THE STANDARDIZATION OF AN APPARATUS FOR THE MIXING OF SOIL SAMPLES FOR NEMATODE EXTRACTIONS

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Accepted: February 1978

ABSTRACT

Quantitative determination of nematode populations in soils frequently necessitates the mixing of representative soil samples to form a homogeneous, compound sample from which the nematodes are extracted. A mixing apparatus was developed and standardized with the aid of a spectrophotometric technique by which the dispersion of a dye through the soil during mixing could be determined. It was found that the mixer is most suitable for use on sandy loam soils. Optimal mixing is obtained at a speed of 15 rpm for 2 minutes. Under these conditions mechanical damage to nematodes during mixing is minimal.

INTRODUCTION

Little information is available concerning standardized sample mixers used in the mixing of soil samples. Cobb (1918) recommended the processing of a mixture to a muddy paste, and then using a hundredth portion as a representative sample. Martin (1962), Ayala *et al.* (1963), Griffin & Darling (1964) and Brodie *et al.* (1969) obtained representative samples by mixing the soil by hand. Barker *et al.* (1968) improved on this by passing the hand-mixed samples four times through a sample divider. Malek (1969) also mixed the samples by hand but, in addition, spread them out on a flat container in order to obtain small random portions which, collectively, formed the representative sample.

Since soil-mixing by hand is difficult to standardize, it was decided to construct a mixer which conformed to the following:

1. It had to be able to mix thoroughly small or large numbers of soil samples. A convenient range is 25 to 175 samples per series with an average size of 40 cm^3 per sample;
2. The mixer had to be able to mix soil volumes with differing moisture contents;
3. The damage to nematodes during mixing had to be minimal.

After testing several unsatisfactory methods for determining the efficiency of the mixer, a spectrophotometric technique was chosen by which the dispersion of a dye through the soil samples during mixing was determined. By using a dye which is adsorbed onto soil particle surfaces it becomes possible to examine the distribution of such labelled particles through an unlabelled sample. The revolution speed and mixing time of the mixer for optimum mixing, as well as its ability to mix soils of differing moisture content and volume were determined.

MATERIAL AND METHODS

The mixer

A steel drum, 45 cm long and 25 cm in diameter having a volume of 21 200 cm^3 was used (Figure 1). The drum, which rotates on a diagonal axis of 2 cm diameter, is provided with a steel sliding door which can be tightly shut. The axis and internal surfaces are smoothly finished without fins or other structures. The drum is mounted on a steel frame. An electric motor provides, through a series of pulleys and belts, the power for a range of drum rotation speeds.

Standardization method

A series of 48 sandy loam samples with a volume of 2 000 cm^3 and moisture content of 7,0 percent was placed in the mixer. Of this mixture 40 cm^3 was removed and well mixed with 20 cm^3 of waxolene red acetone solution. After evaporation of acetone the sample was replaced in the mixer. The mixer was allowed to rotate at a constant speed for a specific time after which the soil was spread out in a flat container from which at least three subsamples of 300g (250 cm^3) were removed. In each of these subsamples the dye was extracted with 200 cm^3 acetone by shaking for 60 seconds. The procedure was repeated twice more with two similar series of 48 samples each for each test speed/time combination. The concentration of waxolene dye in the three replicates of each series was spectrophotometrically determined using a Zeiss PMQ11 spectrophotometer at 515 nanometres. Control solutions were obtained by repeating the mixing experiment once without the addition of waxolene red.

Revolution speed

The speed at which the mixing drum should be rotated for optimum mixing was determined by mixing three series of soil samples separately for 30 revolutions at speeds of 0,21; 0,25; and 0,50 revolutions per second respectively. The basic procedure described under standardization method was followed for each speed.

Revolution numbers

To determine the number of drum revolutions needed for optimum mixing three series of sandy loam soil samples were mixed separately for 30; 60 and 100 revolutions using the optimum speed of 0,25 revolutions per second as previously determined.

Mixing abilities of two soil types

Additional experiments were performed to compare a light sandy loam soil with a heavy clay soil.

Thirty different combinations of soil type (i.e. sandy loam and clay), soil moisture content and volume were tested in the mixer. Each mixing lasted for 120 seconds at a speed of 0,25 revolutions per second. Mixing capabilities with these combinations were determined by the same procedures as before.

FIGURE 1

The apparatus used for the mixing of soil samples.

Condition of nematodes after mixing

A complementary series of experiments to determine the condition of the nematodes after mixing was performed as follows: 4000 cm^3 sandy loam (i.e. two series of 48 samples) containing nematodes was mixed thoroughly by hand. Half of this (2000 cm^3) was placed in the mixer and mixed at the optimum speed and time as previously determined, i.e. 0,25 revolutions per second for 120 seconds. After mixing, six soil samples of 300 g each were removed and separatey extracted by the Oostenbrink flotation technique III (Oostenbrink 1960). The cotton filter part of the process was omitted since it would have held back the dead and damaged nematodes. The nematode suspension was placed in six containers and their extent of injury assessed using a stereomicroscope. For controls six 300 g soil samples were taken from the remaining handmixed soil and similarly extracted. The suspensions were placed in six containers and the condition of the nematodes assessed. The same procedure was followed with clay soil.

TABLE 1

The coefficients of variation and arc sin values of spectrophotometer readings for different revolution speeds.

CV = Coefficient of variation = S/x̄ where S, the standard deviation, is expressed as a fraction of the mean, x̄ (Snedecor & Cochran 1976).

Number of revolutions	Time (seconds)	Revolution speed (r.p.s.)	Replication number	CV	Arc sin
30	140	0,21	1	5,57	13,65
			2	5,93	14,09
			3	6,78	15,09
30	120	0,25	1	2,42	8,95
			2	3,25	10,42
			3	3,66	11,03
30	60	0,50	1	9,40	17,85
			2	6,70	15,00
			3	5,50	13,56

RESULTS AND DISCUSSION

Revolution speed

Since the minimum number of revolutions necessary for satisfactory mixing was still unknown, it was assumed that the sandy loam was sufficiently mixed after the mixer had made 30 revolutions. Table 1 gives the coefficients of variation and the arc sins of the square roots of the coefficients of variation of the spectrophotometer readings performed in triplicate at each speed.

An analysis of variance, performed on the arc sin values, showed a significant difference at the 5% level between the coefficients of variation for the three different revolution speeds. (One way analysis of variance; $df_1 2$; $df_2 6$; $F = 10{,}94$.) The optimal speed for mixing soil samples for 30 revolutions is therefore concluded to be 0,25 revolutions per second.

The above and subsequent analyses of variance were done on the arc sin values to better satisfy the assumptions of normality and homogeneity of variance required for the tests in the analyses of variance.

Revolution numbers

The determination of a suitable revolution speed rested on the supposition that 30 revolutions produced a satisfactory mixing. Since it was determined that the optimum speed was 0,25 revolutions per second, the next step was to determine whether a greater number of revolutions at this optimum speed would produce better mixing.

Table 2 shows the coefficients of variation and the arc sins of the square roots of the coefficients of variation of the spectrophotometer readings for the three repeats for the different numbers of revolutions.

An analysis of variance comparing the coefficients of variation of the three different numbers of revolutions could not be performed since the variance of the three readings at 60 revolutions was found to be significantly higher than those for the two other numbers of revolutions. A t-test comparing the coefficients of variation at 30 and 100 revolutions showed no significant difference at the 5% level. The shortest mixing time, namely 120 seconds (30 revolutions) is therefore preferable since there is less chance of damaging the nematodes.

Mixing abilities of two soil types

The mixing tests so far carried out used sandy loam samples with a moisture content of 7,0 percent and a volume of 2 000 cm^3. The questions then arising were what effect would drier or damper soil and different soil volumes have on the mixing process and how would the mixing ability of the drum compare using two different soil types? To answer these questions experiments were performed using a sandy loam (light soil) and a clay (heavy soil). Table 3 summarizes the particle size distribution of the two samples.

Soil type, soil moisture and soil volume combinations

Table 4 gives the coefficients of variation and arc sins of the square roots of the coefficients of variation of the absorbance values.

TABLE 2

The coefficients of variation and arc sin values of spectrophotometer readings for different revolutions at a constant speed.

CV = Coefficient of variation

Number of revolutions	Time (seconds)	Replication number	CV	Arc sin
30	120	1	2,42	8,95
		2	3,27	10,42
		3	3,66	11,03
60	240	1	6,33	14,57
		2	10,12	18,55
		3	2,80	9,63
100	400	1	2,11	8,35
		2	1,52	7,08
		3	2,55	9,19

TABLE 3

Mechanical analysis of loam and clay soil.

Particle size (mm)	Percentage distribution of soil particles	
	Sandy loam	Clay
2,0 –0,5	24,1	11,2
0,5 –0,2	40,0	9,9
0,2 –0,02	17,6	18,5
0,02–0,002	2,2	11,2
0,002	13,7	50,1

TABLE 4

The coefficients of variation and arc sins of the square roots of the coefficients of variation of the spectrophotometric readings.

T: soil types; 1 = clay; 2 = sandy loam
V: volume, 1 = 1 000 cm^3 (24 samples); 2 = 2 000 cm^3 (48 samples); 3 = 4 000 cm^3 (96 samples); 4 = 6 000 cm^3 (144 samples); 5 = 7 000 cm^3 (168 samples)
M: moisture content; 1 = < 4% (dry soil); 2 = 5–9% (damp soil); 3 = < 10% (wet soil).

REPLICATE / TREATMENT			1		2		3		Sum of treatments (arc sin)
T	V	M	CV	Arc sin	CV	Arc sin	CV	Arc sin	
1	1	1	10,001	18,43	19,389	26.13	6,443	14,65	59,21
1	1	2	18,060	25,18	7,411	15,79	12,408	20,62	61,59
1	1	3	8,150	16,64	7,984	16,43	1,947	8,13	41,20
1	2	1	41,607	40,16	41,131	39,87	26,982	31,31	111,34
1	2	2	8,072	16,54	3,371	10,47	5,883	14,06	41,07
1	2	3	35,892	36,81	49,527	44,71	22,742	28,45	109,97
1	3	1	6,454	14,77	3,346	10,47	5,293	13,31	38,55
1	3	2	7,149	15,45	10,586	19,00	6,041	14,18	48,63
1	3	3	20,583	26,99	28,740	32,39	2,633	9,28	68,66
1	4	1	11,541	19,82	4,662	12,52	6,740	15,00	47,34
1	4	2	8,315	16,74	8,106	16,54	6,740	15,00	48,28
1	4	3	16,800	24,20	45,403	42,36	27,470	31,63	98,19
1	5	1	32,224	34,57	25,492	30,33	18,331	25,33	90,23
1	5	2	17,291	24,58	6,264	14,54	15,824	23,42	62,54
1	5	3	21,973	27,97	25,225	30,13	44,831	42,02	100,12
2	1	1	10,655	19,09	11,281	19,64	14,989	22,79	61,52
2	1	2	26,502	30,98	17,876	25,03	12,001	20,27	76,28
2	1	3	10,141	18,53	18,573	25,55	4,486	12,25	56,33
2	2	1	4,360	12,11	3,031	9,97	4,192	11,83	33,91
2	2	2	1,335	6,55	3,270	10,47	6,724	15,00	32,02
2	2	3	4,726	12,52	5,662	13,81	3,697	11,09	37,42
2	3	1	5,718	13,81	9,428	17,85	3,450	10,78	42,44
2	3	2	6,456	14,77	11,655	20,00	5,237	13,18	47.95
2	3	3	6,524	14,77	4,969	12,92	9,083	17,56	45,25
2	4	1	1,895	7,92	22,358	28,25	11,025	19,37	55,54
2	4	2	2,085	8,33	22,358	28,25	11,025	19,37	55,74
2	4	3	8,725	17,15	9,093	17,56	7,840	16,22	50,93
2	5	1	15,288	23,03	16,116	23,66	10,790	19,19	65,88
2	5	2	17,693	24,88	25,258	30,20	9,085	17,56	72,64
2	5	3	19,249	25,99	49,340	44,60	42,567	40,74	111,33
TOTAL TREATMENTS			—	609,28	—	689,23	—	573,59	1 872,10

An analysis of variance on the arc sin values showed that there were highly significant differences in the mixing abilities of sandy loam and clay soil. In general sandy loam mixes better than clay soil. There was also a significant difference between soil volumes, 4 000 cm^3 mixing, on average, the best and 7 000 cm^3 the poorest. One thousand cm^3 and 6 000 cm^3 do not differ much with respect to their mixing properties; however, the interaction between soil type and volume is very significant. This is most clearly shown for 2 000 cm^3 which is the optimum for sandy loam. The mixing of clay is very irregular because 1 000 cm^3 and 4 000 cm^3 are the two best volumes and 2 000 cm^3 the poorest. Sandy loam mixes more constantly for soil volumes between 2 000 cm^3 and 6 000 cm^3 which in turn mix better than the two outer limits of the series namely 1 000 cm^3 and 7 000 cm^3.

If the 1 000 cm^3 volume is disregarded, there is a linear tendency for sandy loam to be mixed progressively more poorly with increasing volume.

Soil moisture has a very significant effect on mixing with a moisture content of 5 to 9 per cent the optimum, followed by a content of 1 to 4 per cent. There is also a significant interaction between soil type and moisture content. For moisture contents of 1 to 4 per cent and 5 to 9 per cent, sandy loam mixes the best. When mixing clay soil a moisture content of 5 to 9 per cent is recommended.

The soil volume and moisture content interaction is also significant. Soil with a volume of 2 000 cm^3 and a moisture content of 5 to 9 percent is the best combination for efficient mixing. Next is 4 000 cm^3 and a moisture content of less than 4 percent, although soil with a volume of 4 000 cm^3 and a moisture content of 5 to 9 percent is also a good combination for mixing. Excluding the 1 000 cm^3 volume, wet soil with a moisture content of over 10 percent mixes most poorly at all volumes.

It is clear that in the case of sandy loam and to a lesser extent clay, a volume of 2 000 cm^3 and a moisture content of 5 to 9 percent is the most favourable combination for use in the mixer. This mixer is thus efficient in mixing large numbers of samples, especially damp sandy loam.

Condition of the nematodes after mixing

The samples processed through the mixer showed only 1,5 percent dead and damaged nematodes against 1,0 percent in the controls. The percentage of nematodes damaged by the mixing process is thus so small as to be negligible.

ACKNOWLEDGEMENTS

Sincere thanks to Mr G Goosen, Mrs Wanda Aucamp and Miss L Beckley for services rendered and Drs A G de Wet and D Baird for statistical advice. The University of Port Elizabeth is thanked for financial aid in publication.

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