DACRYDIUM (PODOCARPACEAE): A SCANNING ELECTRON MICROSCOPE STUDY OF THE WOOD OF NEW ZEALAND SPECIES

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ABSTRACT

Some anatomical features of the secondary xylem of New Zealand species of *Dacrydium* are described. The nature of the growth rings and cross-field pitting are important for specific xylotomical identification. A key is presented on the basis of microscopic structure. *D. cupressinum* is shown to be unique among the seven species possessing axial parenchyma, callitrisoid thickening and spiral thickening. The possible occurrence of "incipient" spiral thickening in *D. biforme* is recorded.

INTRODUCTION

The genus *Dacrydium* is represented in New Zealand by seven endemic species. The wood anatomy of the New Zealand *Dacrydium* has been described by Orman and Reid 1946, Patel 1967a and Greguss 1955 and 1972.

Orman and Reid, and Patel emphasised the importance of the nature (size, shape and number) of cross-field pitting as a diagnostic feature distinguishing the species. The above studies were carried out by the use of a light microscope. The aim of this paper is to elucidate distinguishing features by means of the scanning electron microscope with particular reference to the nature of cross-field pitting and thereby adding to the identification keys developed by both Orman and Reid, and Patel.

MATERIALS AND METHODS

The species examined were: *D. cupressinum* Lamb., *D. colensoi* Hook., *D. biforme* (Hook.) Pilger, *D. bidwillii* Hook. f. ex Kirk, *D. kirkii* F. Muell. ex Parl., *D. intermedium* Kirk, *D. laxifolium* Hook. f.

The wood samples were obtained from the trunk of morphologically normal trees, either by the use of an increment borer or, as was the case with *D. laxifolium*, by removal of a major limb. Samples of *D. biforme* and *D. intermedium* were

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obtained from recently wind-blown trees in the north of the South Island.

The samples were transformed immediately into containers of formalin acetic-alcohol (FAA) to prevent desiccation and damage to more delicate features such as pit membranes.

Blocks of wood approximately 3 mm per side were cut in transverse section and radial longitudinal section according to the technique of Exley *et al.* (1974 and pers. comm.). Final surface cuts were made using a new razor blade for each face. In *D. laxifolium* a transverse section of the whole stem was used to observe the nature of the growth rings, and cut directly in half to observe the radial longitudinal section.

The block was soaked in 50% sodium hypochlorite (domestic bleach) for two hours to remove the cell contents, and then put through two distilled washes to remove traces of FAA and bleach. Dehydration through an ethanol series followed, with the samples passed through 25%, 50% and 75% solutions for one hour each and 100% for two hours. After one hour in 100% acetone the blocks were dried overnight in a desiccator, glued to a specimen stub using colloidal silver, and coated with approximately 1 μm of gold.

The specimens were examined in a vacuum dry state under a Cambridge Stereoscan 600 scanning electron microscope. Relevant views were photographed by a Nikon F2 35 mm camera attached to the microscope. In selecting the wood sample care was taken to avoid abnormal wood such as knots and compression wood. This was difficult to avoid in the collected material of *D. laxifolium* as this had a much branched and distorted main stem.

Terms used follow the "International Glossary of Terms Used in Wood Anatomy" prepared by the International Association of Wood Anatomists (I.A.W.A. - 1957) and those used by Jane (1970).

RESULTS

The anatomical features of the secondary xylem of *Dacrydium* are described below. Particular reference is given to the nature of growth rings and the pits in the cross-field in each species. These are illustrated in Figs. 1-7 and Figs. 8-14 respectively (Appendix). Features unique to *D. cupressinum, D. intermedium* and *D. biforme* are also described and shown in Figs. 15-19. An identification key is given in Table 1.

D. cupressinum (rimu)

Tree up to 35 m, rarely to 60 m; trunk up to 1.5 m diameter. North, South and Stewart Islands. Lowland and montane forests (Allan 1961).

GROWTH RINGS indistinct to slightly distinct (Fig. 1). TRACHEIDS: fine spiral thickening (Fig. 16) and callitrisoid thickening in isolated tracheids: bordered pits in one row or sometimes oppositely paired on radial walls; tracheid to

TABLE 1. KEY FOR IDENTIFICATION OF *DACRYDIUM* SPECIES.

- AXIAL PARENCHYMA
  - PRESENT
    - *D. cupressinum*
  - ABSENT
    - CROSS-FIELD PITS
      - LARGE, WINDOW-LIKE
        - *D. colensoi*
      - SMALL; NUMEROUS
        - PIT ARRANGEMENT
          - LINEARLY ARRANGED
            - *D. laxifolium*
          - SCATTERED
            - *D. intermedium*
      - LARGE CUPRESSOID, PICEOID, PINOID AND TAXODIOID; USUALLY 1-3
        - SPIRAL THICKENING
          - *D. biforme*
        - GROWTH RINGS
          - NARROW(??)
            - *D. kirkii*
          - WIDE(??)
            - *D. bidwillii*

ray pitting - cupressoid, taxodioid, generally small but occasionally large. Commonly 1 and 2 (sometimes 3) per cross-field; each pit occupies a small proportion of cross-field area (Fig. 8). AXIAL PARENCHYMA abundant, aggregated or isolated (Fig. 15); sieve-like pits on radial walls (Fig. 17).

D. colensoi (silver pine)

Tree up to 15 m; trunk up to 1 m in diameter. North and South Islands. Lowland and montane forests. Lat. 35° to Lat. 44° on west of South Island (Allan 1961).

GROWTH RINGS distinct; average 20-26 tracheids in width (Fig. 2). TRACHEIDS: with bordered pits in one row or sometimes oppositely paired on radial walls; tracheid to ray pitting - large window-like with distinct border and prominent pit membrane; commonly 1 (in the marginal cells sometimes 2) per cross-field; each pit when solitary occupies the majority of cross-field area (Fig. 9). AXIAL PARENCHYMA absent.

D. biforme

Shrub or tree up to 10 m; trunk up to 0.6 m in diameter. North, South and Stewart Islands. Montane, subalpine forest and scrub from Lat. 36°50' southwards, also lowland forest in South and Stewart Islands (Allan 1961).

GROWTH RINGS distinct and sometimes wavy; average 9-11 tracheids in width (Fig. 3). TRACHEIDS: incipient form of spiral thickening in isolated cells (Fig. 18), bordered pits in one row, sometimes oppositely paired or scattered on radial walls; tracheid to ray pitting - large "taxodioid" (narrow border) and large "cupressoid" (wide border); commonly 1 per cross-field but where two are present they are usually one above the other (occasionally 3 and 4); when solitary each pit occupies the majority of the cross-field area (Fig. 10). AXIAL PARENCHYMA absent.

D. bidwillii (bog pine)

Spreading or erect shrub up to 3.5 m (Allan 1961), 7.5 m (Patel 1967a); trunk up to 0.4 m in diameter. North, South and Stewart Islands. Montane to subalpine scrub from Lat. 39° southwards, also lowland in western South and Stewart Islands (Allan 1961).

GROWTH RINGS distinct and sometimes slightly wavy with pronounced late wood; ranging from 14-50 tracheids in width (Fig. 4). TRACHEIDS: bordered pits in one row, sometimes oppositely paired, or scattered on radial walls; tracheid to ray pitting - large "taxodioid" and occasionally large "cupressoid". Commonly 1 and 2 per cross-field; where two are present they are usually one above the other (occasionally 3 and 4); when solitary each pit occupies the majority of the cross-field area (Fig. 11). AXIAL PARENCHYMA absent.

D. kirkii (monoao)

Tree up to 25 m; trunk up to 1 m in diameter. North Island. Lowland forest occurring from Lat. 35° to 37° (Allan 1961).

GROWTH RINGS distinct to indistinct; average 10 tracheids in width (Fig. 5). TRACHEIDS: with bordered pits in one row, sometimes oppositely paired or scattered on radial walls; tracheid to ray pitting - large "taxodioid" and large "cupressoid". Commonly 1 and 2 per cross-field; where two are present they are usually one above the other (occasionally 3); when solitary each pit occupies the majority of the cross-field area (Fig. 12). AXIAL PARENCHYMA absent.

D. intermedium (yellow silver pine)

Tree up to 15 m; trunk 0.3-0.6 m in diameter. North, South and Stewart Islands. Lowland, montane and subalpine forest from 35° southwards; in South and Stewart Islands on western side (Allan 1961).

GROWTH RINGS distinct but sometimes indistinct; ranging from 8-15 tracheids in width (Fig. 6). TRACHEIDS: with bordered pits in one row, sometimes oppositely paired or in groups of three in a triangular formation (Fig. 19); tracheid to ray pitting - small cupressoid, piceoid, pinoid or taxodioid. 1 to 6 per cross-field in no definite arrangement (occasionally up to 8); if solitary occupy small proportion of cross-field area and size of pits within a cross-field variable on a small scale (Fig. 13). AXIAL PARENCHYMA absent.

D. laxifolium (pigmy pine)

Prostrate shrub with branches about 2-5 mm in diameter towards base; up to 1 m or more long. North, South and Stewart Islands. Montane and subalpine scrub and moorland from Lat. 39° southwards, also lowland in Stewart Island (Allan 1961).

GROWTH RINGS distinct to slightly distinct; ranging from 6-21 tracheids in width (Fig. 7). TRACHEIDS: one row of bordered pits, sometimes paired on radial walls; tracheid to ray pitting - small cupressoid or taxodioid (often pinoid); 1 to 10 per cross-field mostly arranged one above the other in rows (occasionally up to 13 or 14); if solitary occupy small proportion of cross-field area, but occasionally odd pits are large and occupy the majority of the cross-field area. Size is variable (Fig. 14). AXIAL PARENCHYMA absent.

DISCUSSION

Dacrydium is anatomically heterogeneous (Phillips 1948) and therefore not readily distinguishable as a whole from other conifers, especially the related genera *Podocarpus* and *Phyllocladus*, with which it is often associated in forests (Orman and Reid 1946). On the other hand, however, the individual species are readily differentiated (Phillips 1948).

Microstructural keys for the identification of the New Zealand Dacrydiums have been compiled by Orman and Reid (1946) and Patel (1967a). Both authors concluded that the *Dacrydium* species form three natural anatomical groups based on the nature and appearance of the pits between the tracheids and the ray cells, the ray heights and the tracheid dimensions. The groups are:

1. *D. cupressinum*
2. *D. colensoi; D. biforme, D. bidwillii, D. kirkii*
3. *D. intermedium, D. laxifolium*

There is considerable disparity in the tracheid dimensions documented in these two keys. This emphasises the necessity for using the nature of cross-field pitting for more specific identification.

D. cupressinum with its many unique features is easily distinguished from the other species. In these features it resembles *Podocarpus* more closely than do the other New Zealand species of *Dacrydium* (Orman and Reid 1946, Patel 1967a).

D. colensoi with large window-like cross-field pitting is easily distinguished from the remaining species. Orman and Reid (1946) imply in their illustrations that the pits of *D. colensoi* closely resemble those of *D. bidwillii*. However, when the pits are viewed on the inner wall of the ray parenchyma there is a pronounced difference. The advantage of viewing a block of wood in three dimensions is shown here. Both sides of the pit pairs can be examined, a technique not possible when using thin sections for the light microscope. Because *D. colensoi* is so significantly different and readily distinguishable from the other species the author believes that *D. colensoi* should be accorded a natural anatomical grouping among the *Dacrydium* species.

D. colensoi resembles *Phyllocladus alpinus* and *P. trichomanoides* in possessing large window-like pits but the *Phyllocladus* species differ in having pronounced late wood and inter-tracheid pitting on the tangential walls (Patel 1968). *Podocarpus spicatus* is similar to *D. colensoi* in possessing large cross field pits but their separation is relatively simple due to the presence of axial parenchyma in *P. spicatus* (Patel 1967b).

D. intermedium and *D. laxifolium* are grouped together anatomically because of the presence of small, numerous and apparently simple pits in the cross-field (Orman and Reid 1946, Patel 1967a). However, large pits are occasionally present and these may occupy a large proportion of the cross-field area. In the two keys constructed on the basis of microstructure by the above authors, the two species are separated using different features. Orman and Reid use tracheid length as the diagnostic feature whereas Patel uses the criterion of pitting on the horizontal walls of ray parenchyma.

The present study has revealed other anatomical differences between the two which may be used to distinguish them. These features are:

1. Sometimes up to 13 pits per cross-field in *D. laxifolium* compared with a maximum of 8 in *D. intermedium*.

2. Ray parenchyma cells in *D. laxifolium* are commonly only three tracheids or less in width whereas those in *D. intermedium* are more often five tracheids or more in width. (Measured on the basis of the number

of cross-fields).

3. The cross-field pits in *D. laxifolium* are mostly arranged one above the other in regular rows, but those of *D. intermedium* appear to be scattered within the cross-field with no regular arrangement.

Phillips (1948) classified cross-field pits of conifers into five types:

1. Large, simple or almost simple, window-like.
2. Pinoid - found in several species of *Pinus*, and characteristically simple or with narrow borders, often variable in size and shape
3. Piceoid - as in *Picea*, with a narrow and often slightly extended aperture
4. Taxodioid - as in *Sequoia*, with a large, ovoid to circular, included aperture that is wider than the lateral space on either side between the aperture and the border
5. Cupressoid - as in *Cupressus*, with an ovoid, included aperture that is rather narrower than the lateral space on either side between the aperture and the border.

The taxodioid and cupressoid pits are relatively small and each is reported to occupy a small proportion of cross-field area. Patel (1967a) noted that the cross-field pits of *D. bidwillii, D. biforme* and *D. kirkii* do not conform to any of these types described by Phillips (1948). He suggested that such pits which are large with each, when solitary, occupying a large proportion of cross-field area, may be considered as large types of taxodioid and cupressoid pits. For the above reason *D. bidwillii, D. biforme* and *D. kirkii* form a distinct anatomical group. Subsequent specific identification has proved difficult but according to Patel (1967a) is practicable.

The problem is substantiated further by conflicting information recorded by Orman and Reid (1946), Patel (1967a) and Greguss (1955, 1972). Orman and Reid used tracheid length as a means of differentiating the three, with *D. bidwillii* having very short tracheids (average 1.0-1.6 mm), *D. biforme* having short tracheids (average 1.1-2.4 mm), and *D. kirkii* having medium length tracheids (average 2.2-3.0 mm) respectively. This seems a clear distinction. However conflict develops in the key compiled by Patel (1967a), who recorded the length of *D. kirkii* tracheids as 1.81-2.75 mm and *D. bidwillii* and *D. biforme* tracheid lengths as 1.12-1.69 mm. The discrepancy is obvious and since both authors examined a wide range of specimens the use of either key would leave one open to errors in identification.

Having separated *D. kirkii* from *D. bidwillii* and *D. biforme* on the basis of tracheid length Patel (1967a) then separated *D. bidwillii* and *D. biforme* using the nature of the growth rings. He noted that *D. bidwillii* has wide rings and *D. biforme* narrow, wavy rings. However, Greguss (1955 and 1972) observed in the limited specimens that he studied that the rings of *D. bidwillii* ranged from 10-50 tracheids in width and those of *D. biforme* ranged from 15-50 tracheids wide. Severe criticism has been levelled at Greguss (Davis and Heywood 1963) for what has been called an "ambitious" attempt at a complete xylotomy of the living conifers, and specific identification by wood anatomy, and on these grounds one may accept Patel's (1967a) classification as more valid. The present study supports the observations of Patel but the presence of slightly wavy rings in *D. bidwillii* emphasises the need for a more vigorous study of these features.

The size, shape and number of pits in the cross-field does little to help the specific identification of *D. bidwillii*, *D. biforme* and *D. kirkii*. All three possess "large taxodioid" and "large cupressoid" pits with variations on these standard types. There is generally one pit per cross-field but occasionally two or three exist and these are almost always one above the other in a regular linear sequence. In each species when a pit is solitary it occupies most of the cross-field area.

Orman and Reid (1946) and more recently Greguss (1972) report a difference in the size of cross-field pits; *D. bidwillii* pits appearing larger than those of *D. biforme*. The existence of varying shapes and sizes of pits in each species makes exact identification difficult. However, since no overlap exists in the range of sizes given by Greguss then one may assume that a pit in the range of 13-15 μm would identify the specimen as *D. bidwillii*. This is by no means convincing in the light of the criticism levelled at Greguss.

The present study has revealed what may be termed an "incipient" form of spiral thickening in isolated tracheids of *D. biforme*. It is equivalent to that described in *D. cupressinum* (Patel 1967a). The value of this feature as a definite means of separating *D. biforme* from *D. bidwillii* and *D. kirkii* can be determined by further study of a wider range of specimens from different localities.

Patel (1967a) recorded the presence of spiral and callitrisoid thickening for the first time in the family Podocarpaceae. Phillips (1948) described callitrisoid thickening, which occurs on the radial walls of tracheids, as pairs of transverse bars overhanging the bordered pits and restricted almost entirely to the genus *Callitris*. This type of thickening occurred in *D. cupressinum* with a much higher frequency than spiral thickening which is confined to small areas in the tracheids ends. Intermediate forms of such thickening were reported by Patel (1967a) and he regarded them as "incipient forms" of both spiral and callitrisoid thickening. This has been observed in the present study and it has been noted that these thickenings were oriented more or less transversely to the long axis of the cell, in agreement with Patel (1963).

Callitrisoid thickening was not found in any of the other species of *Dacrydium*. Spiral thickenings may be present in *D. biforme* in a restricted form but not in the other five species.

Scanning electron microscopy has failed to confirm the observation of Patel (1967a) that the nature of the cross-field pit depends on the position in the length of the tracheid crossed by the ray parenchyma. However, macerations of material from *D. cupressinum* would tend to support this observation, where pits towards tracheid ends tend to lack borders.

In conclusion, scanning electron microscopy has in general verified the observations which Orman and Reid (1946), Phillips (1948), Greguss (1955, 1972) and Patel (1967a) obtained using light microscopy. The present study has not been able to resolve the specific anatomical identification of *D. bidwillii, D. biforme* and *D. kirkii* but the author believes that with further study this problem might be solved. The spiral thickening shown in *D. biforme* may be significant.

The remaining four species pose no real problems to an anatomist. Each possesses features significantly different from the others.

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APPENDIX

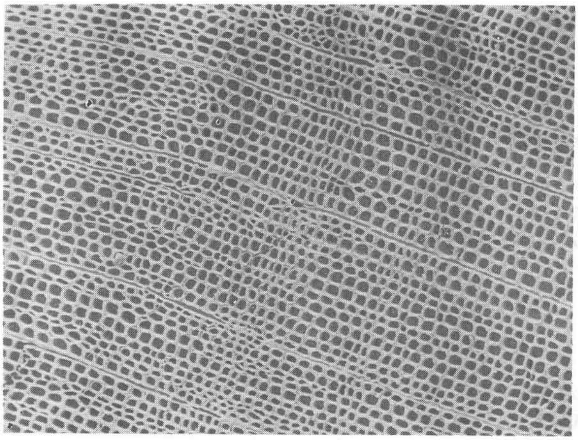

Fig. 1. *D. cupressinum* T.S. Indistinct growth rings (X 100)

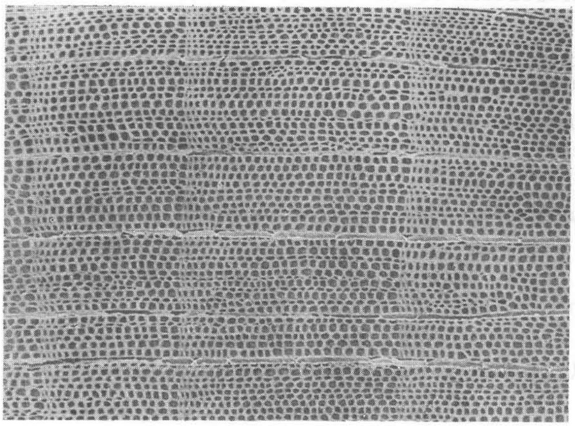

Fig. 2. *D. colensoi* T.S. Distinct growth rings (X 70)

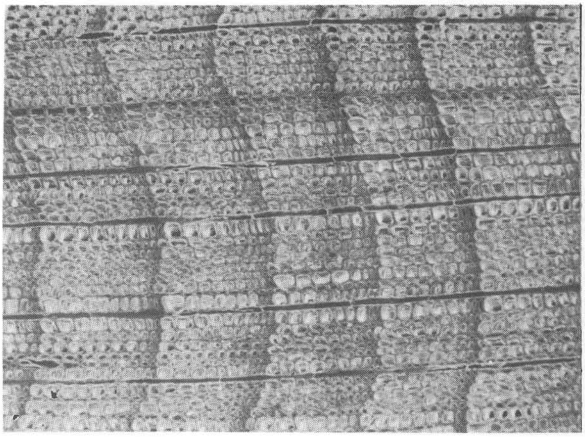

Fig. 3. *D. biforme* T.S. Distinct and wavy growth rings (X 125)

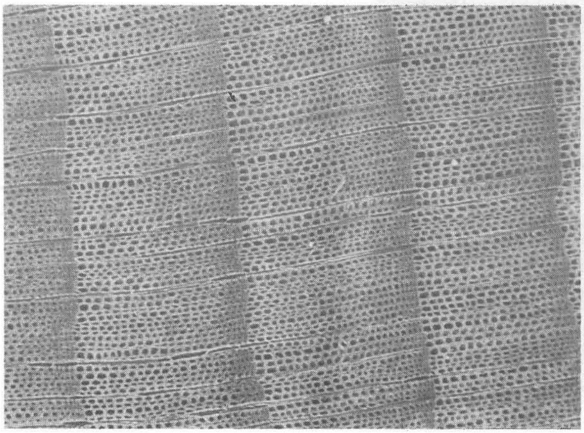

Fig. 4. *D. bidwillii* T.S. Distinct and slightly wavy growth rings. Note the pronounced late wood (X 80)

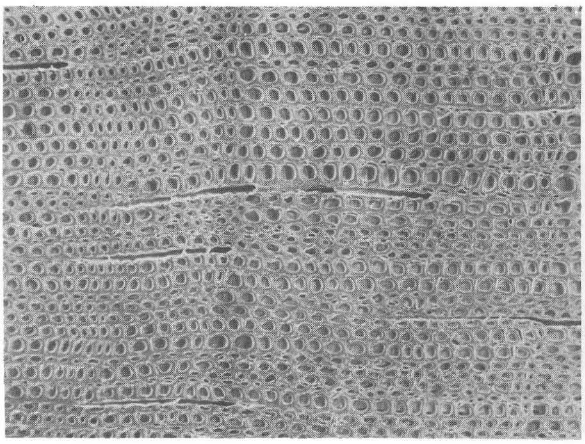

Fig. 5. *D. kirkii* T.S. Slightly distinct growth rings (X 1580)

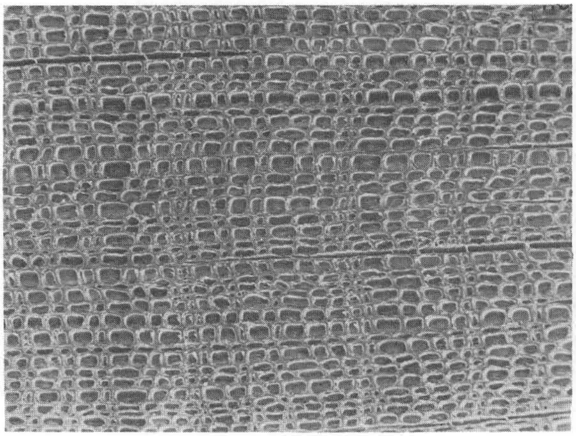

Fig. 6. *D. intermedium* T.S. Distinct and slightly distinct growth rings (X 160)

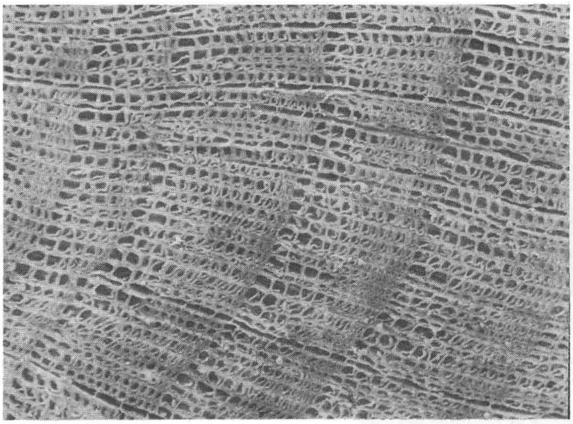

Fig. 7. *D. laxifolium* T.S. Distinct and slightly distinct growth rings (X 160)

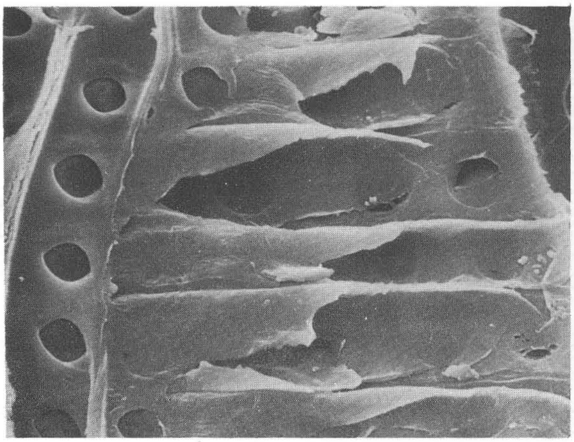

Fig. 8. *D. cupressinum* R.L.S. Cupressoid cross-field pits as seen on the ray parenchyma and tracheid walls (X 1250)

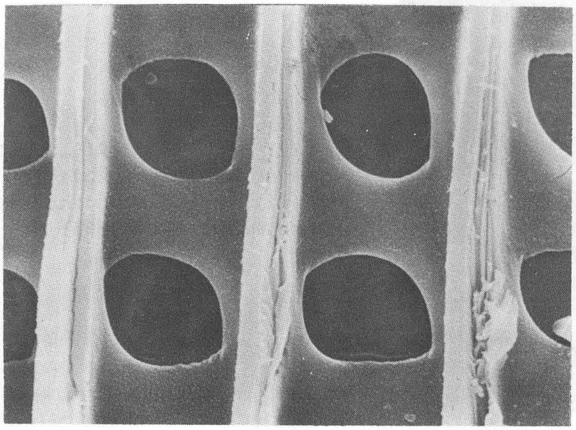

Fig. 9. *D. colensoi* R.L.S. Large window-like pits as seen on the tracheid wall (X 1385)

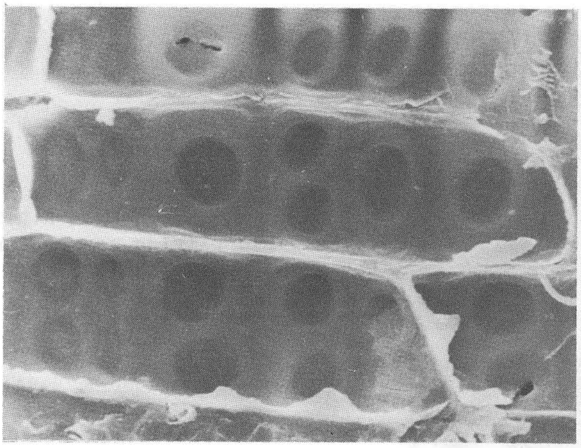

Fig. 10. *D. biforme* R.L.S. Large "cupressoid" cross-field pits as seen on the ray parenchyma walls (X 1170)

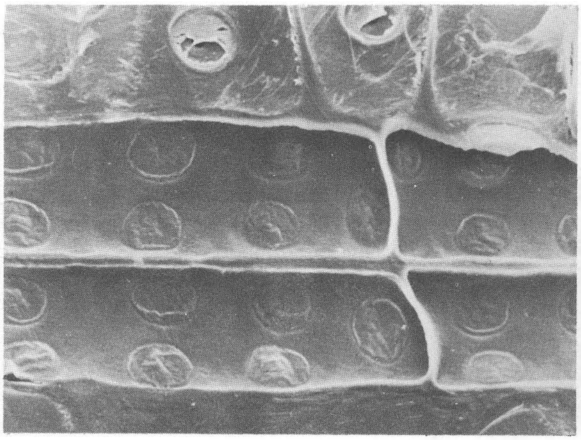

Fig. 11. *D. bidwillii* R.L.S. Large "taxodioid" and "cupressoid" cross-field pits as seen on the ray parenchyma walls (X 1265)

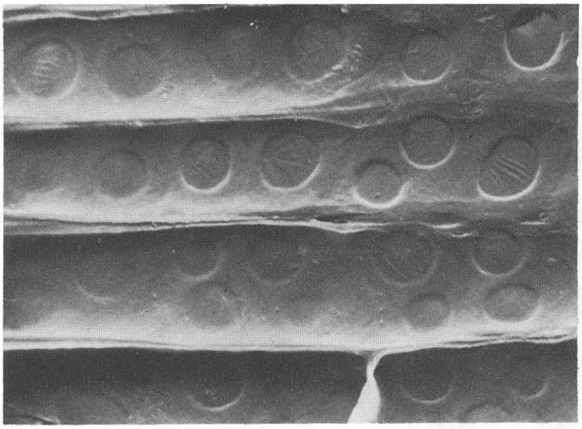

Fig. 12. *D. kirkii* R.L.S. Large "cupressoid" and "taxodioid" cross-field pits as seen on the ray parenchyma walls (X 595)

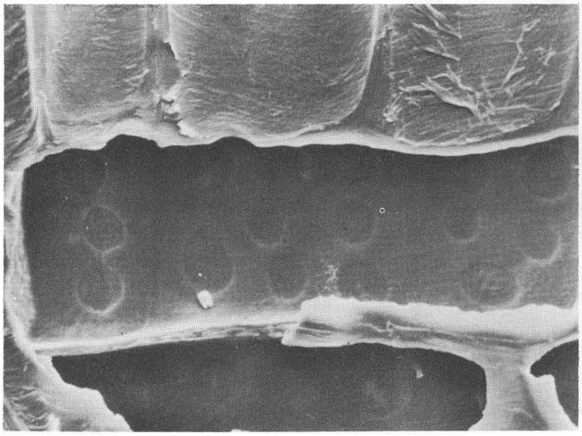

Fig. 13. *D. intermedium* R.L.S. Cross-field pitting as seen on the ray parenchyma wall (X 1580)

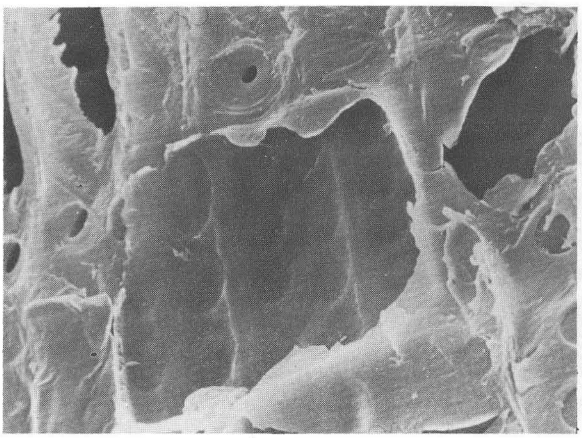

Fig. 14. *D. laxifolium* R.L.S. Small cross-field pits as seen on ray parenchyma walls. Note some larger pits and the length of ray cells in terms of tracheid number (X 1820)

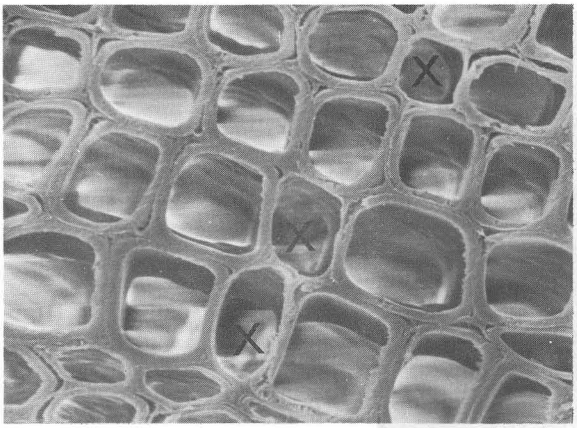

Fig. 15. *D. cupressinum* T.S. Axial parenchyma cells (marked with an X). Note difference in wall thickness between tracheid and axial parenchyma (X 1580)

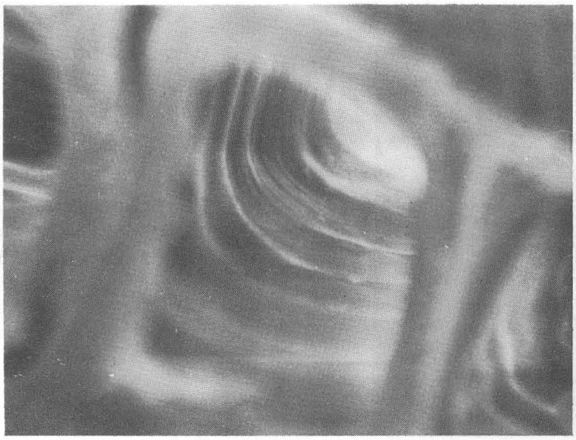

Fig. 16. *D. cupressinum* T.S. Tracheid showing fine spiral thickening (X 3950)

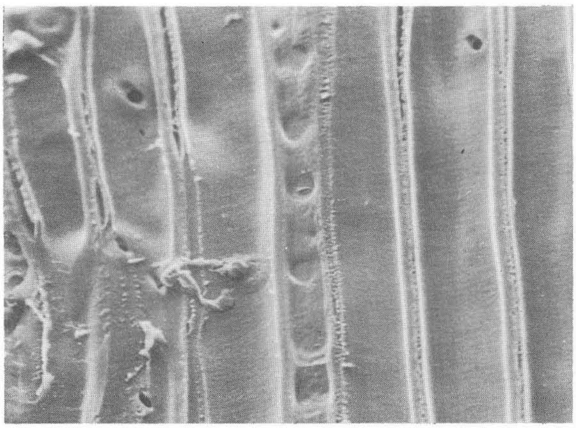

Fig. 17. *D. cupressinum* R.L.S. Sieve-like pits between axial parenchyma and a tracheid (X 325)

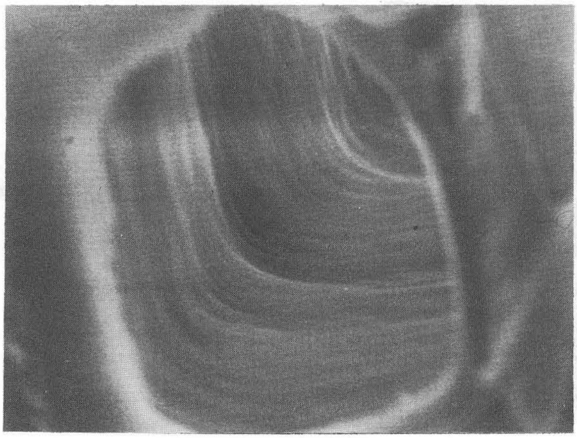

Fig. 18. *D. biforme* T.S. "Incipient" form of spiral thickening (X 9320)

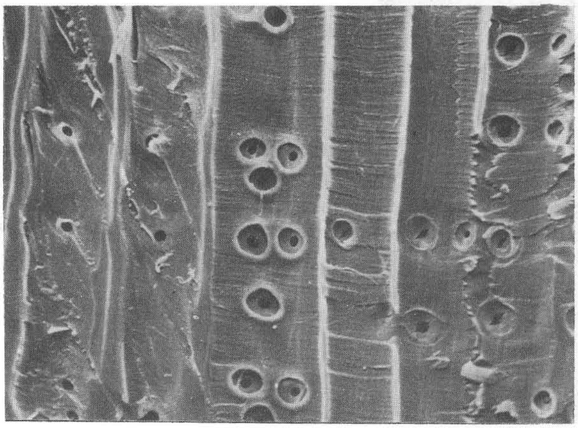

Fig. 19. *D. intermedium* R.L.S. Bordered pits oppositely paired and in groups of three (X 340)