SCANNING ELECTRON MICROSCOPY IN GONDWANA PALAEOBOTANY

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ABSTRACT

The development of Scanning Electron Microscope (SEM) techniques in palaeobotany is briefly reviewed. Special reference is made to the use of these techniques in the investigation of Gondwana plants. Examples are given of their application in recent research on silicified wood from Zambia, pyritised stems and leaves from South Africa and leaf impressions from Antarctica. The limitations and potentialities of SEM in studies of Gondwana fossil plants are summarised.

Key-words — SEM techniques, Silicified wood, Pyritised stems, Leaf impressions, Gondwana.

INTRODUCTION

ALTHOUGH the genus Glossopteris Brongniart has been known for more than 100 years and its many species are widespread throughout Gondwanaland, the preservation of this and other taxa in both Lower and Upper Gondwana floras is most often such as to yield no detailed information on anatomical structure. Leaves and reproductive organs usually occur as impressions or compressions retaining little more than carbon, from which cuticle preparations are not possible. Indeed, as far as Lower Gondwana deposits are concerned, the most rewarding cuticle studies have been almost totally confined to India, where, following the pioneer work of Birbal Sahni (1923) on Glossopteris angustifolia, many other Indian workers (for example: P. N. Srivastava, K. R. Surange, D. D. Pant, J. Sen, Shaila Chandra, M. Banerjee, to name but a few) have made extensive and valuable contributions.

Little cuticle work has been done in other parts of Gondwanaland. In southern Africa the possibility of cuticle studies was first demonstrated by Zeiller (1896) on Glossopteris indica from near Johannesburg and has been confirmed since for other species by Lacey (1961) and Williams (1966) using material from Rhodesia and the Transvaal respectively. Very recently Chaloner, Leistikow and Hill (1979) have succeeded in preparing cuticles of a Permian lycopod from Brazil.

In Upper Gondwana deposits, such as the Molteno of South Africa, the Ipswich of eastern Australia (both of Triassic age) and the Jurassic Rajmahal in India, cuticle-yielding plants are rather more abundant.

Permineralised (petrified) material, usually in the form of silicified (occasionally calc-
fied) wood, is widespread in both Lower and Upper Gondwana deposits, but is often poorly preserved, revealing little or no cellular detail in sections.

The first discoveries of permineralised Glossopteris flora (as distinct from wood) showing internal structure were made in 1965 by the late Professor J.M. Schopf in the Queen Alexandra Range, Antarctica (Schopf, 1970, 1971, 1976) and in 1967 by Gould in Queensland, Australia (Gould, 1970, 1975; Gould & Delevoryas, 1977). More recently, pyritised Glossopteris flora material, also showing internal structure, has been found in Natal, South Africa (van Dijk, Gordon-Gray & Lacey, 1975, 1978).

It remains generally true, however, that in the majority of localities structurally-preserved material is lacking and, of necessity, species have been created on external characters alone, such as, for example, size, shape and venation pattern in Glossopteris leaves. In such circumstances, any new technique which provides additional diagnostic features from otherwise difficult material is welcome. Scanning electron microscopy (SEM) is just such a technique. Originally used in palynology, it has now been applied to studies of the surfaces of fractured permineralised specimens of cuticles prepared from compressions by transfer or maceration methods and of latex casts (replicas) prepared from impressions.

This paper briefly reviews the development of SEM techniques in palaeobotany and gives examples of their application in recent studies of permineralised (silicified) wood from Zambia, pyritised material from South Africa and leaf impressions from Antarctica.

DEVELOPMENT OF SEM TECHNIQUES IN PALAEOBOTANY

The theoretical principles and practical problems of SEM were first described by Von Ardenne (1938a, b) but it was not until about 1960 that commercial instruments became available. The first studies of biological materials were in medicine and entomology.

In palaeobotany the earliest applications were in palynology in the mid-1960s, one of the pioneer studies in this field being the work of Hibbert on Carboniferous spores from North Wales (Hibbert, 1967; Hibbert & Lacey, 1969). Applications to the study of microfossils and some megafossils were reviewed by Taylor (1968), Taylor and Eggert (1969), Taylor and Millay (1969) and Muir (1970a). At about this time Alvin and Muir described a new method of studying lignite and silicified wood by using SEM to examine the surfaces of split fragments (Alvin & Muir, 1969; Muir, 1970b). Subsequently SEM studies were made of the surfaces of fossil leaves (Alvin, 1970), fossil epiphyllous fungi (Alvin & Muir, 1970; Alvin, 1971), fusainized material (Alvin, 1974) and cuticles prepared by maceration (Boulter, 1970, 1971; Thomas, 1974, 1977).

The study of plant impression fossils seems to have been rather neglected until Chaloner and Gay (1973) developed a method of preparing latex casts for examination with SEM, the value of which was further demonstrated by Chaloner and Collinson (1975). Later a slightly modified technique, using low viscosity silicone rubber, was described by Watson and Alvin (1976).

It is interesting now to note that the development of the important latex cast method was foreshadowed in a question and comment from the reviewer of Alvin's 1970 paper (abbreviated here);

"Have any fine-grained impressions been examined for epidermal features....? Because of the large number of...... specimens in which carbonaceous films and cuticles are not preserved, the potential of examining such impressions (by SEM-WSL insert) would be of great help taxonomically".

The early developments of the various SEM techniques listed above were effected mainly using Northern Hemisphere materials but in the last five years the Alvin and Muir (1969) technique for permineralised specimens and the Chaloner and Gay (1973) technique for impressions have been increasingly used in the study of Gondwanan megafossils. Some examples of this are the work of Chaloner, Mensah and Crane (1974) on compressions from Ghana; Smith (1975) on silicified wood from Zambia; Van Dijk, Gordon-Gray and Lacey (1975, 1978) on pyritised specimens from South Africa; Watson and Alvin (1976) on silicified plants from Sudan; Lucas (1977, 1979) on...
leaf impressions from Antarctica; Rigby (1978) on fructification compressions from Australia; and Chaloner, Leistikow and Hill (1979) on stem impressions from Brazil.

**SILICIFIED WOOD FROM ZAMBIA**

Pl. 1, figs 1-4

During the course of research on African fossil plants Smith (1975) examined both ground sections with transmitted light and the surfaces of small fragments by SEM to investigate the structure of silicified wood of *Taxopitys africana* Kräusel & Range. The material came from a locality near Sitwe in the Upper Luangwa Valley, but its exact age (Permian or Triassic) was unknown.

Preparation of the permineralised material was simple. Using a hammer and chisel, small pieces of the specimens were chipped off to expose fresh surfaces in the planes required, mounted on stubs with “Araldite” and coated with gold or gold palladium. Dust was excluded at all stages of the preparation and an even, continuous coating of the specimen and stub was ensured to prevent charging. The scanning electron microscope used was a Cambridge “Stereo­scan” Mark II A.

One of the distinguishing features of *Taxopitys africana* is the occurrence of tertiary spiral (helical) thickening, associated with araucarian pitting, in the secondary wood tracheids. In the ground sections this feature was scarcely visible but with SEM the tertiary spirals were seen very clearly (Pl. 1, figs 2, 3). Additionally, the details of the bordered pit and torus were readily visible and could be studied under high magnification (Pl. 1, fig. 4). These illustrations testify to the value of SEM in the case of *Taxopitys africana* and indicate the potentialities of the method for the study of permineralised Gondwana woods generally.

**PYRITISED MATERIAL FROM SOUTH AFRICA**

Pl. 1, fig. 5; Pl. 2, figs 6-9

In 1975, D.E. van Dijk discovered at Lidgetton, Natal, the type locality for the fructification *Lidgettonia* (Thomas, 1958), *Glossopteris* leaves and sphenopsid stems with small regions of surface replication or deeper infilling with pyrite. This Upper Permian plant material occurs in a very fine-grained shale, the pyritisation being attributable presumably to a strongly reducing environment associated with anaerobic decomposition.

SEM studies were made of surface replicas and of fragments of deeper tissues, both coated and uncoated. In practice coating usually proved unnecessary if colloidal silver was painted from the edge of the specimen to the stub to prevent charging (for technical details, see van Dijk, Gordon-Gray & Lacey, 1975, 1978).

Surface replicas of *Glossopteris* leaf lamina yielded useful electron micrographs of subsurface features, such as epidermal cells, often seen from the inside looking outwards (Pl. 1, fig. 5). A deeper infilling of *Glossopteris* midrib yielded specimens showing considerable detail of the vascular elements (Pl. 2, figs 6-8).

Compressions of sphenopsid axes were sometimes fractured in such a way as to show pyritisation of tissues at different levels, presumably representing epidermis, cortex and vascular region (Pl. 2, fig. 9).

It is clear from the preliminary work already published (van Dijk, Gordon-Gray & Lacey, 1975, 1978) and from the illustrations provided here that, although the Lidgetton fossils do not yield cuticles by oxidative maceration, they nevertheless hold the promise of much information through the examination of the locally pyritised areas by SEM.

**LEAF IMPRESSIONS FROM ANTARCTICA**

Pl. 3, figs 10, 11

In his studies of Permian Antarctic fossil plants Lucas (1977, 1979) used the latex cast technique with the dual purpose of (i) obtaining illustrations of *Glossopteris* leaves which were difficult to photograph in the normal way due to the lack of contrast between the leaves and the rock matrix, both being dark-coloured, and (ii) of obtaining, if possible, additional information on epidermal features that might prove of value in the taxonomy of this difficult genus. Both of these objectives met with an encouraging degree of success.
The leaf impressions were preserved in very hard shales and sandstones of fine texture. Such preservation was ideal for the preparation of good quality latex casts for examination with SEM.

The technique employed, essentially that of Chaloner and Gay (1973), was as follows: A coat of 50% latex/water mixture was applied to the impression with a fine brush, followed by several coats of a latex/filler paste mixture, allowing drying between successive coats. The first cast thus prepared picks up dust and surface debris and was discarded. A second latex cast, prepared as above, was retained for examination, selected portions being mounted on stubs with Bostick adhesive, coated with gold palladium and examined with a Cambridge "Stereoscan" Mk. IIA scanning electron microscope. Plate 3, fig. 10 shows at low magnification the vein pattern and suggestion of epidermal cell pattern on the abaxial surface of a Glossopteris cf. conspicua leaf from the Theron Mountains. Plate 3, fig. 11 illustrates at higher magnification a distinct epidermal pattern on the adaxial surface of a Glossopteris browniana leaf from the Transantarctic Mountains.

It is apparent that the latex cast/SEM technique has potentiality for providing supplementary information on epidermal features which may have taxonomic value in the study of Glossopteris leaves preserved only as impressions, provided that the latter are in a very fine-grained hard matrix which faithfully reproduces the original epidermal pattern and whose surface particles are not easily detached by the latex transfer process or large enough to produce a pseudo-cellular pattern in the replica.

CONCLUDING REMARKS

The SEM techniques described here have the advantages of quick and easy preparation of material and immediate photography of interesting features. They are thus of great value in assessing the suitability of specimens for further study, as well as in offering the potential of providing additional microscopic detail for use in taxonomy. The scanning electron microscope is, however, expensive in use and time is necessary to acquire dexterity in its operation.

In studies of permineralisations the experience of various workers (Alvin & Muir, 1969; Smith, 1975) shows that there is no predicting from light microscope work what results will be obtained by SEM. Even allowing for the fact that splitting of the petrifaction fragments is rather haphazard, sometimes necessitating several preparations, it does not follow that good results will always be obtained by SEM. Nevertheless, the SEM studies of surfaces of silicified wood fragments and of pyritised specimens described here have yielded much more information than was available by light microscope examination of ground sections or polished surfaces.

In the latex cast technique there are additional problems, related to the nature of the fossil itself and of the matrix in which it is embedded. At the outset (and this is not confined to SEM studies) it is necessary to know whether the adaxial or abaxial surface of the specimen is exposed and, in addition, whether the specimen under examination is the counterpart (negative impression) of the plant organ or the surface of the organ itself. Thus a latex cast from a negative impression will produce a positive replica of the original fossil, but a cast from the fossil material itself will produce a reversed effect, with originally raised features appearing as depressions and vice-versa. Experience has shown (Chaloner, 1973; Chaloner et al., 1974, 1979; Lucas, 1977, 1979) that the most useful results are obtained from latex casts prepared from the counterparts, (negative impressions) of plant organs preserved in a hard fine-grained matrix. Compressions with much carbon remaining or impressions in soft sediments (even, though very clear, such as those in the South African Vereeniging deposits) do not yield latex replicas suitable for SEM study. Finally, the high magnification possible with SEM techniques introduces the problem (by no means uncommon in electron microscopy generally) of interpreting what is seen. In palaeontological work there may be a difficulty, for example, in distinguishing between an original organic feature and one reflecting the grain size or mineral pattern of the rock matrix. While a magnification of up to ×4,500 has been used to advantage with permineralised wood, it seems likely that for epidermal features in latex casts the upper magnification limit is nearer ×1000.
Despite what has been said above, it is certain that the advantages of SEM techniques outweigh the limitations and the application of these techniques on a wide scale cannot but advance the knowledge of Gondwana fossil plants.

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REFERENCES


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EXPLANATION OF PLATES

(All figures are scanning electron micrographs)

PLATE 1

Silicified and pyritised material from Africa


1. Radial view of several tracheids, showing contiguous and distant pits and traces of spiral thickening. x 900.

2. Radial view showing, at centre, the inside of a tracheid with pit pores and parts of tertiary spiral thickening passing over the pit borders. x 1865.

3. Tangential view of parts of two tracheids; one, at centre, showing clear tertiary spiral thickening (note also, upper right, a fragmented pit in the radial wall exposing the pore and torus); the second tracheid, at right, in the form of a cast of the cavity with the spiral thickening appearing as depressed, not raised, bands. x 1865.

4. Two bordered pits in radial view, the upper with torus intact, the lower lacking torus. x 4500.


PLATE 2

Pyritised material from Africa

(from the Permian of Lidgetton, Natal. Photographs: D. E. van Dijk)

6. Part of midrib of a Glossopteris leaf, showing tracheids with scalariform thickening. x 600.

7. As fig. 6, scalariform tracheids enlarged. x 1200.

8. Another part of a midrib of Glossopteris leaf showing tracheids with bordered pits. x 600.

9. Compression of a sphenopsid stem which has split to show cellular pattern at three levels, probably representing epidermis (slightly elongated cells at extreme right), cortex (isodiametric cells right and left) and vascular strands in the centre. Node with two branch scars at the bottom. x 15.

PLATE 3

Compression material from Antarctica

