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RESEARCH MEMORANDUM

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METHODOLOGICAL AND VALIDATION STUDY OF SEED RESERVES IN DESERT SOILS

> D.W. Goodall, S. Childs & H. Wiebe



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1971 PROGRESS REPORT

METHODOLOGICAL AND VALIDATION STUDY OF SEED RESERVES IN DESERT SOILS

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Utah State University Logan, Utah

APRIL 1972

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ABSTRACT

The development of a method for separating seeds from desert soils, and enumerating them, is described.

In the Great Basin desert, species differed greatly in their depth distribution, some having a peak at or just below the surface, while others were still abundant below 5 cm. Much higher seed densities were found beneath the canopies of shrubs and of tussock grasses than in the interspaces; no consistent differences were found, however, associated with the species of the canopy plant, or with distance from its center.

Tentative estimates are given of the seed population in the four validation sites in Curlew Valley. 2.2.1.2.-2

INTRODUCTION

The purpose of this study is to provide data on a part of the biomass on the validation study areas which has often been neglected in ecosystems studies. The population of seeds in the soil and on its surface may be of importance in ecosystem dynamics in two ways:

- a) As the source of new seedlings; and
- b) As food for the many species of mammals, birds and insects which largely depend on them.

For the first purpose, information is needed about their vitality and state of dormancy, as well as their biomass and composition.

Numerous studies have been made of the seed reserves in arable soils and in improved grassland, but very little information is available on reserves in desert soils, either in North America or elsewhere. In any case, inventories of the seed reserves are clearly needed as part of the data for the Desert Biome validation studies.

The present progress report is limited to work completed during 1971, and in practice (through personnel difficulties) to the last four months of that year. This work was largely exploratory in nature, directed to developing methods and acquiring a "feel" for the problems rather than to the actual collection of data.

OBJECTIVES

- 1. To develop techniques for inventory of the seed populations of desert soils, including their spatial distribution and their viability.
- 2. To apply these techniques to obtain inventories of each of the validation sites on one or more occasions.

METHODS

Some studies have relied on germination tests applied to soil samples with the contained seeds, either *in situ* or transported, as a means of inventory for seed reserves (e.g., Brenchley and Warington 1930; Major and Pyott, 1966; Roberts, 1958). This would clearly be unsatisfactory for the present purpose. Seed dormancy is very prevalent in desert species an inventory based on germination would include only that part of the seed population which happens to respond to the range of conditions used. For future germination potentiality, and for their use by herbivores (for which non-viable seed may be equally suitable) a more complete record is necessary.

Attempts must be made to separate and count the seed population as a whole. Methods for doing this depend mainly on distinctions in size and density between the seeds and the much more numerous particles of the mineral soil. The soil, and particularly the litter, contain remains of plants and animals similar in size range and density to seeds and consequently not separable from them by mechanical means. The final step in separation must accordingly be visual.

Preparation of Seed Samples.

General Considerations. As a preliminary to sieving and flotation, measures to disperse soil aggregates may be necessary. Sodium hexametaphosphate ("Calgon") solution was found satisfactory for this purpose. Ultrasonic vibration was also tested, but no advantages were found. The possible value of hydrochloric acid treatment in soils with caliche or concretionary/layers is being borne in mind, but the need for it has not arisen so far.

Flotation liquids used in the literature (i.e., Awano and Lizumi, 1956; Kropac, 1966; Malone, 1967) have mainly been concentrated salt solutions. Tests were made with sodium thiosulfate, calcium chloride, and potassium carbonate, the densities of which respectively are about: 1.64, 1.52 and 1.56. The possibility of separating seeds from soil by these flotation liquids was studied for a variety of commercially available seeds in the size range 0.5 to 2.0 mm., together with some native and introduced species present in the study areas.

The results showed that a satisfactory separation of all species of seed tested could be obtained with concentrated potassium carbonate solution as the flotation liquid. As samples of seeds of other species occurring in the study area come to hand, their density will be tested; if any are found to exceed 1.56, the flotation liquid used will be changed -- probably to concentrated zinc chloride solution (density 2.07).

Flotation is supplemented by sieving. Various types of metal sieve were tested, both wet and dry, but the most convenient method found involved sieving through the commercially available cloth organza, with a mesh of .12 mm in one dimension, .16 mm in the other. Metal sieves were also used to separate the coarser and finer fractions of organic material, and thus facilitate subsequent work under the microscope.

Recovery of added seeds. Recovery tests have been performed on soil samples to which known numbers of seeds of various species have been added by another operator. Table 1 shows the results of a series of recovery tests on eight soil samples, some from the Great Basin (Curlew Valley), others from the Mohave Desert (Rock Valley). It will be seen that results differ considerably between species. For some species recovery is consistently close to 100%, in other cases barely half the seeds may be recovered. Efforts are being made to improve recovery, but complete success is too much to expect. Accordingly, the number of seeds actually found will have to be multiplied by a factor to allow for losses. For the present, a factor based on the average of the nine species in Table 1 is being used. When sufficient stocks are available of the seeds, and correction factors appropriate to each particular species will be calculated and applied to the field data.

	Total seeds in		
Species	Added	Recovered	% Recovery
Castilleia sulphurea	13	11	85
Daucus carota	30	29	97
Delphinium bakeri	11	11	100
Descurainea richardsonii	61	54	89
Eragrostis lehmanniana	73	34	47
Gilia pulchella	57	33	58
Sporobolus airoides	73	61	84
Sporobolus flexuosus	60	52	87
Trifolum repens	30	27	90

Table 1. Recovery of added seeds.

Procedure. The procedure accordingly adopted is as follows:

- A sample divider is used to reduce the air-dried soil to a sub-sample of about 100 g.
- 100 g of the soil sample is weighed into a square of filter cloth, wrapped in it, and immersed in a solution of 1% sodium hexametaphosphate for 1 hour. This disperses the larger soil aggregates.
- 3. The remaining particles within the cloth are washed out under a gentle stream of water, the contents of the cloth being gently kneaded meanwhile.
- 4. The contents of the cloth are washed into a beaker with a stream of concentrated potassium carbonate solution. After 2 minutes, the floating material is decanted through a metal sieve with a mesh of 1 mm.
- 5. The two floated fractions (that retained on the sieve, and that which passes through it) are washed with water, dried on pieces of organza, and subsequently examined under a binocular dissecting microscope (the magnification most commonly used is x 10).

Identification.

A reference collection of seed samples is being built up; at present, it consists mainly of Great Basin species. Some of these samples come from stocks at the Crop Research Laboratory of the A.R.S., Logan; others have been removed from specimens in the Intermountain Herbarium. The majority, however, have come from material collected in the Curlew Valley, near the validation study areas. A valuable collection of Mohave Desert seeds has also been provided by Dr. Janice Beatley of the University of California, Los Angeles.

As extracted from the soil, the seeds often differ considerably in appearance from those in the seed herbarium. They become abraded, or seeds with sticky surfaces may be associated with fine soil particles which are difficult to separate from them. The seeds recovered are separated by morphology, and are then compared with herbarium samples and with published illustrations (e.g., Hitchcock, 1950; U.S.D.A., 1952; Holmgren and Andersen, 1969, 1970; Musil, 1963). Illustrations, however, are often inadequately representative of the range of variation to be of much use. If these methods of identification fail, or are ambiguous, morphological examination must be supplemented by germination tests. Information on germination behavior will be required for other aspects of the project, but for the present purpose quantitative measures of germination success are of no interest. All that is needed is a few seedlings which can be grown on until they are mature enough for unequivocal identification. In some cases, no identification has yet been possible, though the seed types are distinct enough morphologically. These are recorded by symbols. In other cases, two or more species are so similar morphologically that reliable discrimination is not possible. These are lumped for inventory purposes.

Field Sampling: Spatial Distribution of Seeds.

The distribution of seeds on and under the soil surface may be expected to be highly heterogeneous. The initial pattern of distribution of the larger seeds depends on the distribution of the parent plants, that of the smaller seeds on wind and eddy air movement. This pattern is subsequently modified by water movement on the soil surface, by wind, and by the activities of animals. The seeds may also be buried to various depths — again partly through animal activities, partly through the impact of raindrops, and partly through soil cracking during drying and freezing processes. In consequence of all these sources of

heterogeneity, it is clearly necessary to determine the spatial distribution of the seeds, both laterally and vertically, and develop a sampling procedure which will enable an unbiased estimate of the seed population to be obtained, with minimum error for a given sampling effort. The pattern found may itself be relevant to ecosystem dynamics, determining as it does the spatial distribution of seedlings, and also modifying animal activity.

Since the organic material in each soil sample requires hand-sorting under the microscope, each sample takes from 1 to 4 hours for analysis. It was consequently possible to handle only a rather small number during the period covered by this report while laboratory techniques were also under development. At this stage in the work it was considered more useful to cover a wide range of sample types without effective replication than to analyze a more limited series of samples in replicate. Accordingly, no estimates of error are offered. This deficiency will be made good in future work.

Vertical distribution. A number of sets of samples were collected in proximity to the Curlew Valley validation sites, with a view to answering these questions. All these samples were taken to a depth of 10 cms. and were divided as follows:

Surface litter layer:

0 to 1 cm. 1 to 2 cms. 2 to 5 cms. and

5 to 10 cms.

A summary of the results for different depths, averaged over all samples, is given in Table 2.

	On Surface	Per cent 0-1 cm.	t of seeds 1-2 cm.	at different 2-5 cm.	depths 5-10 cm.	Total (*) per sq dm
Agropyron cristatum	65.2	15.0	16.1	3.7	0.0	10.8
Atriplex confertifolia	13.8	21.1	33.6	17.7	13.8	100.0
Bromus tectorum	54.5	17.4	13.1	2.5	12.5	20.0
Camelina microcarpa	2.3	15.5	65.1	10.9	6.2	4.8
Collinsia parviflora	38.3	21.7	22.0	7.4	10.6	39.7
Halogeton glomeratus	32.0	37.4	10.9	9.9	9.8	13.6
Phlox gracilis	21.9	47.5	13.3	7.3	10.0	8.2
Poa nevadensis	54.1	10.7	0.0	35.2	0.0	1.9
Polygonum douglasii	2.6	10.7	21.4	38.4	26.9	21.2
Sitanion hystrix	56.1	17.4	5.0	8.5	13.0	25.4

Table 2. Distribution of seeds by depth.

* Mean for the samples in which this species is present.

2.2.1.2.-6

It will be noted that the different species are by no means uniform in their depth distribution. Some, like all the grasses and *Collinsia parviflora*, have most of their seeds on the soil surface, while for others, like *Polygonum douglasii*, more than half the seed reserve lies below 2 cm.

These differences doubtless depend partly on seed morphology, partly on animal activity, partly (since the seeds found may represent the residues of several years' crops) on differing germination and dormancy behavior. Evidence so far does not suggest that depth distribution for any particular species differs from sample to sample; but this is a possibility which will constantly be kept in mind, and for which further tests will be performed from time to time.

In converting the seed content per unit weight of soil to an area basis, which is necessary in order to make the figures for different depths comparable, the assumption has been made that the samples as collected represent the volume of soil vertically below the area outlined on the surface, between the depths specified. The method of collection ----by spade and trowel --- may to some extent falsify this assumption, and steps are being taken to obtain bulk destiny figures for the soils at different levels, and to use these for improving the conversion. The possibility of using fixed-area collection devices will also be explored, but on some of the soils exact horizontal division of samples would be difficult, and bulk density data may provide a more reliable approach. The surface litter has in any case been sampled by area, so no question of conversion arises.

Horizontal distribution. Since the main features modifying seed distribution are likely to be associated with the presence of perennial elements of the vegetation, samples were taken at different distances from the center of shrubs and of grass tussocks of different species (Table 3).

	Distance	(cmi) of <u>5</u>	Sample Center	from base of <u>25</u>	plant <u>35</u>	<u>45</u>
Agropyron cristatum	Plant A	102.5	84.6	-	12	-
	Plant B	57.9	105.5			-
	Plant C	5.0	6.9	9.8		-
Artemisia tridentata	Plant A	-	223.9	135.2	89.2	59.3
	Plant B		59.3	86.7	113.9	149.5
Atriplex confertifolia		-	231.1	89.4	263.9	120.1

Table 3. Estimated total seeds per sq. dm., to depth of 1 dm.

From these results no clear picture emerges of the radial distribution of seeds beneath the canopy of an individual shrub or tussock. These studies will be pursued further, but, in the meantime, there seems little ground for sample stratification within a canopy area.

Table 4 indicates the mean population of seeds beneath the canopy of certain perennial species in Curlew Valley. It is clear that bare ground between the shrubs and grass tussocks has a sparse population of seeds, but that the other categories (with the exception of the unreplicated tussock of Agropyron cristatum at the northern sites) do not differ substantially or consistently in their seed population.

Canopy Species	North	ern Sites	Southern Sites			
	Number of Canopies	Mean seeds per sq. dm.	Number of Canopies	Mean seeds per sq. dm.		
Agropyron cristatum	1	7.2	2	87.6		
Artemisia tridentata	3	78.2	2	89.5		
Atriplex confertifolia	i de en la compañía de la compañía d	a shift of the second	3	112.7		
Chrysothamnus nauseosus	2	61.8		and the second		
Poa secunda	2	80.2	-			
Sitanion hystrix	-		3	51.1		
None (Bare ground)	3	6.5	2	0.0		

Table 4. Mean seed population under different canopies.

FINDINGS

Speed Inventories in Curlew Valley.

In all, 163 soil samples from Curlew Valley have been examined — 4 or 5 horizons at each of 19 sites near the northern plots; 18 sites near the southern plots. The total numbers of seeds of different species actually counted in the sub-samples analysed are given in Table 5.

Table 5.	Total	numbers	of	seeds	counted	in	the	soil	sub-samples.

	Northern Sites	Southern Sites
Agropyron cristatum	7	52
Atriplex confertifolia	0	620
Bromus tectorum	240	6
Camelina microcarpa	5	9
Collinsia parviflora	207	0
Gillia sp.	1	0
Halogeton glomeratus	5	78
Phlox gracilis	57	0
Poa nevadensis	4	2
Polygonum douglasii	92	0
Sitanion hystrix	0	91
Sitanion jubatum	2	0
Unidentified A	8	2
В	2	0
С	1	0 ·
D	5	0
Е	11	0

There are some noteworthy absentees from this list, and some of those present are poorly represented. No seeds of *Artemisia tridentata* were found, for instance, though a number of empty achenes occurred in the samples from the northern sites; none of *Chrysothamnus* were found, either. No seeds of *Descurainea richardsonii* were in the samples, despite its abundance — admittedly patchy. The record of seeds from some of the grasses do not seem to match their abundance in the vegetation. These discrepancies may in some cases reflect a failure to set seed, in others efficient seed-harvesting activity by animals.

It is clear that the number and sizes of samples taken is inadequate to do more than provide a broad picture of the seed reserves in the validation sites in Curlew Valley. However, taking the northern and southern sites separately, stratifying by shrub canopy, tussock-grass canopy, and bare ground, and using the proportions of area falling into these categories as revealed in the validation study, very tentative estimates may be formed as tabulated in Table 6.

Table 6. E	stimates of	seed reserves	per sq. m.	in the	Curlew	Valley va	lidation areas
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Species		North	ern Sit	es	Southern Sites				
	No.	Native g. dry weight	Re- No.	seeded g. dry weight	No.	ative g. dry weight	Re- No.	seeded g. dry weight	
Agropyron cristatum		0	140	.25		1	760	1.35	
Atriplex confertifolia				-	4800	8.74	2600	4.73	
Bromus tectorum	-	-	930	2.05	20	.04	40	.09	
Camelina microcarpa	30	.01	12.1	indexes a	140	.05	70	.02	
Collinsia parviflora	760	.97		-		-	-		
Halogeton glomeratus	160	.04	1.1-17	1 1 e 1	50	.01	1000	.22	
Phlox gracilis	720	.85	-	-		-	-	-	
Poa nevadensis	10	.01	40	.02	10	.01	-	-	
Polygonum douglasii	1500	.76	90	.05		-	-	-	
Sitanion hystrix	-	-	-	-	820	2.06	650	1.63	

DISCUSSION AND EXPECTATIONS

The results reported here refer only to the Great Basin sites in Curlew Valley; it is necessary to obtain at least preliminary estimates, comparable with those in Table 6, for the other desert types. An extensive series of samples was taken from Rock Valley in December 1971 and is now being analysed. A small set of samples was collected from the Silver Bell site early in 1971, and these still await analysis. It is intended to collect sets of samples from the Silver Bell and Jornada sites comparable to those from Curlew Valley and Rock Valley during the coming months. As indicated above, some of the conclusions reached regarding horizontal and vertical distribution in Curlew Valley are regarded only as tentative, and attempts will be made to provide a sounder basis for them. The present intention, for the inventory, is to stratify only by the three main cover types, not to divide by depth, to bulk a number of replicate samples in each category, and then to sub-sample the bulk for analysis. This should enable considerably better estimates to be obtained for a given expenditure of time and effort in field and laboratory. Information about vertical distribution of different species will clearly be needed, but is better obtained by *ad hoe* studies.

Apart from the total seed populations, information is also needed on their viability and germination performance. Some germination tests on the Curlew Valley seeds have already been performed, and records are also being kept of seedlings actually emerging from the soil *in situ*. This work on germination, with tests (by the tetrazolium method) of seed vitality, will be greatly extended later.

ACKNOWLEDGMENTS

Some of the early work on seed separation described in this progress report was performed by Miss Jana Lhotova. For seed samples, we are indebted to Dr. Janice Beatley of the University of California, Los Angeles; to Mr. Alvin Bleak of the Agricultural Research Service; and to Professor Arthur Holmgren, Director of the Intermountain Herbarium. The last two were also extremely helpful in identification, as was Mr. Richard Wilson, of the U.S.D.A.

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