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Wet-sieving and Decanting Techniques for the Extraction of Spores of Vesicular-arbuscular Fungi

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I. Introduction

The extraction of small living structures from soil and other substrata originally presented a problem for nematologists. The methods they developed have been adapted to enable the separation of spores of Endogonaceous fungi, the structures of which were initially confused with cysts of *Heterodera*, a common pest of roots. Both spores and cysts can be separated by flotation methods (Triffitt, 1935). One such method was successfully developed by Gerdeman and Nicolson (1963) and this is now the most widely used procedure for the study of spores of Endogonaceous fungi in soil. The method is described here and a few modifications are proposed to improve its effectiveness.

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II. Description of the technique

A suspension of soil in water is passed through a series of metallic sieves arranged in decreasing order of mesh width. The method is illustrated in Fig. 1. The steps are as follows:

1. Suspend 250 ml of soil in 1 litre of water, or more if necessary.
2. Wait for heavier particles to settle.
3. Pass the suspension through a sieve with a 1 mm wide mesh, saving the filtrate.
4. Debris retained by this sieve must be re-suspended into the saved suspension, stirred again, or washed under a stream of water, saving the suspension.

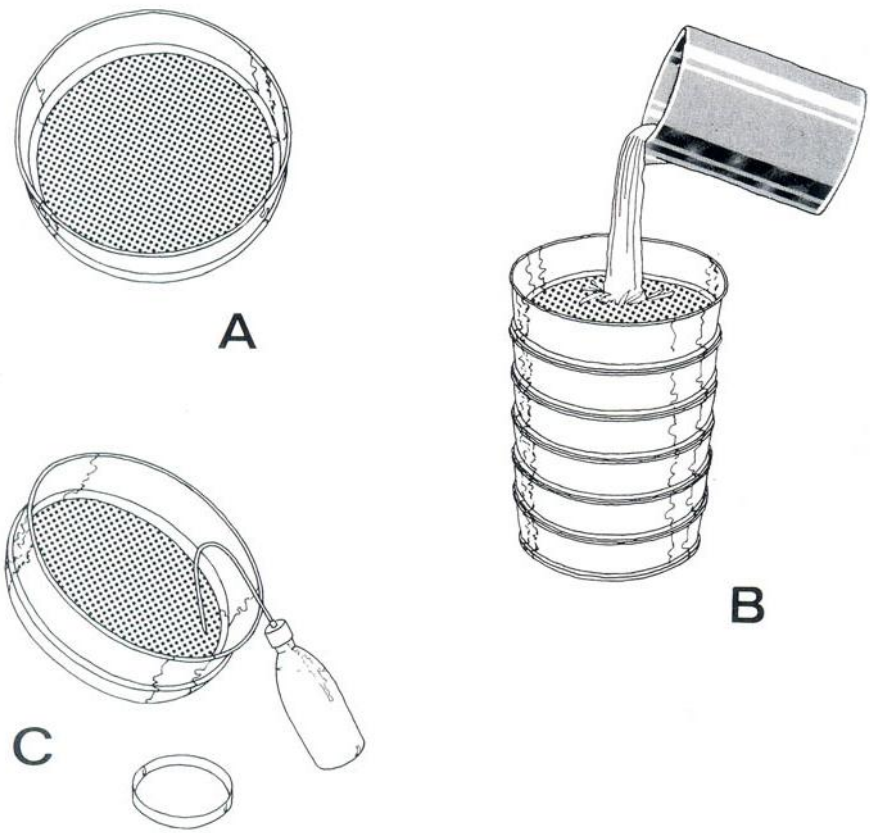

Fig. 1. Steps in the wet-sieving technique: (A) metallic sieve; (B) decanting through a sieve series; (C) removal of debris to a Petri dish.

5. Decant all suspensions through a sieve series ranging from 1 mm to 40 μm mesh.
6. With the aid of a jet of water directed at both sides of the sieve, the contents of each sieve is transferred to a Petri dish.
7. The shallow suspension can be observed with a stereo-microscope and spores and microsporocarps picked up with a flattened needle or a Pasteur pipette.
8. Small concave watch-glasses or slides can be used for further observations.

III. Modifications and improvements

Experience has led to the introduction of some modifications to the original method.

A. Preparation of the suspension

The following modifications to the method for preparing the suspension give better results:

(1) An optimal ratio of soil/water was found to be 1/10, that is 100 ml of soil in 1 litre of water.
(2) The suspension should be stirred with a magnetic stirrer or by hand using a rod. Magnetic stirring gives better results, using a water-driven stirrer that does not heat. Stirring time varies according to the nature of soil—usually 10 min. is sufficient.
(3) To avoid foam that could retain debris, add an anti-foam agent, for example Tween 80, 0.1–0.5%.
(4) If the soil is a clay that yields a suspension which blocks the sieve, precipitate the particles in 0.1 M sodium pyrophosphate (Fogel and Hunt, 1979).

The last two procedures are sometimes indispensable when using the nylon filters recommended by Pacioni and Rosa (1985). Particularly when working with either humic or clay soils, the efficiency of the filter can be altered by the presence of foam or suspended particles.

B. Filtering apparatus

The removal of spores from metallic sieves can be difficult and, particularly when working with soil where a predominance of single spores occurs, significant losses of spores can occur. An effective

modification of the filtering apparatus was introduced and satisfactorily tested by Pacioni and Rosa (1985).

Nylon filters (supplied by RI.MA., Via Gramsci 2/C, Bologna, Italy, fax. 39-51-553761) with standard meshes of the kind used in palynology or with cellular cultures for the separation of protoplasts were used. The method is illustrated in Fig. 2.

(1) A series of filters, decreasing in pore size (1 mm to 40 μm), are attached to each other by plastic tubing of 20 cm diameter, cut in lengths of 20 cm.
(2) Once filled, the filters are placed onto a transparent plexiglas grid-lined sheet and placed under a stereoscope.

The spores are supported by the filter meshes where they can be counted accurately and manipulated easily. Using a transmitted light stereoscope, the transparency of the supports (nylon filter and plexiglas sheet) allows easy observation of spores.

C. Technique for a large amount of soil

The techniques described above are suitable for small-scale studies, involving up to 100 g of soil at a time. If kilogram quantities of soil are to be examined, a drum, for example a petrol barrel without cover but with a lateral over-flow pipe, can be utilized. The filtering apparatus is made up of a metallic sieve with meshes of 1 mm for retaining larger debris and a nylon filter bag held by a fixing band. The size of the mesh is selected to be appropriate for the dimensions of the spores required. The method is illustrated in Fig. 3.

(1) Place a plastic pipe or insert a fixed water source at the bottom of the drum.
(2) Place the filtering apparatus to catch the water suspension.
(3) Fill the drum, suspend the soil, stir with a rod, treat as necessary and then turn on the water, leaving the suspension to flow through the filtering apparatus.

IV. Conclusion

Of the various methods proposed for the recovery of Endogonaceous spores from soil, the wet-sieving and decanting technique is the simplest and most effective. However, using the procedures described spores with a diameter less than 40 μm will be lost. Unfortunately these smaller spores often occur in compact soils, such as clays, that quickly block a

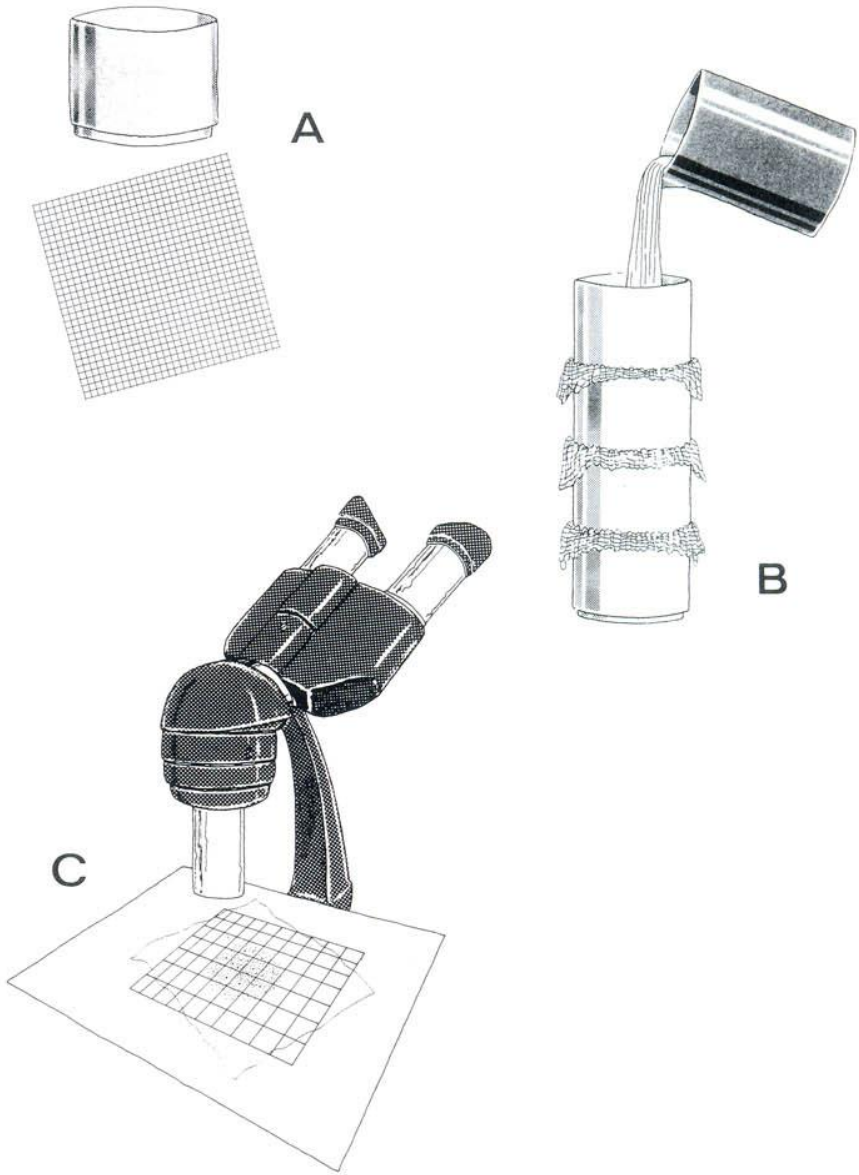

Fig. 2. Steps in the modified wet-sieving technique: (A) section of wide plastic tubing and nylon filter; (B) soil suspension being poured onto a column containing nylon filters; (C) filter on plexiglas grid-lined sheet under stereoscope.

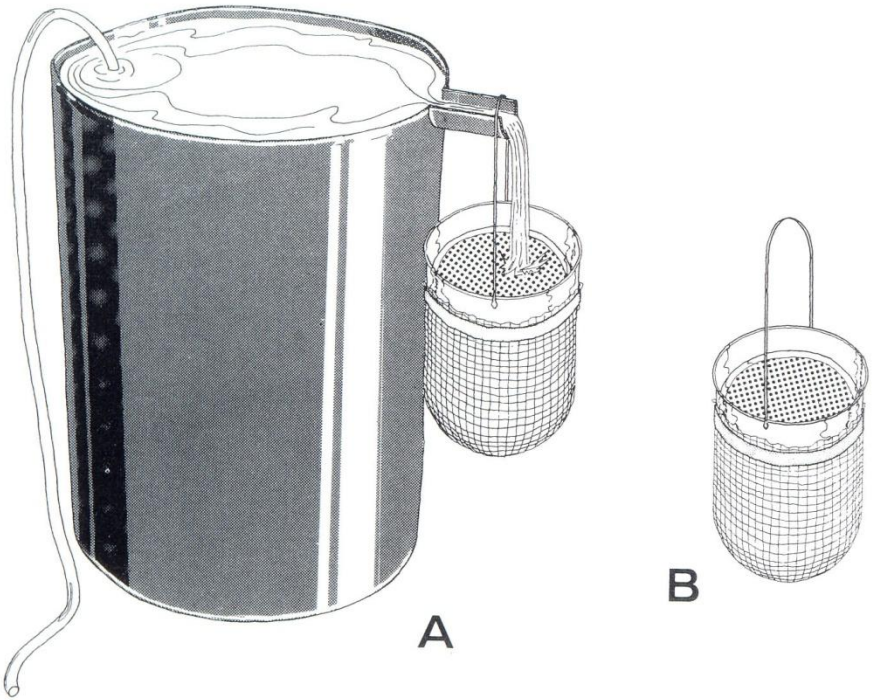

Fig. 3. Wet-sieving technique for a large amount of soil: (A) drum with soil suspension; (B) filtering apparatus.

cellulosic filter (Pacioni and Puppi, 1989). If the presence of these smaller spores is suspected, save the passed suspension, precipitate the clay particles still in suspension and pass again through a cellulosic filter under vacuum. Observe the filter directly under a stereoscope with indirect light.

References

Fogel, R. and Hunt, G. (1979). *Can. J. For. Res.* **9,** 245–256.
Gerdeman, J. W. and Nicolson, T. H. (1963). *Trans. Br. Mycol. Soc.* **46,** 235–244.
Pacioni, G. and Puppi, G. (1989). *Micol. Veg. Medit.* **3,** 133–142.
Pacioni, G. and Rosa, S. (1985). *Bull. Br. Mycol. Soc.* **19,** 66–68.
Triffitt, M. J. (1935). *J. Helminth.* **13,** 59–66.