Plant and Soil 121, 211–215 (1990).
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PLSO 8244

An instrumental modification of Gerdemann and Nicolson's method for extracting VAM fungal spores from soil samples

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Received 16 May 1989. Revised September 1989

Key words: VAM fungal spore extraction, water stream sieving

Abstract

An easily constructed apparatus for extraction of VAM fungal spores from soil samples by a modification of Gerdemann and Nicolson's wet sieving and decanting method is described. For the soils employed in this study, it proved considerably more effective and more precise than either the original wet sieving and decanting method or differential water/sucrose centrifugation. The apparatus gave results that were less subject to interference from extraneous particles than the wet sieving and decanting method. The effect of prior soil dispersion is also reported.

Introduction

Since Gerdemann and Nicolson (1963) published their wet sieving and decanting method, most of the effective techniques for extracting vesicular-arbuscular mycorrhizal (VAM) fungal spores have either, like the Gerdeman-Nicolson method, involved flotation in water (Sutton and Barron, 1972), or have been based on differential sedimentation in a gelatine column (Mosse and Jones, 1968), differential water-sucrose centrifugation (Allen *et al.*, 1979), sucrose gradient centrifugation (Ianson and Allen, 1986), or air-stream fractionation (Tommerup, 1982). Being a standard step, wet sieving and decanting is a convenient starting point for routine spore extractions from soil samples. However, this method has losses of spores attached to soil aggregates or debris (Ianson and Allen, 1986), and is also subject to considerable operative-induced variations. These are due, among other factors, to variation in the intensity with which the soil is shaken before sieving, and the force with which the soil suspension is poured onto the sieve. In order to reduce the variability and improve the efficiency of the wet sieving and decanting method, we have devised an easily constructed apparatus in which what is essentially this procedure is carried out using a continuous stream of water. We call the improved technique Water Stream Sieving (WSS).

Material and methods

Soil samples were taken from sites on Mount Pedroso and near Monforte (N. W. Spain); their relevant characteristics are listed in Table 1. Fresh soil samples were homogenized, air-dried, and sifted through a 2-mm sieve. On drying, the high-clay Monforte soil formed large, hard aggregates, making it necessary to steep samples in water for 3 hours before counting by any of the methods employed. After that, 5-fold wet sieving and decanting was carried out as normal using 500, 250, 125 and 65-μm sieves.

The Pedroso soil contained spores of *Acaulospora laevis* Gerdemann et Trappe, and the Monforte soil contained spores of *Glomus macrocarpum* Tul. et Tul. Spores of both species were collected on the 125 μm sieve.

Table 1. Characteristics of the soils used

Soil	pH		O.M. (%)	Texture			Aggregate stability[a]	Presence of carbonates[b]
	H_2O	KCl		Clay	Loam	Sand		
Pedroso	5.36	3.85	12.05	11	42	47	8	–
Monforte	7.53	7.02	7.30	53	31	16	4	+

[a] Stability class according to Emerson's test (1967).
[b] Detected from effervescence on addition of 0.1 *M* HCl.

Description and use of the WSS apparatus

The WSS apparatus (Fig. 1a) centres on a 1-litre plastic separation bottle (4) through whose neck (5) a 10 g soil sample is introduced. A constant stream of water flows into the base of the bottle through two 6 mm internal diameter inlet tubes (3) at a flow rate indicated (proportionally) by the height to which water rises in a vertical column (2) connected to the tube (1) feeding these inlets, which are arranged so as to give rise to a spirally ascending water current inside the bottle (Fig. 1b). The outlet is a 10 mm internal diameter tube leading from the upper region of the bottle to a column of sieves with pore sizes ranging in descending order from 500 to 63 μm. In order to prevent stoppage of the outlet by vegetable debris, the soil sample to be analysed is fed into the separating bottle under gravity (through funnel 9) from a plastic flotation/adhesion bottle (8) where the sample has been decanted for about 40 s; under these circumstances, unwanted vegetable debris tends to adhere to the walls of bottle 8.

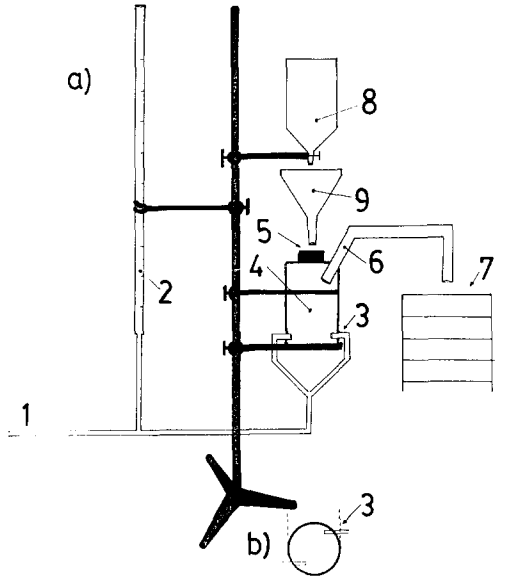

Fig. 1. **a)** The WSS apparatus, and **b)** a horizontal section through the base of the separating bottle: **1** water feed; **2** flow rate indicator column; **3** inlets; **4** shaking bottle; **5** neck; **6** outlet; **7** sieve column; **8** flotation/adhesion bottle; **9** funnel for introducing of sample.

Before use, the apparatus was calibrated by measuring the numbers of spores collected on the 125 μm sieve using different water flow rates and separation times.

Evaluation of WSS

The number of *A. laevis* and *G. macrocarpum* spores collected by WSS under optimal flow rate and separation time conditions was compared with the results of: 1) 5-fold wet sieving and decanting (Gerdemann and Nicolson, 1963), henceforth WSD; 2) differential centrifugation in water/sucrose as described by Allen *et al.* (1979), henceforth DC; 3) wet sieving and decanting followed by centrifugation of the fraction retained on the 125-μm sieve (WSD + DC); and 4) WSS (under optimal conditions) followed by differential centrifugation of the fraction retained on the 125-μm sieve (WSS + DC). Spores were counted in the fractions finally obtained by observation on a Doncaster (1962) disc under a dissection microscope with a magnification of 20–30×. The total dry weight of material retained in the 500–63 μm sieve column after processing 10 g soil samples by the WSS and WSD methods was also determined.

To determine the effect of prior soil dispersion on spore recovery by the various methods, we stirred the 10 g samples for 15 min with 100 mL of a 0.05% (w/v) aqueous solution of Calgon®; a screw stirrer was used because magnetic stirring ruptured numerous spores.

The data obtained were subjected to analysis of variance after passing the Kolmogorov-Smirnov and/or χ^2 normality tests. Least significant differences were estimated using Tukey's W test.

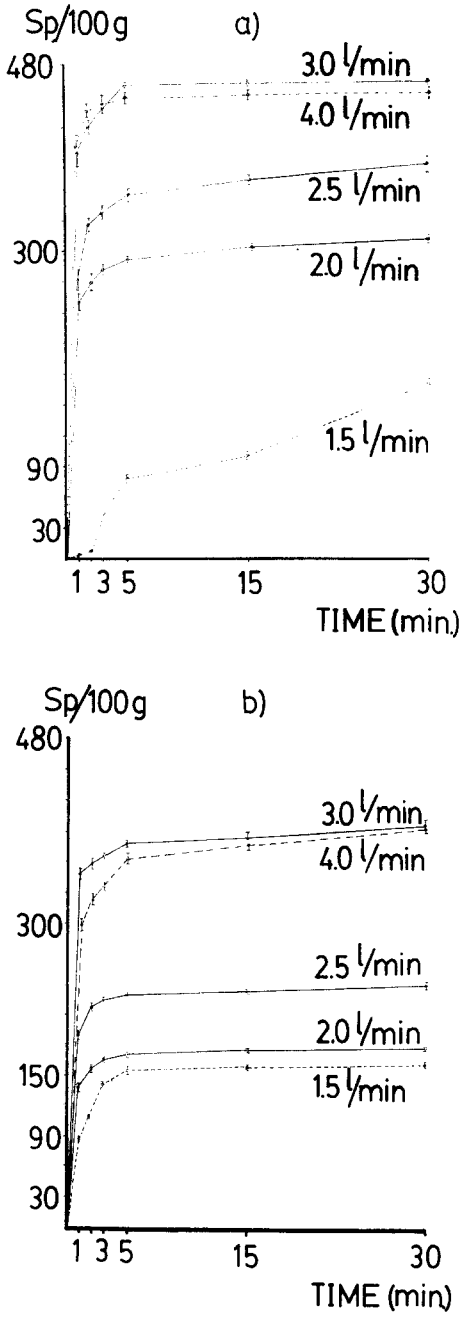

Fig. 2. Number of spores per 100 g of soil collected at various times after commencement of WSS, for various flow rates: **a)** Pedroso soil; **b)** Monforte soil.

Results

For both soils, the number of spores collected by WSS increased with increasing separation time, and also with flow rate for rates of up to 3 L min^{-1} (Fig. 2). Counts made at 4 L min^{-1} were very similar to the 3 L min^{-1} figures, and for the Monforte soil were significantly lower for the first 3 min. All the collection-time curves except the 1.5 L min^{-1} curve for the Pedroso soil tend to level off after 5 min. The 1.5 L min^{-1} Pedroso curve is roughly sigmoid, with less than ten spores per 100 g of soil being collected in the first 2 min, as against 90 for the Monforte soil.

WSS was more effective than WSD, DC and the different combinations of these techniques, showing the least coefficients of variation (Table 2). Prior soil dispersion with Calgon® increased collection by all five methods, especially for the Monforte soil. WSS + DC was more effective than WSD + DC.

The quantity of undesired material collected on the sieves was significantly less for WSS than for WSD, and significantly reduced by dispersion with Calgon® whichever method was used, especially for the Monforte soil (Fig. 3).

Discussion

The effectiveness of WSS depended on both the flow rate and the separation time employed. Consideration of the effect of the former (Fig. 2) suggests that though weak streams are sufficient to float free spores or sporocarps, stronger streams are necessary to break up soil aggregates and release trapped spores or spores adhering to vegetable matter. This explanation is also supported by the difference in behaviour between the two soils: the class 4 aggregates of the high-clay Monforte soil disintegrated at once on exposure to the lowest flow rate, 1.5 L min^{-1}, while the class 8 Pedroso aggregates required longer and/or stronger treatment. The slight fall in spore count that occurred on increasing the flow rate from 3 to 4 L min^{-1} is attributed to the spores being retained in the separation bottle by turbulence. Taken together, the results of Fig. 2 indicate that the optimal operating conditions for the apparatus are a flow rate of 3 L min^{-1} and a separation time of 5 min.

Prior dispersion of the soil sample with Calgon® considerably improved spore collection rates, particularly for the Monforte clay soil. The fact that the samples of this soil were previously steeped in water for 3 hours suggests that the effect of the

Table 2. Numbers of spores extracted (Sp; mean ± standard error) and percentage coefficients of variation (CV) for the various extraction techniques

Techniques	Pedroso				Monforte			
	N		D		N		D	
	Sp[a]	CV	Sp	CV	Sp	CV	Sp	CV
WSD	218 ± 12a	12	265 ± 9c	8	241 ± 19bc	18	408 ± 13d	7
DC	255 ± 20ab	17	277 ± 15c	12	192 ± 9a	10	213 ± 8ab	9
WSS	461 ± 4e	2	496 ± 7e	3	374 ± 6d	4	555 ± 7e	3
WSD + DC	223 ± 16ab	16	260 ± 15bc	13	203 ± 19ab	19	232 ± 23abc	22
WSS + DC	362 ± 14d	9	388 ± 16d	9	278 ± 20c	16	340 ± 15d	10

N = Soil not treated with dispersant.
D = Dispersant-treated soil.
DC = Differential water/sucrose centrifugation (Allen *et al.*, 1979).
WSD = Wet sieving and decanting (Gerdemann and Nicolson, 1963).
WSS = Water Stream Sieving.
[a] For each soil, labelling with different letters indicates significant difference at the $p < 0.01$ level.

dispersant in this case was not to increase the number of spores borne to the sieves, but rather to increase the detection rate by freeing them of clay and other particles adhering to them.

With or without dispersant, WSS proved better than the other methods, both in detecting a greater number of spores and, perhaps more importantly, in giving a smaller coefficient of variation. Regardless of which soil was analysed, it also approximately halved the quantity of non-spore soil particles collected by the sieves (largely due to the retention of vegetable matter in the flotation/adhesion bottle), thus greatly facilitating spore counting on the Doncaster disc.

The fact that DC and WSD afforded very similar spore counts for the loamy sand Pedroso soil is in keeping with Ianson and Allen's (1986) findings for sandy soils and may probably be attributed to the large number of free spores. Our results are nevertheless at variance with theirs in that we found WSD to be more effective than DC for the Monforte soil, whereas Ianson and Allen reported DC to be more effective for clay soils. On the whole, the results suggest that the efficacy of any method depends more on the nature and stability of soil aggregates than on soil texture.

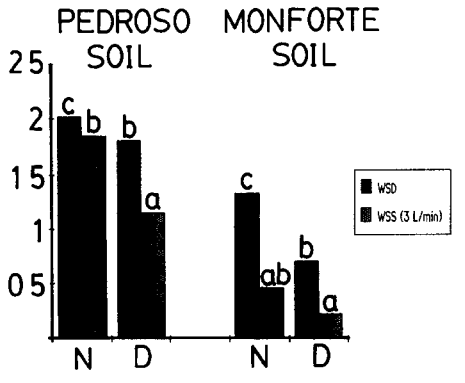

Fig. 3. Dry weight of residue collected on sieves by WSS and conventional wet sieving and decanting (WSD): **N** = untreated soil; **D** = dispersant-treated soil. For each soil, labelling with different letters indicates significant differences at the $p < 0.01$ level.

Combination of either WSD or WSS with DC reduced their effectivity. When McKenney and Lindsey (1987) applied DC to samples previously sieved through 38 μm, they found that the number of spores in the aqueous supernatant was similar to that in the subsequent sucrose supernatant. We obtained similar results, and attributed this to sifting having removed cementing material and causing a loss of spore retention.

In conclusion, spore extraction by WSS after dispersant treatment has the following advantages over other methods: 1) variability originated by the operator is virtually eliminated; 2) the quantity of undesired material collected on the sieves is greatly reduced; 3) more spores are extracted; and 4) the spores extracted are considerably cleaner.

References

Allen M F, Moore Jr T S and Christensen M 1979 Growth of vesicular-arbuscular mycorrhizal and nonmycorrhizal

Bouteloua gracilis in a defined medium. Mycologia 71, 666–669.

Doncaster C C 1962 A counting dish for nematodes. Nematologica 7, 334–337.

Emerson W W 1967 A classification of soil aggregates based on their coherence in water. Aust. J. Soil. Res. 5, 47–57.

Gerdemann J W and Nicolson T H 1963 Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 46, 235–244.

Ianson D C and Allen M F 1986 The effects of soil texture on extraction of vesicular-arbuscular mycorrhizal fungal spores from arid sites. Mycologia 78, 164–168.

McKenney M C and Lindsey D L 1987 Improved method for quantifying endomycorrhizal fungi spores from soil. Mycologia 79, 779–782.

Mosse B and Jones G W 1968 Separation of Endogone spores from organic soil debris by differential sedimentation on gelatin columns. Trans. Br. Mycol. Soc. 51, 604–608.

Ohms R E 1957 A flotation method for collecting spores of a phycomycetous mycorrhizal parasite from soil. Phytopathology 47, 751–752.

Sutton J C and Barron G L 1972 Population dynamics of Endogone spores in soil. Can. J. Bot. 50, 1909–1914.

Tommerup I C 1982 Airstream fractionation of vesicular-arbuscular mycorrhizal fungi: concentration and enumeration of propagules. Appl. Environ. Microbiol. 44, 533–539.