifolius L.	7	7	1.0	0	0	0
Spergula arvensis L.	11	8	1.4	171	114	1.5
Veronica persica Poir.	6	3	2.0	2	1	2.0
Total	**40**	**36**	**1.1 ns**	**320**	**225**	**1.4*****
Summer grasses						
Digitaria sanguinalis (L.) Scop.	84	33	2.5	9	9	1.0
Panicum dichotomiflorum Michx.	347	291	1.2	208	167	1.2
Total	**431**	**324**	**1.3 ns**	**217**	**176**	**1.2 ns**
Winter grass						
Poa annua L.	87	64	1.4**	61	74	0.8 ns

[A] Determined by soil extraction method.
[B] Number of seedlings that emerged in the glasshouse, total from seven incubations.
[C] Ratio of number of seeds extracted to seedlings emerged. The asterisks refer to the overall significance of the weed group using Fisher's test on the ratio of two methods.

Results and discussion

Comparison of techniques

For the seedling emergence method it was found that >95% of the weed seedlings emerged within the first seven incubations, which in each case covered the period between spring and autumn. The results presented are from these first seven incubations.

The seed extraction method found potentially viable weed seeds from a total of 28 species in quantities ranging from 1 to 1030 kg^{-1} dry soil while the seedling emergence method resulted in 26 different species in quantities ranging from 1 to 860 kg^{-1} dry soil. However many of the species found were in quantities that were too small to analyse. The 13 most common and abundant species are listed in Table 1 in four groups. 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 and James 1993).

Of the four groups listed in Table 1, the summer broadleaf weeds were found in greater quantities by the seed extraction method compared to the seedling emergence method. 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 appears to be 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 than for the broadleaf weeds.

The ratios of the number of seeds extracted to the number of seedlings emerged are also presented in Table 1. When the ratio is different from 1.0, it suggests that one method estimated the numbers differently to the other. However, if this ratio remains the same over a number of experiments, it is likely that there is a

constant relationship between the two methods for the given weed species. Overall, we recorded a high level of germination of seedlings in the glasshouse in both soil types for the 13 most common and abundant species. 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 in some soils.

Although the seedlings had to be grown in the trays for up to one 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 (Figure 2). The only species to emerge in significant numbers after two incubations were the winter weeds *Poa annua* and *Juncus bufonius* L., which are responsible for the upward trend in Figure 2 due to their autumn germination (February–March). This point is highlighted in Figure 3 through examples representing one species from each of the four groups. The seedlings of the summer weeds, *Amaranthus* spp. and *Digitaria sanguinalis* emerged during the first two incubations, while those of the winter weeds viz., *Coronopus didymus*, and especially *Poa annua*, continued to emerge in later incubations.

The weed seed extraction method is a fast and efficient technique that provides an immediate result but requires specialized expertise and equipment. While seeds smaller that 0.25 mm could be lost in the initial wash, this method would extract all the larger seeds and with adequate sampling would provide a reliable estimate of the seedbank size for the important weed species. The seedling emergence method is more time consuming, and specific growing conditions are required for maximum emergence of some species. As most seedlings emerged with the first two incubations, for practical purposes, it may be possible to estimate the total seedbank from these numbers.

The four different growing conditions used to compare seedling emergence gave different results for both the number and abundance of species present (Table 2). Chilling the soil for two weeks before placing it in the glasshouse did not significantly increase germination, possibly because chilling had already occurred in the field during winter months. The controlled environmental conditions 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 in terms of seedling germination. It is obvious that the conditions for germination need to be selected carefully to suit the particular groups of weed species present. For example, if summer growing weeds such as *Digitaria sanguinalis* and *Datura stramonium* L. are anticipated in soil samples, higher temperatures are required for incubation. However, our results suggest that temperature may not be so critical in the case of winter weeds. Variations in other growing conditions such as soil depth, soil amelioration and watering method have also been found to affect the emergence of some weed species (Hartley and Rahman 1995).

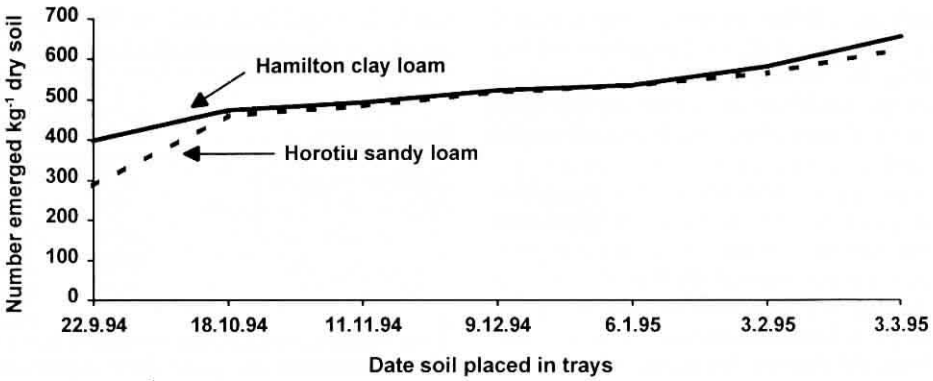

Figure 2. Cumulative number of emerged seedlings in the two soil types from each successive incubation (soil mixed between incubations).

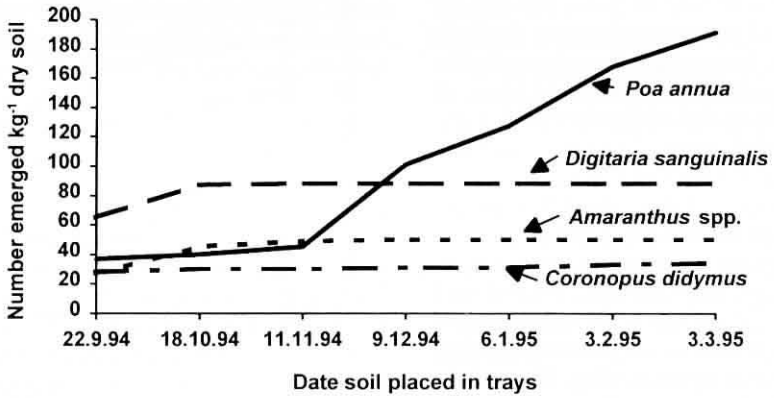

Figure 3. Cumulative number of emerged seedlings (total of five sites) of four weeds from successive incubations (soil mixed between incubations).

Table 2. Number of weed seedlings emerged from 1 kg of Horotiu sandy loam soil under different growing conditions.

Weed group	No of seedlings kg⁻¹ soil^			
	Glasshouse	Glasshouse after chilling	Controlled environment	Outside (shadehouse)
Summer broadleaf	72	79	57	55
Winter broadleaf	152	180	141	143
Summer grasses	952	908	54	743
Winter grasses	189	238	72	181

^ Least significant ratio (5%) between any two columns is 1.3.

Spatial distribution of weed seedbank

In this study also both the methods used for weed seedbank estimation gave similar results. At three of the six sites, the outer areas of the fields contained significantly ($P<0.05$) more seed and seedling numbers than the inner areas, but at one site the results were reversed. Thus over all the six sites, there were no significant differences in the total number of seeds or seedlings between samples collected from the outer and inner areas of fields. Comparing the numbers for some of the major weed species, totalled over all sites, the two grass weeds tended to show higher values in the outer area than in the inner area, but numbers for the broadleaf weeds were similar in both zones (Table 3). Analysis of the data also showed that in five of the six sites, significantly more weed species were found in the outer area using the seedling emergence method, although this difference was apparent in

only one of the six sites using the seed enumeration method. The reason for this could be the larger size of samples used for the seedling emergence method (two to three times of that used for seed extraction).

In field surveys of cereal crops in Britain, Wilson and Aebischer (1995) found that numbers of species declined significantly between the crop edge and 4 m into the crop and that numbers remained stable as distance increased beyond 4 m. The total number of seedlings of common broadleaf weeds also decreased significantly as distance from the crop edge increased. Other workers (e.g. Marshall 1989, Rew *et al.* 1992) have also recorded declining densities of most, but not all, weeds with increasing distance from the field edge. In our study the outer area chosen for sampling may have been too wide (20 m), but as the headland area is cultivated, drilled, sprayed and harvested differently and sometimes has a poorly established crop, it was thought that this zone would possibly be distinct.

No obvious relationships were identified between weed density or weed seed numbers and soil type across sites, despite the range of soils surveyed both in terms of texture and organic matter. This may be a result of continuous maize cropping and the associated use of herbicides on the sites included in this study. However, there were large differences between samples within each site (which was reflected in standard deviations of up to 60%) despite the 60 cores taken along several transects for each sample. This shows a large degree of inherent variation in the weed seedbank of annual cropping systems. It has been suggested that such considerable variation over small distances could be a consequence of the distribution of seed-parents in previous seasons (Bigwood and Inouye 1988). Based on sophisticated statistical analyses, Dessaint *et al.* (1990) indicated that hundreds of cores may be necessary to adequately sample seedbanks. It has been suggested by others, however (e.g. Forcella *et al.* 1992) that for practical purposes, 10–20 cores, each 50 mm diameter, are probably sufficient for a single field or management unit. In a recently completed study we have found that approximately 25 cores, each 25 mm diameter, should provide a sufficient sample for fields of annual crops such as maize (Rahman *et al.* 1997).

A comparison of the two methods of estimating weed seedbanks again showed a positive relationship ($r^2 = 0.92$) between seedling emergence and seed numbers. This confirms the results of technique comparisons given earlier and demonstrates the potential for using the weed seed content of the soil to predict future weed problems, although correlations of individual species frequencies with field emergence patterns still need to be established.

Table 3. Total seed and seedlings counted in all six sites of some major weed species found in the inner and outer areas.

Weed species	Total seeds kg^{-1} soil			Total seedlings kg^{-1} soil		
	Inner	Outer	SEM	Inner	Outer	SEM
Digitaria sanguinalis	11.2	33.2	6.9[A]	8.5	12.8	2.2
Panicum dichotomiflorum	3.7	11.3	2.5[A]	3.9	9.4	1.4[B]
Chenopodium album	68.7	73.2	3.9	74.1	88.1	8.8
Solanum nigrum	12.8	8.9	2.3	12.0	9.9	1.2
Trifolium repens L.	2.5	4.0	0.6	0.4	0.4	0.2
Rumex spp.	7.9	6.6	2.1	8.4	4.0	2.1

[A] Inner and outer areas are significantly different at the 10% level using a two sided test.
[B] Inner and outer areas are significantly different at the 5% level using a two sided test.

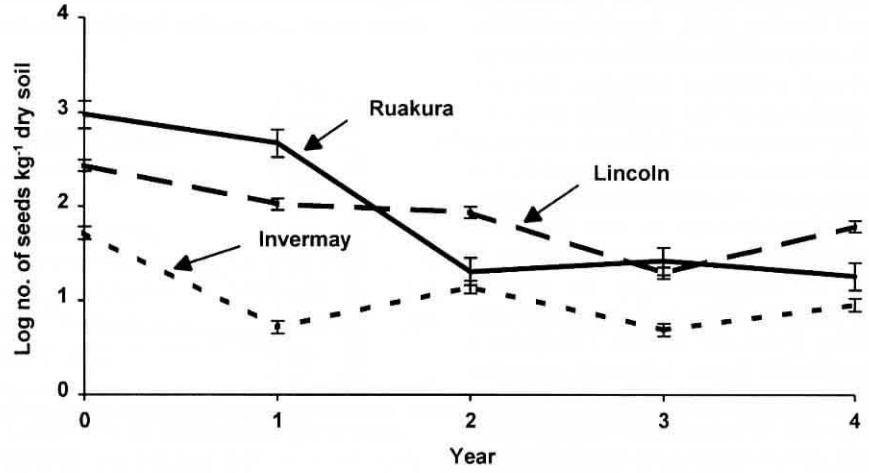

Figure 4. Decline in total weed seed numbers at the three sites following monthly cultivation over a period of four years. Error bars are ± SEM.

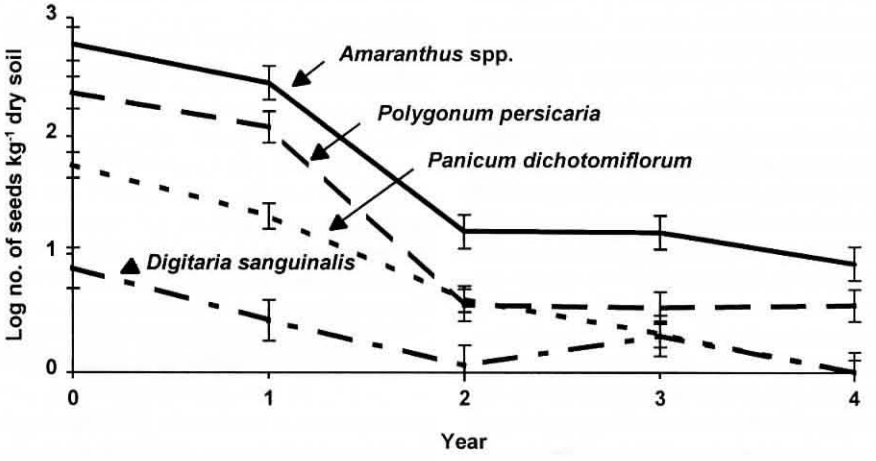

Figure 5. Decline in seed numbers of four weed species at the Ruakura site following monthly cultivation over a period of four years. Error bars are ± SEM.

Rate of decline of weed seedbanks

At the beginning of the experiment (i.e. year 0) seeds of 32, 20 and 18 weed species were found in the soil samples from Lincoln, Ruakura and Invermay, respectively. No additional species were recorded in the subsequent years. Although the number of species was high at Lincoln, nearly half of them averaged only one or less seed per kg dry soil. The seedbank composition was similar at Lincoln and Invermay, the two South Island sites, with the same winter and summer broadleaf weeds and *Poa annua*. A different spectrum of broadleaf weeds was found at Ruakura, the North Island site, and the two warm zone grasses, *Digitaria sanguinalis* and *Panicum dichotomiflorum*, were also significant contributors to the seedbank.

In all three sites the size of seedbank dropped in the first year, down to 56, 43 and 39% of the initial value at Ruakura, Lincoln and Invermay, respectively (Figure 4). The reduction in the second year

was again very large at Ruakura, possibly due to its large seedbank compared to the other two sites. Smaller reductions continued in year 3 at all sites, but the numbers went up slightly in year 4 at both Lincoln and Invermay. This could be a result of the inherent problem of accurate sampling of small seedbanks as has been discussed above. Further, in the case of Lincoln, one species contributed to nearly half of the increase observed in the fourth year, viz. *Capsella bursa-pastoris* (L.) Medik., the seeds of which are known to remain alive in the soil for several years, with a few germinating each year. At the Ruakura site, which had a large seedbank and was in a protected location, no reversal of the seedbank decline was noted at any stage.

The data in Figure 4 were subjected to regression analyses which showed that although the rate of seed decline was rapid, there was a significant departure from pure exponential decay pattern for all sites. As the Ruakura site had the largest seedbank, the rate of seed decline of four major weed species (which contributed nearly 95% of the seedbank) at this site was plotted (Figure 5) and analysed. Not surprisingly, the two dominant species, *Amaranthus* spp. and *Polygonum persicaria*, both showed a significant departure from pure exponential decay. These results indicate a slower initial rate of decline than that reported by previous workers, including Roberts and Dawkins (1967) and Roberts and Boddrell (1984) who showed that number of weed seeds in the soil and of weed seedlings both declined exponentially from year to year. In the only similar study conducted in New Zealand, Popay *et al.* (1994) reported for one location that for most weed species, decline in the size of seedbank was much larger under regular deep (250 mm) cultivation than under shallow (10 mm) or no cultivation. The relatively fast rate of decline of the seedbank in our study is in agreement with their results for deep cultivation. As the plots in our study were cultivated every month, this would have accelerated the seedbank decline as the rate of decline is known to increase with the frequency of cultivation (Roberts and Dawkins 1967).

Results presented in Figure 5 show that in the first year the seed numbers of the two grass weeds declined by 78 and 63%, while those of the two broadleaf weeds declined by 45 and 34%. By the end of the fourth year, the numbers had declined to 1–2% of the original seedbank of these four weeds in the soil. The soil used in the Ruakura experiment has a bulk density of 0.73 kg L^{-1}, so one seed per kg of soil would give a population of 730 000 seeds ha^{-1} to a 100 mm depth in the field. This would mean after four years there were still 6.4 million seeds *of Amaranthus* spp., 2.6 million seeds of *Polygonum persicaria* and 0.2 million seeds each of *Digitaria sanguinalis* and *Panicum dichotomiflorum* per hectare in the soil. This was in a situation where soil was regularly cultivated and no new seed was added. Barralis *et al.* (1988) have found that weed seed numbers in the soil declined less rapidly if wheat or barley were sown and herbicides applied than if the soil was cultivated, but no crop planted, and weed seedlings killed with paraquat. This clearly shows that reducing weed seed numbers by natural depletion will take a long time.

Overall, the results of the experiments presented here show that the two methods of seedbank estimation evaluated provided similar values for some grass weed species, but generally not for broadleaf species. Further comparative work could determine whether there is a constant relationship between the numbers obtained by the two methods for those species which they estimated differently. Both methods have advantages and shortcomings, but it is clear that conditions for germination in the seedling emergence method need to be selected carefully to suit the range of weed species likely to be encountered. The large degree of inherent variation in the weed seedbank of annual cropping systems observed here and by other researchers presents a real challenge for adequate soil sampling procedures to achieve credible estimates of the total seedbank population, both in terms of density and the composition. Further work is underway to confirm the number and size of soil cores required to adequately estimate the weed seedbank.

The high ratio of seedling emergence to the total seedbank recorded for the most abundant weed species in all our experiments suggests that reliable relationships could be established between the weed seedbank and emergence of individual weed species. This demonstrates the potential for using the weed seed content of the soil to predict future weed emergence, but correlations with field emergence still need to be established. It is also obvious from the results that a long period of time is required for natural depletion of the seedbank for most species despite the favourable climate for germination through most of the year in New Zealand. Furthermore, new weed seeds are inevitably added to the soil surface on a continuous basis so that weeds are never eliminated. Satisfactory management of weeds would therefore continue to require approaches that either leave most weed seeds buried in the soil (e.g. conservation tillage) or involve the use of herbicides or shallow cultivation to destroy weed seedlings in early stages of growth.

Acknowledgments

We would like to thank Judy Mellsop, Geoff Hurrell and Ron Abernathy for technical assistance; Official Seed Testing Station staff for seed extractions and Dr. John Waller for statistical analyses.

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