SCANNING ELECTRON MICROSCOPY OF LATEX CASTS OF FOSSIL PLANT IMPRESSIONS

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ABSTRACT. Rubber latex casts of fossil lycopod stem impressions in fine-grained matrices may be subjected to scanning electron microscopy to reveal details of the original epidermal structure. This technique offers the potential of obtaining microscopic detail from plant impression fossils even if the cuticle is not preserved.

IMPRESSIONS of plant surfaces on a rock matrix are generally regarded as a rather poor and uninformative type of fossil. Where some of the coalified plant material has survived, from which a cuticle may be prepared by maceration (a 'compression fossil'), then this may be subjected to microscopic examination, and the value of the fossil to a palaeobotanist is proportionately greater. In reviews of methods of investigating fossil plants, it is generally suggested that impression fossils will reveal only the outline of the plant material, and perhaps in the case of leaf impressions, the venation pattern. This paper is an account of a method of further investigating such impression fossils by preparing a rubber latex cast of the surface, and photographing the microtopography of the cast by scanning electron microscopy (SEM). Latex replicas have been in use in palaeontology for some years, for preparing casts and moulds of fossils (Rigby and Clark 1965). The application of SEM to such replicas appears to have considerable potential for revealing epidermal features—cell outlines, position and orientation of stomata, hairs, etc., in a class of plant fossils which is not generally rated as susceptible to microscopic examination. The use of SEM in palaeobotany has recently been thoroughly reviewed by Taylor (1968), Muir (1970*a*, *b*), and Snigirevskaya (1971). These and other authors have emphasized the value of SEM in studies of spores (Leffingwell and Hodgkin 1971; Reyre 1971), of wood (Alvin and Muir 1969), and of cuticles (Boulter 1971), but its application to plant impression fossils does not seem to have been exploited.

MATERIAL AND METHOD

A typical plant 'impression fossil' shows on the rock surface a mould of the outer surface of the organ, with a microtopography which is 'negative' with respect to the original surface, so that stomatal cavities appear as small protrusions, and so on. A rubber latex cast of such an impression or mould gives a replica of the original plant surface. The quality of such a plant impression fossil appears to depend largely on:

1. The extent to which the original plant tissue surface showed a topography reflecting underlying epidermal or subepidermal features. In some cases, as cell contents collapsed post-mortem, the outer surface of the cell walls may even show more of the underlying cell arrangement than was the case in life.
2. The rapidity with which the plant material became incorporated and the accruing

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sediment formed a mould in juxtaposition to it, before microbial activity or diagenesis caused collapse and loss of structure of the outer surface of the plant tissue.

3. The particle size of the matrix (whether clastic, or more or less syngenetic in character); the smaller the effective particle size, obviously the greater the fidelity of the mould to the minutiae of the original microtopography. In the latex replicas of plant impression fossils in fine-grained matrices that we have examined, the SEM even at magnifications of up to 10 000 times has revealed surprisingly little detail of the particulate nature of the matrix, and does not resolve any texture induced by the character of the latex itself. The latex, once dried at room temperature for 24 hours, withstands both the exposure to vacuum involved in specimen coating and the electron beam itself. Shrinkage of the latex cast appears to be insignificant; specimens up to 6 months old showed a linear shrinkage of less than 3%. No special study of the possibilities of differential contraction was attempted, but clearly if size or shape differences of this order were consequential, this aspect would need further consideration.

In a preliminary investigation, we have found that Carboniferous argillaceous sediments may retain a high degree of epidermal detail of lycopod stems, capable of being picked up on a latex replica. The most satisfactory results have been obtained from specimens from which the coaly plant material has either been burnt off or removed from the mineral matrix by weathering. Either of these processes reveals a surface of matrix with the highest possible fidelity to the original plant surface microtopography. A less satisfactory result is obtained where the impression has been exposed by a fracture plane running more or less along the interface between matrix and coaly material.

The advantages of the method of SEM examination of latex replicas are:

1. The method reveals epidermal characters on specimens which, lacking a cuticle, would not previously have been rated capable of yielding such detail.

2. In addition, this procedure may reveal cellular character on surfaces which have never had a cuticle (e.g. lycopod leaf abscission scars)—see Pl. 79, fig. 2.

EXPLANATION OF PLATE 79

Replicas in latex of leaf cushions of *Lepidodendron* (fig. 6), showing epidermal detail under the scanning electron microscope (figs. 1–5).

Figs. 1–4, 6. *Lepidodendron subdichotomum* Sterzel, *sensu* Thomas, from old tip heap, Radstock Colliery (Nat. Grid. ref. 696 554), British Museum (Natural History) V 67053. Specimen probably from Radstock Group (Westphalian D) or possibly from the underlying Farrington Group.

Fig. 1. Leaf cushion with scar, ×28.

Fig. 2. Detail of leaf scar surface, with vascular scar, and on either side the two parichnos, ×68.

Fig. 3. Detail of ligule pit, the fissure abutting obliquely on upper edge of leaf scar. (Hole to right of ligule pit is artifact caused by an air bubble in the matrix), ×205.

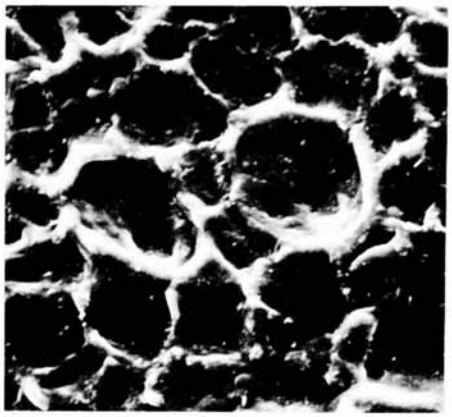
Fig. 4. Stomata on lower field of the leaf cushion: note clarity with which surrounding epidermal cell walls appear on the latex surface, and the stomatal apertures within the two stomatal depressions, ×900.

Fig. 5. Single stoma from the leaf cushion just above the leaf scar on a specimen of *Lepidodendron veltheimii* Sternberg (I.G.S., Kidston Collection No. 5115) from the Edge Coal Group, Stirling, Scotland, ×600.

Fig. 6. A photograph, with oblique illumination, of a white latex rubber cast ('positive') prepared from the same specimen of *Lepidodendron subdichotomum* as figs. 1–4, ×10.

Figs. 1, 4, and 5 were taken on a Cambridge S600; Figs. 2 and 3, on a Cambridge Stereoscan.

1



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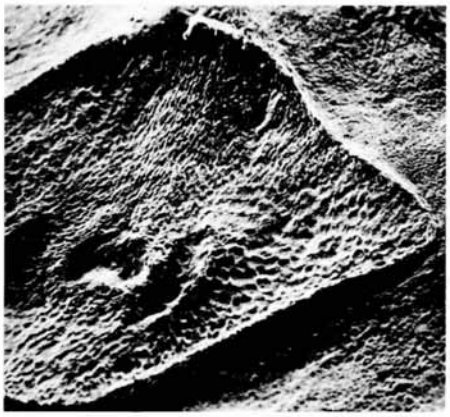

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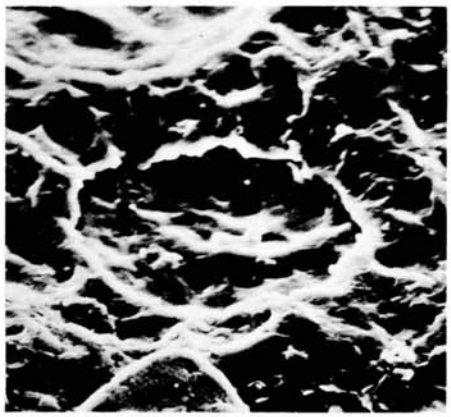

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3

6

CHALONER and GAY, latex casts of fossil plants

3. The fossil is left completely intact, which is an obvious advantage in a figured or type specimen.

4. A latex cast may be prepared from a relatively large specimen (e.g. in a museum), which could never itself be subjected directly to scanning microscopy by any other means. The resulting cast may readily be removed, or sent through the post, without needing to move the original specimen.

5. An incidental advantage is the homogeneity of the latex cast, in terms of its secondary electron emission (forming the SEM image). This contrasts with a clastic matrix which, if of petrologically heterogeneous nature, will give a varied secondary emission under the SEM unrelated to the microtopography.

TECHNICAL DETAILS

Casting. We have prepared casts from a number of Devonian, Carboniferous, and Permian plants. The surface of Lepidodendrid stems shows a surprising amount of detail; this is evidently because partial collapse of the original epidermal cells of the leaf cushion of these plants produces a surface topography reflecting their underlying structure. General information on the preparation of rubber latex casts is given in Rigby and Clark (1965). We have found that the product marketed as 'Revultex' (Bellman, Ivey, and Carter Ltd., 385b Grand Drive, London, S.W. 20) gives satisfactory results. A first coat is applied using the liquid diluted with an equal volume of water, and is worked into the surface of the fossil with a fine brush to ensure contact with depressions in the surface. Subsequent layers should be applied only after each coat has dried completely. The setting of the latex may be accelerated by placing a table lamp immediately above the latex-covered fossil. For a large surface (greater than 10×10 cm) the latex may be strengthened with gauze bandage or other textile applied to the wet surface of the third or subsequent layer of latex. In some cases (especially with museum specimens) it was found that the first cast carried a good deal of air-borne dust and other extraneous matter, and a second or even third cast gave a cleaner subject for SEM study. However, it was also found that once dry, a latex cast could be washed free of much of this debris with soap and water without loss of cellular detail.

Coating. We found that latex with white pigment (supplied by the manufacturer) gave particularly good results; the white surface is more satisfactory for examination by light microscopy prior to SEM study. Suitable pieces of the latex replica were cut out and mounted on stubs with 'Durofix' cement, painted around the margin with 'silver dag', and coated using carbon-paladium rods in an arc in an Edwards 'Speedivac' 12E6 high vacuum coating unit. Multi-directional coating was achieved by rotating the stubs on an improvised disc mounted eccentrically on the rotating spindle of the unit, set at 6 cm from the arcing source. The stubs were given two coatings in this way, being rotated through about 45° individually (with respect to the rotating disc) between each coating operation. Fifteen 1-second bursts of arcing were found to give an adequate coating under these conditions.

RESULTS

We have used this method successfully on a number of Palaeozoic lycopod stem impressions (*Lepidodendron*, *Sigillaria*, *Lepidodendropsis*, and *Lycopodiopsis*). The type of detail revealed is shown for two species of *Lepidodendron* in Pl. 79, figs. 1–6. The depressed stomata characteristic of the Lepidodendrids (Thomas 1966, 1970) show as a particularly clear feature of microtopography on these specimens—probably rather more so than would be the case with other plant groups. A specimen of *Lepidodendron subdichotomum* from the Westphalian C or D of Radstock forms the basis of figs. 1–4 and fig. 6. This specimen had evidently been burnt off, probably by combustion of the tip heap on which it was collected. A white latex cast of part of the stem surface showing leaf scars and cushions is shown in fig. 6, taken with

oblique illumination. Figs. 1–3 show the leaf cushion, the leaf scar, and part of the adjoining cushion surface under SEM at successively higher magnification: these show detail of the cellular pattern on the abscission surface (fig. 2) in addition to the parichnos and vascular cicatricule; the ligule pit is seen as a small fissure abutting on the upper edge of the leaf scar (fig. 3). Stomata show clearly among the epidermal cells on the leaf cushion surface: the orientation of their long axes is evident, and in some cases the stomatal aperture appears in the cast (fig. 4). A single stoma from another species, *L. veltheimii* is shown in fig. 5 for comparison. (This specimen, in the Kidston Collection, No. 5115, is cited in Crookall 1964, p. 302). While this technique has proved successful with a limited number of Palaeozoic plant fossils, it could equally be applied to many types of animal fossils where microtopography is likely to be preserved, such as the chitinous covering of arthropods and the calcareous shells and tests of other invertebrates. No doubt other means of preparing replicas of the surface may give equally good results, but latex has the ability to accommodate to large re-entrant features in the topography, while picking up with equally high precision the microscopic details of the surface.

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REFERENCES

ALVIN, K. L. and MUIR, M. D. 1969. Scanning Electron Microscopy—a new method of studying lignite. *Rev. Palaeobotan. Palynol.* **9,** 115–118.

BOULTER, M. C. 1971. Fine details of some fossil and recent conifer leaf cuticles. *In* HEYWOOD, V. H. (ed.), Scanning Electron Microscopy, Systematic and Evolutionary Applications. *Systematics Ass. Spec. Vol. No. 4*, 211–235.

CROOKALL, R. 1964. Fossil plants of the Carboniferous rocks of Great Britain. *Mem. Geol. Surv. Gt. Brit.* **4** (3), 217–354.

LEFFINGWELL, H. A. and HODGKIN, N. 1971. Techniques for preparing fossil palynomorphs for study with the scanning and transmission electron microscopes. *Rev. Palaeobotan. Palynol.* **11,** 177–199.

MUIR, M. 1970*a*. A new approach to the study of fossil wood. *Proc. 3rd Annual Electron Micr. Symp.* 129–136.

—— 1970*b*. Scanning electron microscopy in palynology. *Rev. Palaeobotan. Palynol.* **10,** 85–97.

REYRE, Y. 1971. Interpretation Botanique des Pollens Inaperturés du Mésozoique Saharien. Essai de Classification d'apres l'Observation en Microscopie Electronique à Balayage. *In* HEYWOOD, V. H. (ed.), Scanning Electron Microscopy, Systematic and Evolutionary Applications. *Systematics Ass. Spec. Vol. No. 4*, 145–154.

RIGBY, J. K. and CLARK, D. L. 1965. Casting and Moulding. *In* KUMMEL, B. and RAUP, D. (eds.), *Handbook of Paleontological Techniques*, 389–413. San Francisco and London, Freeman & Co.

SNIGIREVSKAYA, N. S. 1971. Application of the Scanning Electron Microscope in Botany. *Botanicheski Zhurnal*, **56,** 549–558. (In Russian.)

TAYLOR, T. N. 1968. Application of the scanning electron microscope in paleobotany. *Trans. Amer. Microsc. Soc.* **87,** 510–515.

THOMAS, B. A. 1966. The cuticle of the Lepidodendroid stem. *New Phyt.* **65,** 296–303.

—— 1970. Epidermal studies in the interpretation of *Lepidodendron* species. *Palaeontology*, **13,** 145–173.

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