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# STUDIES ON *HEXAGONIA POLYGRAMMA* MONT. IN RELATION TO ITS HOST *DIOSYPYROS EMBRYOPTERIS* PERS.

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The external symptoms of infected host trees *(Diospyros embryopteris)* and the extend of decay of the same by the pathogen *(Hexagonia polygramma)* have been fully described. The texture of the wood below the bark has been found to be considerably light, soft and spongy. The fibres have lost their toughness and become 'brash'. The bark has been heavily cracked both transversly and longitudinally through which the basidiocarps of the fungus have developed. The transeverse and longitudinal sections of the infected branches have shown rot pockets of various sizes which are filled up with mycelia and dusty fibres. The pathogen has possibly gained entrance through short branch stubs which usually act as portals of entry in the field condition. Considerable discolouration of the rotted wood have also been noticed. The microscopic detail of the rot caused by the pathogen in early stages and advance stages have been studied in the laboratory and in the field condltion respectively. The fungal hyphæ have been found to be confined to the vessels and wood parenchyma. The hyphæ run more or less longitudinally but their branches pass in transeverse directions through simple pits and directly through the cell walls following bore-holes. The fibres and all other wood elements have also been severely attacked. Microchemical tests have been performed in order to find out the effects of decay on the principle chemical components of the wood elements.

## INTRODUCTION

The species *Diosypyros embryopteris* Pers. belongs to the family Ebanaceæ and it is of considerable economic importance for its fruits and also as a source of commercial timber. In West Bengal, it has been found in various parts of the state. The trees are often found to be heavily damaged due to attack by a wood-rotting basidiomycetes *viz., Hexagonia polygramma* Mont. The trees which are particularly found to grow in shady places suffer mostly causing heavy losses.

Investigation on the study of biology of wood-rotting fungi in relation to tree diseases and timber decay are of immense importance in this country. Bose (1919, 1936), Bagchee (1954), Bakshi *et al* (1971), Banerjee (1955) and Banerjee *et al* (1954, 1960) have studied in this lines of research.

In this present investigation attempts have been made to throw some light on the association of the above mentioned host and parasite.

**Isolation of the pathogen and its identification from the rotted tissues**

In order to study the various aspects of host-pathogen relationship and to establish the pathogenic nature of the organism, it is necessary to isolate the organism from the rotted (infected) host tissues.

Isolation of the pathogen has been done following the technique by Banerjee (1955). Several isolations have been made from different rotted areas of the infected branches. All the isolates, thus obtained, have been examined microscopically and found to be of similar nature in so far as the hyphal characters are concerned. These cultures have been subcultured in *malt agar* slants and preserved for future study.

**Symptoms**

The symptoms of the disease are few but conspicuous. The infected trees can be easily identified in the field condition showing die-back branches projecting through handsome lustrous green conopy of the foliages. These branches have been found to be heavily covered with braket-shaped fructification of *H. polygramma* and with cankers of various sizes here and there. On closer examination it has been observed that the basidiocarp of the fungus come out through the cracked bark of the host tree (Plate I, Fig. 1). In the apparently healthy part of the plant these fructifications are, however, not uncommon, but always they are solitary. The trees with heavy infection in the advance stage bear abundant fructifications in clusters not only over the surfaces of the dried up branches but also on the main trunk. Some of the areas of these branches become somewhat swollen with fructifications thereon. The texture of the wood below the bark has been examined by means of a scalpel and found to be considerably light, soft and spongy and the fibres breaking-off into short pieces over the scalpel points. This indicates that fibres have lost their toughness and have become 'brash'. The bark when removed shows the presence of patchy whitish mycelium here and there over the surface of the sapwood. The bark has been heavily cracked both transversely and longitudinally. Through these cracks as has already been stated, the fructifications brust open in the surrounding atmosphere.

The gross characters of the rots show that in all cases the decay has progressed considerably within the wood. For obvious reasons early stages of decay and mode of infection cannot be determined. From the longitudinal section of the wood (Plate I, Fig. 2) it appears that the organism under consideration must have gain entrance through short branch stumps which usually act as the portal of entry. After killing the cambium it must have gained entrance into the sapwood as it has been found that the rots progress from periphery towards the centre of

the wood. The rotted area at first appears as isolated patches which eventually coalesce laterally forming wider areas. As the decay progress it finally attacks the small central heartwood. The cross section (Plate I, Fig. 3) ends of the branch cylinders show rot-pocket of various sizes which are filled up with mycelium and dusty fibres. The bleaching of the wood is gradual, the reddish-brown colour gradually fading to pale yellowish to whitish tinge. In longitudinal section (Plate I, Fig. 4) the colouration is rather patchy and the colour of the streak-like heartwood shows considerable decolouration.

**Microscopic detail of the rot**

In order to determine the effect of mycelium on the host tissues, many infected young and stouter branches have been collected from the field, and short cylinder of these have been preserved in Formal-acetic-alcohol (70% alcohol 85 ml., glacial acetic acid 5 ml. and formalin 10 ml.) for future use. Microscopic examination of transverse sections from different regions of these materials has revealed that the decay has progressed considerably in the various tissues and they show different stages of deterioration. Therefore, for obvious reasons, the early stages of decay cannot be determined from field collections. To study the early stages of decay the method suggested by Banerjee and Sinha (1954) has been followed. The sound sapwood of *Diosypyros embryopteris* has been cut into several small rectangular blocks (5 cm. × 2.5 cm. × 1 cm.) sterilized and exposed aseptically to both primary and secondary mycelia of *H. polygramma* growing separately on 2.5 percent *malt agar* in Kolle flasks and incubated at 30°C in complete darkness for 30, 60, 90 and 120 days. The sapwood before introducing into the Kolle flasks have been thoroughly water-soaked in order to keep the moisture-content of the test pieces much above the 'fibre-saturation-point'. This has been calculated to be about 50-60% of the originally dry weight of the wood. At intervals of 30 days the test blocks have been taken out of the flasks and preserved in formal-acetic-alcohol, as stated before.

Before sectioning a special treatment (Anonymous, 1946) has been done to soft the wood pieces. They have been allowed to soak in distilled water and boiled gently in a litre flask till sank to the bottom. For studying the advanced stages of decay this process have been found to be necessary in cases of the infected test pieces. This has been done by transferring the wood blocks to mixture of equal volume of glycerine and methylated spirit and allowed to stand in this condition for about 72 hours before sectioning. Healthy test blocks have also been treated in the same way and these have been served as controls. Transverse, radial-longitudinal and tangential-longitudinal sections have been made both free hand and with microtome. Free hand sections have been found to be more suitable as the mycelium has remained intact and without much destortion. As such, the microtome sections (15 – 20$\mu$ thick) have been discarded. The sections

have been subjected to various types of differential staining methods. The combination stains Safranine and Picro-aniline blue (Cartwright, 1929), and safranine and Fast green (Anonymous, 1946) have been found to more suitable for detecting hyphæ in the woody tissues. In the former case, the lignified tissue have been taken up the red stain and while the fungal hyphæ the blue stains. In the latter case the fungus mycelium has taken up the green stain and appeared clearly differentiated from the red-stained wood elements.

**Early stages of decay**

The fungal hyphæ have been found to be mainly confirmed to the vessels and wood-parenchyma (Plate I, Fig. 5). The hyphæ are mostly thin walled, hyaline, closely and distinctly septate, sparsely and profusely branched. In case of secondary mycelium the clamp-connexions are abundantly found, but these are absent in sections showing the primary mycelium. The hyphæ are more conspicuous within the vessels, being $1 - 1.5\mu$ in diameter, and run more or less longitudinally (Plate I, Fig. 6). The branches (about $0.5 - 0.7\mu$ in diameter) are rather narrow but more frequent within other host tissues. Due to the presence of dense cytoplasmic contents they are readily stained. The hyphal branches pass in transverse directions mostly through simple pits (Plate I, Fig. 7). In some cases the wood-fibres remain practically unaffected. A few hyphæ have been detected within the lumina of some of the vessels but in certain cases, a dense tangle of hyphæ fills up the lumina of the vessel. The dissolution of the vessel walls has taken place partially and irregular gaps have appeared here and there due to breaking down of wood parenchyma (Plate I, Fig. 8). The medullary rays also have suffered considerably but the tracheids and wood-fibres remained practically unaffected even after 4 months.

**Advance stage of decay**

The decay is more prominent in the advanced stages. The fibres and all other wood elements have been severely attacked (Plate II, Fig. 9). The hyphæ usually have grown in more or less longitudinal direction just after entering through the pits from the adjacent elements. The hyphæ have all been found to be hyline, branched and with abundant simple clamp-connexions, *i.e.*, they are in the secondary stage of development. The walls of the wood-elements have become thinner (Plate II, Fig. 10) and in certain cases they have been completely broken down (Plate II, Fig. 11). Thinning of the walls of the vessels, wood-parenchyma and fibres have been found to be gradual and the removal of gummy materials from the majority of the medullary ray cells have appears to be a notable features (Plate II, Fig. 12). Finally, the tissues have lost their strength and broken down at places forming isolated gaps (Plate II, Fig. 13). The compactness of the wood has been found to be lost in a very late advanced stages of decay making the wood unsuitable for sectioning.

**Modes of penetration**

Numerous bore-holes have appeared in the advanced stages of decay (Plate II, Fig. 14). They are wider in diameter than the hyphæ passing through them. This is possibly due to the enzymatic action of the hyphæ at the point of contact. The hyphal penetration takes place not only through the pits but also through the cell walls forming bore-holes. The much branched finer hyphæ in passing through the cell-walls show little or no diminution in diameter, but the large ones become somewhat attenuated or constricted at the apices (Plate II, Fig. 15). The hyphæ which pass through the pits appear to enlarge their diameters gradually after emergence. The bore-holes are large, circular, oval or irregular in outline with smooth and moulded contours and not with splintered walls (Plate II, Fig. 16). This support Proctor (1941) view of cell-wall penetration due to enzymatic activity of the casual organism. Schmid *et al* (1964) while working with beech spruce-wood decay by *Polyporus versicolor* L., has shown that in the early stage of decay, hyphæ penetrate the vessels via pits and rarely directly through cell-walls.

**Microchemical studies**

In order to gain some preliminary knowledge on the amount of depletion of lignin and cellulosic substances, microchemical tests, that are commonly performed, have been done. Hirt (1927), Harlow (1928) and others are of opinion that these, being stain-reactions, cannot be regarded as the absolute indicators of the degree of utilization of lignin and cellulosic substances by the pathogen during wood-decay. But these methods are still in vogue and have therefore, been performed in order to determine qualitavively the presence or absence of lignin and cellulosic substances in the wood samples.

Transverse, tangential and radial longitudinal sections of the infected wood-samples have been treated with Phloroglucin-Hcl and Chloro-Zinc-Iodine for the detection of llgnin and cellulosic substances respectively. The lignified walls have exhibited different shades of red colouration when treated with the former while the latter turns the walls various shades of blue colouration due to the presence of cellulosic substances.

The diff-rential stains Safranine and Fast Green (Anonymous, 1946) have also been tried to differentiate lignified (red stained) and non-lignified (green-stain) tissues. For further varification "Maule treatment" (for detection of the presence of lignin) as well as Iodine, Potassium iodide (for the detection of cellulose) and 72% Sulphuric acid test have been performed.

The results of various staining reactions have been given in the Tables 1 – 5.

Phluroglucine-Hcl and Chloro-zinc-iodine tests reveals that both lignin and cellulosic substances are present in normal and partially decayed wood in sufficient quantity. Safranine and Fast Green stains show that both cellulosic substances and lignin are closely combined in some of the wood elements. In normal wood

the staining reaction exhibits a good colour reaction, whereas, partially decayed wood fails to show the same degree of colour reaction due to gradual depletion of both lignin and cellulosic materials during 4-months decay under laboratory conditions.

**72% Sulphuric acid test**

Following Ritter (1925) these test has been perfored to established the fact that considerable amount of cellulosic material still remain in the wood element even in advance stage of decay. Sections of both normal and decayed wood have been placed on slides and treated with 72% sulphuric acid. Immediately the sections of both types of wood not only show considerable swelling but also show notable tissue distortion. The middle-lamellæ become seperated and the elements appears in isolated groups, while the medullary ray cells become much convoluted. This violent reaction indicate the presence of considerable amount of cellulose in the cell walls of both normal and decayed wood. As a result of swelling of cellulose preceeding dissolution, the element of the wood are forced apart. This test though not conclusive itself, indicates that considerable amount of cellulose is present in the cell walls of partially decayed wood.

Table 1. *Results of staining in normal and partially decayed sapwood of* Diospyros embryopteris *with Phloroglucin-Hcl for the detection of lignin.*

| Wood element | Normal wood | Partially decayed wood |
|---|---|---|
| Ray cells | Reddish tinge on the primary wall ; secondary walls light pink. | Reddish tinge on the primary walls ; pinkish on the secondary wall. |
| Parenchyma | Red colour on the primary wall ; light red colour on the secondary wall. | Pale pink to colourless both on the primary and secondary walls. |
| Fibres | Red colour on the primary walls ; Pink to light red colour on the secondary walls. | Pinkish to pink on the primary walls ; light pinkish colour in patches on the secondary walls. |
| Tracheids | Deep red colour of the primary wall ; light red to pinkish on the secondary walls. | Light red colour of the primary walls, pink colour on the secondary walls. |
| Vessels | Deep red colour on the primary walls ; light red colour on the secondary walls. | Light red colour on the primary walls ; pirk colour on the secondary walls. |

Table 2. *Results of staining in normal and partially decayed sapwood of* Diospyros embryopteris *with Chloro-zinc-iodine for the detection of cellulosic substances.*

| Wood element | Normal wood | Partially decayed wood |
|---|---|---|
| Ray cells | Light bluish green. | Almost colourless. |
| Parenchyma | Light blue colour on the secondary walls. | Light blue to colourless on the secondary walls. |
| Fibres | Light blue colour to colourless on the secondary walls. | Almost colourless secondary walls. |
| Tracheids | Light blue colour on the secondary walls. | Light blue to almost colourless secondary walls. |
| Vessels | Bluish patches on the secondary walls. | Almost colourless secondary walls. |

Table 3. *Results of staining in normal and partially decayed sapwood of* Diospyros embryopteris *with Iodine-Potassium-iodide for the detection of cellulosic substances.*

| Wood element | Normal wood | Partially decayed wood |
|---|---|---|
| Ray cells | Yellow colour primary walls ; pale bluish green colour secondary walls. | Yellow colour primary walls ; pale yellowish colour secondary walls. |
| Parenchyma | Deep yellowish brown colour primary walls ; pale blue colour secondary walls. | Deep yellow colour primary walls ; light yellow colour secondary walls. |
| Fibres | Deep brown colour on the primary wall ; blue colour on the secondary walls. | Brownish colour primary walls ; yellowish green secondary walls. |
| Tracheids | Brownish coloured, primary wall ; secondary walls mainly pale blue coloured. | Yellowish coloured primary walls ; yellowish green secondary walls. |
| Vessels | Golden brown coloured primary walls ; pale greenish blue secondary wall. | Yellowish coloured primary walls ; yellowish green coloured secondary walls. |

Table 4. *Results of staining in normal and partially decayed sapwood of* Diospyros embryopteris *due to Maule's test for the detection of lignin.*

| Wood element | Normal wood | Partially decayed wood |
|---|---|---|
| Ray cells | Pale orange colour in patches. | Pink colour primary walls ; pale pink tinge. |
| Parenchyma | Light red colour on the primary walls ; secondary walls pinkish. | Pink colour primary walls pale pink to colourless secondary walls. |
| Fibres | Deep red coloured primary walls ; light red to pale pink coloured secondary walls. | Pink coloured primary walls ; light pink to colourless secondary walls. |
| Tracheids | Red coloured primary walls slightly pinkish coloured secondary walls. | Pinkish red coloured primary walls ; secondary walls pale pink to colourless. |
| Vessels | Deep red coloured primary walls ; pale pink to almost colourless secondary walls. | Pinkish to colourless primary walls ; secondary walls colourless. |

Table 5. *Results of staining in normal and partially decayed sapwood of* Diospyros embryopteris *with Safranine and Fast Green for the detection of both lignin and cellulosic substances.*

| Wood element | Normal wood | Partially decayed wood |
|---|---|---|
| Ray cells | Deep red coloured primary walls ; secondary walls light red. | Primary walls light red coloured ; secondary walls pink to colourless. |
| Parenchyma | Red coloured primary walls ; greenish or light red mixed with patches of light green on the secondary walls. | Light green coloured on the primary walls ; secondary walls greenish. |
| Fibres | Deep red colour primary walls ; secondary walls red or light red, where lumina are large secondary walls entirely light green. | Red colour primary walls ; secondary walls red or light red. |
| Tracheids | Red coloured primary walls ; secondary walls light red. | Primary walls red ; pink coloured secondary walls. |
| Vessels | Deep red coloured primary walls ; secondary walls light red. | Pink to colourless primary walls ; light pink to colourless secondary walls. |

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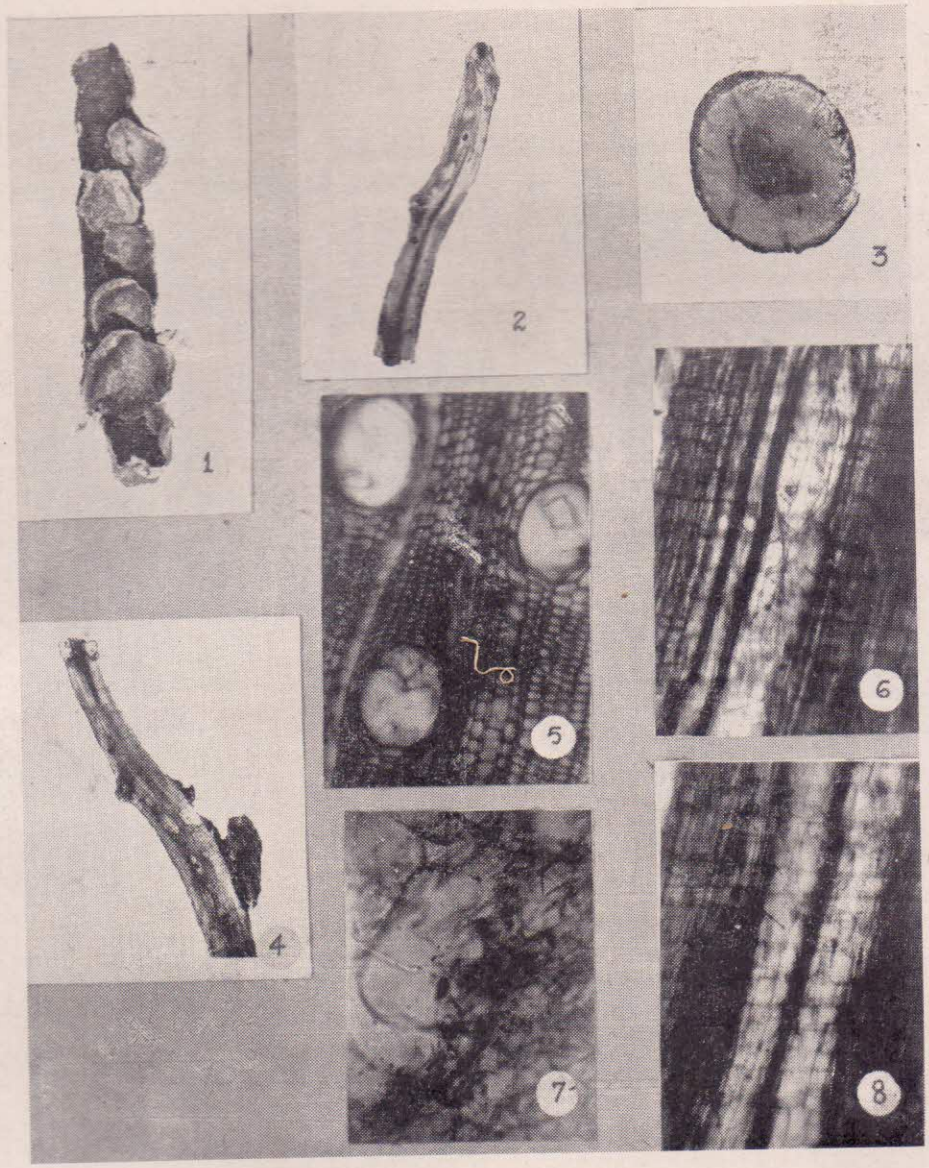
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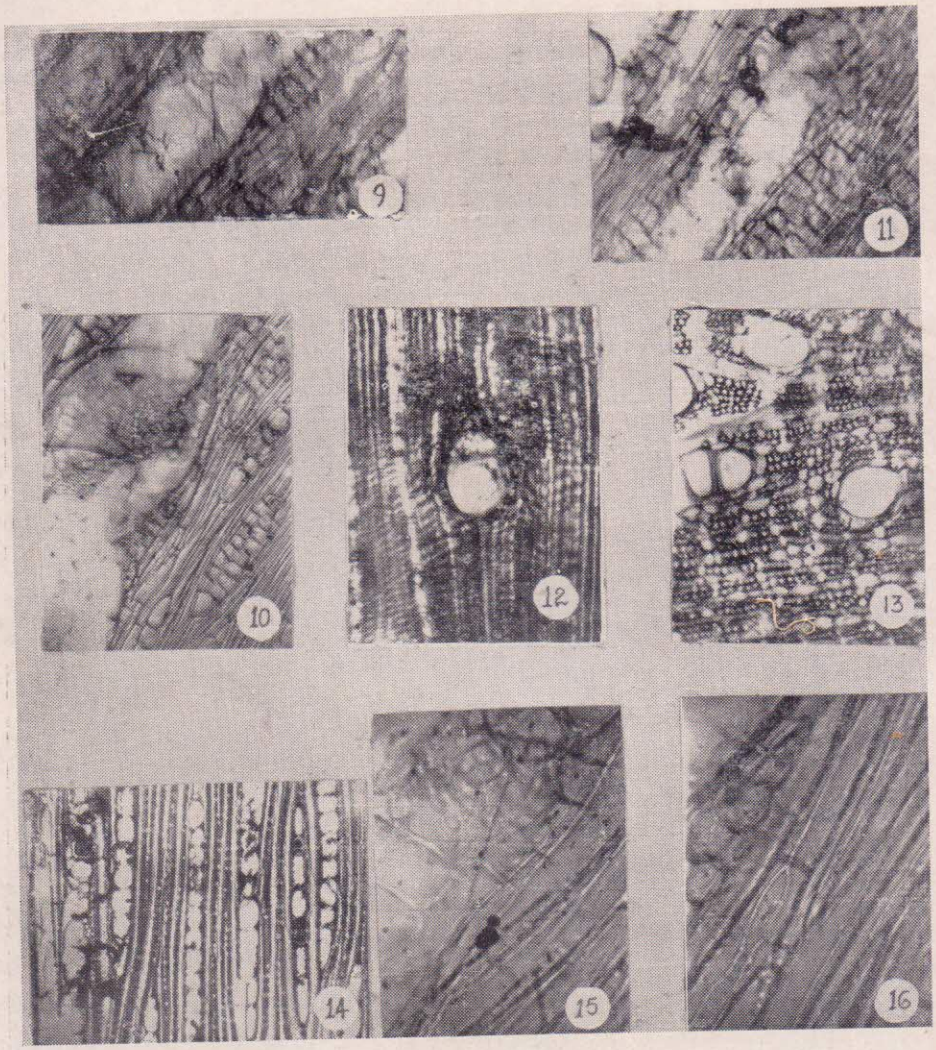
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## EXPLANATION OF PLATES

### PLATE I

Fig. 1. A portion of a branch of *Diospyros embryopteris* showing cracked bark with basidiocarps and a decayed branch stub which has possibly served as portal of entry of the pathogen.
Fig. 2. A longitudinal section of a portion of decayed branch of *D. embryopteris* showing the central sound wood separated from the peripheral zone of decayed wood.
Fig. 3. A transverse section end of a portion of decayed branch of *D. embryopteris* showing characteristics rot-pockets and bleaching of the wood.
Fig. 4. A longitudinal section through a portion of a highly decayed branch of *D. embryopteris* showing discolouration of the wood.
Fig. 5. Photomicrograph of transverse section (Part) of sapwood (4 months decayed) of *D. embryopteris* showing the distribution of hyphæ in the vessels and wood parenchyma (×750).
Fig. 6. Photomicrograph of a longitudinal section (Part) of host wood (4 month decayed) of *D. embryopteris* showing the running of fungal hyphæ in longitudinal direction within the vessels (×750).
Fig. 7. Photomicrograph showing the presence of fungal hyphæ within the vessel even after severe destruction of the host-wood elements (naturally decayed) ×1250.
Fig. 8. Photomicrograph of a longitudinal section (Part) of host-wood (4 month decayed) of *D. embryopteris* showing the breaking down of the cell walls of the wood Parenchyma due to attack by *Hexagonia polygramma* (×750).

### PLATE II

Fig. 9. Photomicrograph of a longitudinal section (Part) of host-wood (naturally decayed) of *D. embryopteris* showing severely affected fibres of wood elements (×750).
Fi. 10. Photomicrograph of a tangential section (Part) of host-wood (4 month decayed) of *D. embryopteris* showing the thining of the wall of wood elements due to fungal attack (×750).

## PLATE II

Fig. 11. Photomicrograph of a tangential section (Part) of host-wood of *D. embryopteris* (naturally decayed) showing complete destruction of cell-walls due to fungal attack (×750).

Fig. 12. Photomicrograph of a transverse section (Part) of host-wood (4 month decayed) of *D. embryopteris* showing the removal of gummy materials from the medullary rays (×750).

Fig. 13. Photomicrograph of a transverse section (Part) of host-wood (naturally decayed) of *D. embryopteris* showing the formation of isolated gaps due to dissolution of host-wood elements (×750).

Fig. 14. Photomicrograph of a longitudinal section (Part) of host-wood (naturally decayed) of *D. embryopteris* showing the formation of nemerous bore-holes (×750).

Fig. 15. Photomicrograph of longitudinal section (Part) of host-wood (4 month decayed) of *D. embryopteris* showing the attenuation of the fungal hyphæ during passing through the bore-holes (×750).

Fig. 16. Photomicrograph of a longitudinal section (Part) of host-wood (4 months decayed) of *D. embryopteris* showing the somewhat circular large bore-holes (×750).