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Harley J L & Waid J S. A method of studying active mycelia on living roots and other surfaces in the soil. *Trans. Brit. Mycol. Soc.* 38:104-18, 1955.
[Department of Botany, University of Oxford, England]

Serial washing of roots and other surfaces in measured volumes of sterile water allows one to check for the complete removal of spores. Applied to roots, mycorrhizas, or petioles, this method can be used to study patterns of mycelia on them and the differences between mycelial and spore populations. [The *SCI*® indicates that this paper has been cited in over 115 publications.]

J.L. Harley
The Orchard
Old Marston
Oxford OX3 0PQ
England

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It has always been a bugbear of work on root-infecting fungi that isolation of either organisms that cause disease or mycorrhizas is bedevilled by the presence of "contaminants" on the root surface. Through the 1930s much work on mycorrhizas was vitiated by such contaminants. Moreover, the separation or recognition of fungi present in soil habitats as mycelia and as spores was difficult. From 1938 to 1939 I attempted to clean the surfaces of ectomycorrhizas using a method[1] in which mycorrhizal roots were hung in a tube through which sterile water flowed while the whole apparatus was shaken mechanically. The method, although reasonably successful for isolation of mycorrhizal fungi, was tedious and the apparatus reminiscent of those of Heath Robinson. It was called "Alph" by laboratory wags after "Alph, the sacred river" that "ran through caverns measureless to man, down to a sunless sea," as described by the poet Coleridge.[2]

After 1939 there was a pause whilst war intervened; when it was over I decided that simpler methods could be used to achieve the same objective. At that time, the study of rhizosphere populations was proceeding strongly, especially in the US and Canada where R.L. Starkey and G. Lockhead were active. A graduate student of mine, J.L. Harper (now a well-known ecologist), took up the study. Its potential relevance to mycorrhiza became apparent to me when writing the review "Mycorrhiza and soil ecology."[3] It seemed obvious that serial washing of roots in measured volumes of water could indicate not only the extent to which the surfaces become clean, but also the numbers and kinds of fungi present as spores and the kinds of mycelia present and their distribution on the washed surfaces. This indeed proved to be so, indicating that the species present as spores were not necessarily the same as those present on the surfaces as mycelia.

The work was done with the help of an excellent ex-army corporal, technician John Brewer, who later became a head technician in universities at Nottingham and in Zimbabwe. It was a time of maximum strain for me, for I had classes every day except Thursday, so Brewer had to prepare all the material for the experiments. He had a system of putting notices on my laboratory door when we were busy; on Thursdays it was a red notice. Even the head of the department respected the red notices; only once did a student barge in. His departure was precipitate, and it did not happen again.

The extreme simplicity of the technique and its effectiveness were such that we regarded it as hardly worth publishing, for it seemed a plain commonsense extension of previous methods. However, when John Waid (now head of microbiology at La Trobe University in Australia) joined me as a graduate student, he used the method in his work for his BSc and DPhil degrees. So we decided to publish jointly, illustrating the method's potential with results drawn from his work and from my own. We also published a second joint paper that used the method.[4] Subsequently, I did not continue this line of work because I became more interested in the physiology of salt absorption by mycorrhizas, but Waid did. Indeed, the method proved to be of value to many others, and quite soon it was quoted as a useful method.[5] It is still mentioned in experimental papers and reviews.[6,7]

We suppose that the reason the method is so frequently cited is that it is simple, inexpensive, and an effective way of separating and isolating fungi that are present as spores or as active mycelia on washable surfaces. It has been used both in rhizosphere and phyllophase studies.

1. **Harley J L.** Beech mycorrhiza: re-isolation and the effect of root extracts upon *Mycelium radicis fagi* (chan). *New Phytol.* 38:352-63, 1939.
2. **Coleridge S T.** Kubla Khan. (Empson W & Pirie D, eds.) *Coleridge's verse: a selection.* New York: Schocken Books, 1973. p. 177-9.
3. **Harley J L.** Mycorrhiza and soil ecology. *Biol. Rev. Cambridge Phil. Soc.* 23:127-58, 1948.
4. **Harley J L & Waid J S.** The effect of light upon the roots of beech and its surface population. *Plant Soil* 7:96-112, 1955.
5. **Johnson L F, Curl E A, Bond J H & Fribourg H A.** *Methods for studying soil microflora. Plant disease relationship.* Minneapolis: Burgess, 1959. 168 p. (Cited 155 times.)
6. **Lynch J M.** Biological control within microbial communities of the rhizosphere. (Fletcher M, Gray T R G & Jones J G, eds.) *Ecology of microbial communities.* New York: Cambridge University Press. (In press.)
7. **Subramarian C V.** *Hyphomycetes: taxonomy and biology.* London: Academic Press, 1983. 502 p.

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