

necessitate storage in the refrigerator to prevent darkening and deterioration.

The following technique is remarkably simple and effective. Freshly prepared squashes are thoroughly irrigated with 45 per cent acetic acid to remove all excess stain. (This may be facilitated by wiping the edges of the coverglass, holding the latter securely at one corner.) A small drop of 10 per cent glycerine in 45 per cent acetic acid is then placed at the margin of the coverglass and allowed to flow under as the aqueous portion evaporates. Should bubbles appear after a day or two, another drop of the glycerine solution may be added.

Advantages of the method are: (1) It is very rapid. (2) It is truly permanent. Even though large areas under the coverglass become filled with air, the glycerine remains in the individual cells and prevents their distortion. (3) Should darkening occur, 45 per cent acetic acid may be added at the edge of the coverglass and the slide gently heated until correct differentiation is achieved. (4) If care is exercised to avoid adding too much glycerine, the coverglass is held so firmly in place (by the evaporation of the acetic acid) that it may be rubbed quite vigorously without danger of displacement when removing oil or dirt. (5) The glycerine is so inert and non-volatile that there is no problem of artefact formation or of the medium becoming discoloured or brittle. (6) The refractive index of glycerine is sufficiently close to that of balsam to give a good microscopic image. It has the further advantage of complete transparency to ultra-violet and near-ultra-violet light.

The method may be extended to other types of microscopic preparation where the final mounting medium is soluble in water, and where pressure of the coverglass is not deleterious or the tissue too thick. It has been used for many years in this Department, for example, for preparations of macerated cells.

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Sectioning of Soil

MANY workers interested in soil microbiology or soil structure have attempted to impregnate soil with materials such as balsam in the hope that the soil could be hardened sufficiently to enable sections to be prepared by normal geological techniques. Few of these attempts met with much success. Kubiena¹ made considerable progress by introducing the use of a thermolabile plastic material, and was able to prepare sections showing the soil structure in many different soil types. However, Kubiena's technique does not seem to have given satisfactory results in the hands of other workers. Haarlov and Weis-Fogh² have developed a method for sectioning litter, using a hardened agar, but their method is unsatisfactory for mineral soil. More recently, Alexander and Jackson³ have described the use of a marco-resin for impregnating the soil and preparing thin sections.

We have found that the procedure can be greatly simplified by using a 'Bakelite' polymeric resin S.R. 17497 and its associated catalyst and accelerator. The resin has a low viscosity, good wetting powers and sets at room temperature in 12-24 hr. to a hard

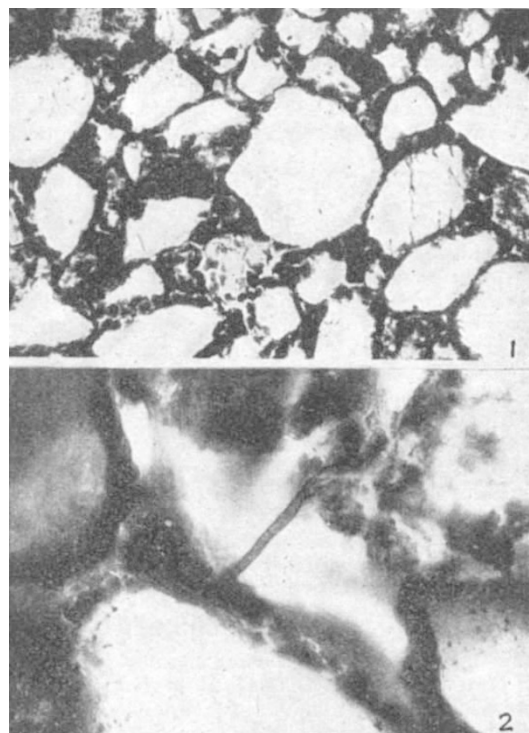


Fig. 1. B_1 horizon of a podzol, sand grains cemented with sesquioxide and humus. $\times 60$ approx.

Fig. 2. Fungal hypha in pore space of the B_1 horizon. $\times 300$ approx.

water-white solid which can be ground with carborundum. The setting time can be controlled by varying the amounts of catalyst and accelerator.

A piece of air-dry soil about 2 cm. \times 1.5 cm. \times 0.5 cm. is placed in a pool of resin on a slide. The resin readily fills the soil spaces, and after it has set the soil can be treated like a rock chip. The upper surface is ground to give the maximum surface area and then polished. The soil is taken off the slide and remounted in resin, polished side downwards. After the resin has set, the soil is ground down to a thin film and polished. Finally, it is mounted in resin under a cover glass. 'Carborundum' 280-grade has been used for the grinding and a No. 200 hone for polishing. Sections 50-60 μ thick have been found suitable for the examination of the fungi in soil.

This method has been developed primarily for the study of fungal hyphae in the soil; but mites, nematodes and thecate amoebae have also been examined.

Fig. 1 shows the general distribution of quartz grains and the cementing sesquioxide and humus in the B_1 horizon of a podzol. In Fig. 2 a fungal hypha can be seen passing from the humus coating on one sand grain across the soil pore to another grain.

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¹ Kubiena, W. L., "Micropedology" (Ames, Iowa, 1938).

² Haarlov, N., and Weis-Fogh, T., *Oikos*, 4, 44 (1953).

³ Alexander, F. E. S., and Jackson, R. M., *Nature*, 174, 750 (1954).