Indian J. mycol. Res., 7 (2), 61—69 (1969)

FLAMMULA DILEPIS B. & BR. AND THE ASSOCIATED BAMBOO—ROT

By

BALEN NANDI

Department of Botany, University of Burdwan, Burdwan, West Bengal, India.

(WITH PLATE I)

The present investigation has been undertaken to study the biology of *Flammula dilepis*, a common yellowish-brown spored member of Agaricaceæ causing decay of *Bambusa bambos* during rainy months in Calcutta and suburbs. History, synonymy, distribution and host-range of the fungus have been discussed. Its distribution has been found to be restricted only in the tropics. The morphology and anatomical structures of the basidiocarp of the fungus have been fully described. The basidia are tetrasterigmatic and quadrisporous. The oval, yellowish-brown basidiospores have verrucose wall with an excentric hilum. Gross characters of the rot produced by the fungus have been noted. The decay is mainly restricted to the softer areas at first but later other parts become affected as well Histopathological studies reveal the presence of abundant, thin-walled hyaline hyphæ with frequent clamp-connexions. In a very advanced stage of decay, tissues of the host show appearence of cracks due to dissolution of cell-walls. Microchemical tests of the decayed host tissues reveal that the test-fungus can consume cellulose at a much higher rate than lignin. Decay resistance tests of three local varities of bamboos 'Basni', 'Talla' and 'Volka' under a set of controlled conditions of 30°C. in complete darkness show that the loss due to decay is maximum in 'Talla' followed in order by 'Basni' and 'Volka'. Oxidase tests show that *F. dilepis*, being a 'white rot' fungus, develops deep brown oxidase rings when grown on *malt-agar* medium containing 0.5% gallic or tannic acid.

INTRODUCTION

Flammula dilepis B. & Br., a common brown-spored member of the family Agaricaceæ, is found to grow luxuriantly on stumps, poles and posts of bamboo (*Bambusa bambos* Druce) in Calcutta and suburbs causing their decay. Besides bamboo, it has been reported to grow also on stumps of *Borassus flabellifer* L., *Cocos nucifera* Linn., and logs of *Shorea robusta* Gærtn. *f.* (Bose, 1920 ; Banerjee, 1947). Berkeley and Broome (1871) first described the fungus based on collections of Thwites (1869) from Peradeniya, Ceylon. Besides Ceylon the fungus has so far been reported only from Bengal (Banerjee and Ghosh, 1942-43). The occurrence of the fungus in Ceylon and Bengal and the absence of its occurrence from temperate countries, suggest its possible distribution only in the tropics. Recently, Singer (1962) in his classification of Agaricales records this species as *Gymnopilus dilepis* (B. & Br.) Sacc. (*Flammula* Sacc.) belonging to the family Cortinariaceæ.

It appears, from the available literature that so far nothing has been done on *F. dilepis* in relation to bamboo-rot. In the present paper an attempt has, therefore, been made to study its nature and the extent of damage it causes to the bamboo, one of the chief forest products of India.

Source of Material

The fructifications of *F. dilepis*, at various stages of development, were collected during the months of July to October, while growing luxuriantly on stumps and poles of bamboos in bamboo-groves and timber-yards of Calcutta and its suburbs (Plate I, Fig. 1). Shady places with minimum amount of light and maximum humidity provide an environment most suitable for the growth of the fungus. Bamboo showing various stages of decay together with fructification of *F. dilepis* were also collected and the macroscopic characters of the rot were noted. Microscopic examination of the rots revealed the presence of numerous, much branched, separate hyphæ with fruquent clamp-connexions within the different tissue elements. Spore-deposits were taken on dry slides from fresh fruit-bodies since dried specimens failed to discharge spores even after soaking. Several polysporous and monosporous cultures were made from the these spores on 2.5% *malt-agar* slants and incubated at 30°C. The polysporous cultures on microscopic examination revealed the presence of much branched, septate mycelium with abundant clamp-connexions which were found to be lacking in the mycelia of monosporous origin.

The Basidiocarp

Fructification : Centrally stipitate, sometimes excentrically so ; about 1.5—3 (6) cm. high. *Pileus* : Broadly convex, umbonate, then expanded and depressed, somewhat orbicular or irregular in outline, fleshly to coriaceous, 1—3 (5) cm. across, 0.5 – 0.7 cm. high when convex. *Upper surface* : Minutely scaly throughout, (Plate I, Fig. 2) more abundant around the centre, easily rubbed off, colour Mellow glow or Golden yellow at first, becoming Rawsiena or Gold brown towards the periphery with age or in patches in older specimens. Margin thin, entire and straight at first, becoming uneven or upturned and lacerated with age, incurved on drying, concolourous with the upper surface. *Flesh*: Thin, soft but with a staff cuticle, Italian straw or Amber white in colour, 0.5 – 1 mm. in thickness. Consisting of thin walled or slightly thick-walled hyphæ, sparingly branched, closely or distantly septate, yellowish in colour when comparatively narrow hyphal cells are more or less uniformly cylindrical and with frequent clamp-connexious. In wider hyphæ, hyphal cells slightly or irregularly inflated, constricted at the septa and clamp-connexions lacking, with dense or sparce granular contents, with variable widths, ranging from 5.5 – 12.5μ. *Hymenial surface*: Lamellate (Plate I, Fig. 3); gills adnate or subdecurrent, soft, subdistant or close, ventricose, Princeton orange or Golden poppy in colour, sometimes Orange Rufous, changing to Titian, Amber brown or Gold pheasant when bruished, 0.5 – 1.5 mm. broad, edge entire, splits with age. *Hymenium*: Consisting of basidia and cystidia, basidia showing different generations, basidial layer with cystidia upto 27μ thick. *Basidia*: Clavate, tetrasterigmatic and quadrisporous, each with a simple clamp-connexion at the base, dimension about 17 – 21.5 × 6.5 – 10.5μ at maturity; sterigmata about 5.5 – 7.5μ long. *Basidiospores*: Yellowish brown, wall thick and varrucose, more or less oval when viewed from front or back, in side view slightly depressed at one side, with an excentric hilum, full of granular contents, dimension about 7.5 – 8.5 × 5.5 – 6.5μ. *Cystidia*: Large, ventricose-rostrate, thick-walled, brownish in colour, present on both sides of the gill and not at the edge, projecting upto 6.5μ beyond the basidial layer, subhymenial in origin, dimension about 20.5 – 27.5 × 7.5 – 12.5μ. *Subhymenium*: Consisting of one or two layers of somewhate circular, vacuolated hyphal cells with granular contents. *Trama*: Consisting of loosely arranged interwoven hyphæ, hyphal cells long, somewhat cylindrical or irregular in shape, with granular contents, about 225μ wide. *Stipe*: Central or slightly excentric, fleshy to fibrous, at first solid, (Plate I, Fig. 4) becoming hollow in older specimens, uniformly cylindrical throughout, with a slightly bulbous base or gradually dilated upwards into the pileus, at first smooth, sometimes becoming finely striate in larger specimens, not separable from the substance of the flesh, surface Mellow glow or light coloured, assuming darker tint when bruished, internally pale coloured, about 1 – 2.5 (5) cm. high and 0.15 – 0.2 (0.5) cm. in diameter. Hyphal composition similar to that of the flesh.

DECAY OF BAMBOO

Gross symptoms

The only externally visible symptom in the field is the appearence of groups of numerous yellowish brown pustules of different sizes, covering the surfaces of the stumps, posts and poles of bamboos. These pustules are the precursors of fructifications of the test-fungus and are formed as a result of condensations of internal mycelium. The transverse and longitudinal sections of these infected bamboos, however, show considerable decay of distinctive character (Plate I, Figs. 5 and 6). In early stages of decay the rot is rather patchy being confined to the softer areas but later other parts become affected. The bamboo, which is internally whitish in colour, gradully becomes light yellow and eventually assuming a dark brown tint. The softer tissues are gradually destroyed, the vascular bundles separate as distinct strands and a typical 'fibrous rot' develops. The bamboo finally looses its toughness, becomes soft, dries up, cracks lenthwise into pieces and is rendered useless for commercial uses. It has been noticed that the decay progresses gradually from the centre to the periphery and even in an advanced stage the peripheral portion of bamboo retains its toughness for a considerable period but is ultimately destroyed.

Isolation and identification of casual organism

Isolations of the fungus were made on *malt-agar* slants from different regions of the infected posts and poles of bamboos bearing the fructifications, following Banerjee (1955). These inoculated tubes were then incubated at a constant temperature of 30°C. in complete darkness and allowed to grow for a week. On critical examinations, both macroscopically and microscopically, these isolations not only resembled one another in all essential details but were found to be identical with the polysporous mycelia and tissue-cultures from the fruit-bodies of *F. dilepis* already made available for the purpose.

Histopathology

In the study of histopathology, infected bamboo showing various stages of decay were fixed and preserved in Formal-Acetic-Alcohol (10 : 5 : 85c. cm of 70% alcohol). Early stages of decay were studied from decaying test-blocks of bamboos, subjected to the attack of the fungus for a period of four months in the decay-resistance-tests.

Before sectioning, small pieces of sound and decayed bamboo were softened in boiling water and then treated in a mixture of glycerine and methylated spirit for three to four days. Transverse, radial longitudinal and tangential longitudinal sections 15-20μ thick were cut. Of the various differential staining methods, the combination stains with Saffranine and Picro-Aniline Blue (Cartwright, 1929) proved to be the best for detecting the fungal hyphæ within the host-tissues.

In the early stages of decay the distribution of hyphæ is more common in the ground parenchyma. A few hyphæ are also noticeable in other tissues. The hyphæ are hyline, thin-walled, sparsely branched, distinctly septate, 3-5μ wide, with frequent clamp-connexions and full of granular contents which take up the blue stain (Plate I, Fig. 7). In later stages of decay the hyphæ accumulate within vessels as interwoven mycelial wefts (Plate I, Fig. 11). As the decay progresses they also invade the tracheids and sclerenchyma cells. The epidermis, hypodermis and peripheral bundles, however, remain unaffected. Finally, in highly advanced stages of decay, all the tissues of the host are attacked. In no case appreciable thinning of cell-walls can be detected in early stages. The walls of the parenchyma cells gradually break down at places and this tissue appears as isolated groups (Plate I, Figs. 8 and 9). As a result of this dissolution, the vascular bundles become isolated and form strands. Eventually, the elements of the vascular bundles become separated mostly due to decay of xylem parenchyma and cracks appear in the bundle sheath (Plate I, Fig. 10).

The hyphæ in passing through parenchyma at first pass through the simple pits but later penetrate the cell-walls directly forming bore-holes. In case of vessels and tracheids the penetration is mainly through the pits. The bore-holes are more or less circular to oval and have smooth contours which indicate dissolution of the wall by enzymic activity (Proctor, 1941).

Microchemical studies

The change in chemical contituents of the different elements of the host-tissue due to decay can be most conveniently studied qualitatively by means of micro-chemical tests. Tests for cellulose and lignin have been performed with both sound and partially decayed bamboo. Phloroglucin-HCl and Chlor-zinc-iodine test have been employed to detect the presence of lignin and cellulose respectively. The intensities of reaction in sound and partially decayed bamboo have been compared and the results noted. 'Maule treatment' and Iodine-potassium iodide-sulphuric acid tests have been used to corroborate Phloroglucin-HCl and Chlor-zinc-iodine tests respectively.

The results of Phloroglucin-HCl and 'Maule treatment' shows the presence of considerable amount of lignin in all the elements excepting the parenchyma cells of the sound host-tissue. On the other hand, in partially decayed tissue, the intensity of colour reaction is lower indicating thereby that gradual delignification has taken place.

From the tests for cellulose, it becomes apparent that the cellulosic materials from the parenchyma elements have been consumed considerably during the process of decay and the destruction is much more severe than that suffered by the lignified tissues. From results it is evident that the fungus can consume cellulosic materials at a much higher rate than lignin.

Decay-resistance tests

Decaying capacities of different fungi vary greatly, being partly dependent on the power of resistance offered by the timbers attacked. In order to find out the natural resistance of bamboos to the attack of *F. dilepis*, decay-resistance tests under controlled conditions has been performed in the laboratory. The method as suggested by Banerjee (1955) has been mainly followed with some modifications.

Three local varieties of bamboo (*Bambusa bambos*), viz., 'Basni', 'Talla' and 'Volka' were employed for this experiment. Small rectangular blocks ($2'' \times 2'' \times 1\frac{1}{2}''$) were cut from healthy internodes, serially numbered, dried in an oven at 60°C. to constant weights and their initial dry weights were recorded. These blocks were then soaked in distilled water under reduced pressure with the help of a suction pump for facilitating ready soaking not only saved time but also prevented leaching of nutrients and other substances from the blocks which would otherwise render them poorer from nutritive stand point. The moisture content of the test-blocks was raised above the 'fibre saturation point' (Cartwright and Findley, 1946) for normal activity of the test-fungus. After soaking, the blocks were sterilized in an autoclave at 15 lbs. pressure for 15 minutes following Chidester (1937, 1939). The sterilized test-blocks of the three varieties were then aseptically and separately introduced into Kolle flasks, six in each, and placed over the actively growing primary and secondary mycelia of *F. dilepis*. A set of Kolle flasks without the fungus but containing the test-blocks was kept as control. The flasks were then incubated at a constant temperature of 25°C. and diffuse light inside a temperature controlled room. The percentage of the moisture-content of the wood-blocks was calculated from their dry weights following Savory (1954). The test-blocks were exposed to the fungal attack for a period of four months in order to obtain significant loss in weight of the blocks in the decay-resistance tests.

After the experimental period, the blocks were taken out of the Kolle flasks. The superficial mycelia from the test-blocks were carefully removed. The test-blocks were immediately weighed in order to determine the moisture content of the blocks after the experiment. These were then dried in an oven at 60°C. and the loss in dry weights were finally determined.

The results obtained has been given in Table 1.

Table 1. *Data showing comparative loss in dry weights (%) of 'Basni', 'Talla' and 'Volka' varieties of bamboo after four months' exposure to the mycelia of* Flammula dilepis.

Nature of mycelium	Bamboo variety					
	Basni		Talla		Volka	
	Range	Mean	Range	Mean	Range	Mean
Primary	5.45-9.78	7.18	10.18-22.85	18.10	2.55-5.92	5.25
Secondary	4.24-6.89	5.51	7.72-18.04	12.14	3.37-5.28	4.08

A comparative study of the losses in dry weights of the three varieties of bamboo reveals that the losses in 'Talla' variety due to attack by primary and secondary mycelia are maximum being 18.10% and 12.14% respectively. In 'Volka' and 'Basni' varieties, on the otherhand, the losses are comparatively less being 3.95% and 4.08% in the former and 7.18% and 5.15% in the later. It has further been noticed that the decay due to the attack of primary mycelium is greater in 'Talla' and 'Basni' varieties than due to secondary mycelium. But reverse condition exists in case of 'Volka' variety.

Following Findlay's classification (1938), the 'Talla' variety appears to be 'non-resistant' (10-30%) to the attack by *F. dilepis* while 'Basni' and 'Volka' varieties are 'moderately resistant' (5-10%) and 'resistant' (upto 5%) respectively.

The average moisture-contents of the test-blocks before the experiment were determined to be above 80% in case of 'Talla' variety and 70% in cases of 'Basni' and 'Volka' varieties. After the experiment, in all the three types of blocks, the moisture contents remained above 60% *i.e.*, much above the 'fibre-saturation point'. The normal activity of the fungus in causing decay, therefore, did not hamper during the experimental period.

The results clearly indicate that *F. dilepis* can damage the two local varieties of bamboo *viz.*, 'Talla' and 'Bansi' under saprophytic conditions and there is a definite relationship between the type of bamboo and the nature of mycelium.

Oxidase tests

Oxidase tests, following Bavendamm (1928) were performed in order to determine whether *F. dilepis* is a 'white rot' or a 'brown rot' fungus. Accordingly, both primary and secondary mycelia were grown separately on 2.5% *malt-agar* medium containing 0.5% gallic or tannic acid in Petri-dishes (100 mm. in dian). These were than incubated at 30°C. in complete darkness. After 24 hours of growth dark brown zone appeared around the inoculum which is characteristic of a 'white-rot' fungus. This positive reaction is due to the fact that the test-fungus capable of secreating extracellular oxidase enzyme and hence can destroy lignified portion of the host-tissue by oxidation process (Nobles, 1958).

DISCUSSION

A detailed study on the biology of *Flammula dilepis* B. & Br. make it possible to discuss, in general, some of the salient features regarding the life-history and the relation that exist between the fungus and the host. The fungus has been found to grow on different local varieties of (Talla, Basni and Volka) of bamboo

(*Bambusa bambos* Druce) which are of great economic value. Its yellowish-brown stipitate fruit-bodies have been found to appear luxuriantly during the rainy season on poles of bamboos.

An infected bamboo shows the characteristic 'fibrous rot' when it loses its toughness, becomes soft and cracks lengthwise. The decay is at first restricted to the softer parenchymatous tissue but as the decay progresses the hyphæ spread through the sclerenchyma vessels and tracheids. The penetration of the hyphæ is at first accomplished through the pits but later directly through the cell-walls forming bore-holes bue to enzymic activity of the fungus. Microchemical tests for detecting qualitatively the utilization of the chief chemical components of the tissue-elements in both sound and partially decayed bamboos reveal that considerable consumption of cellulosic materials with simultaneous delignification takes place. The fungus is distinctly a 'white rot' one as it gives strong positive reaction to oxidase tests. Further, decay-resistance-tests in the laboratory also reveal that the fungus can cause considerable damage to sterilized test-blocks of 'Talla' and 'Basni' varieties of bamboo, of which the former is 'non-resistant' (more than 10% loss) and the latter 'moderately resistant' (more than 5% loss). The 'Volka' variety, on the other hand, is resistant as it undergoes less than 5% loss in dry weights of the blocks during 4 months' test.

ACKNOWLEDGEMENT

The author expresses his deep gratitude to Dr S. N. Banerjee, M.Sc., Ph.D., for his kind guidance and valuable suggestion in the present investigation.

REFERENCES

Banerjee, S. N. (1947). Funguous flora of Calcutta and suburbs, *Bull. Bot. Soc. Beng.*, **1** : 37-54.

——— (1955). A diseases of Norway spruce *(Picea excelsa)* associated, with *Stereum sanguinolentum* and *Pleurotus mitis Ind. J. mycol Res.*, **1** : 1-80.

——— and Ghosh, T. (1942-43). Preliminary reports on the occurrence of higher fungi on bamboos in and about Calcutta, *Science and Culture*, **8** : 194.

Bavendamm, W. (1928). Neue Untersuchungen uber die Lebense-bedingungen holzzertorerder. *Pilze. Zbl. Bakt.*, 11, **76** : 172-277.

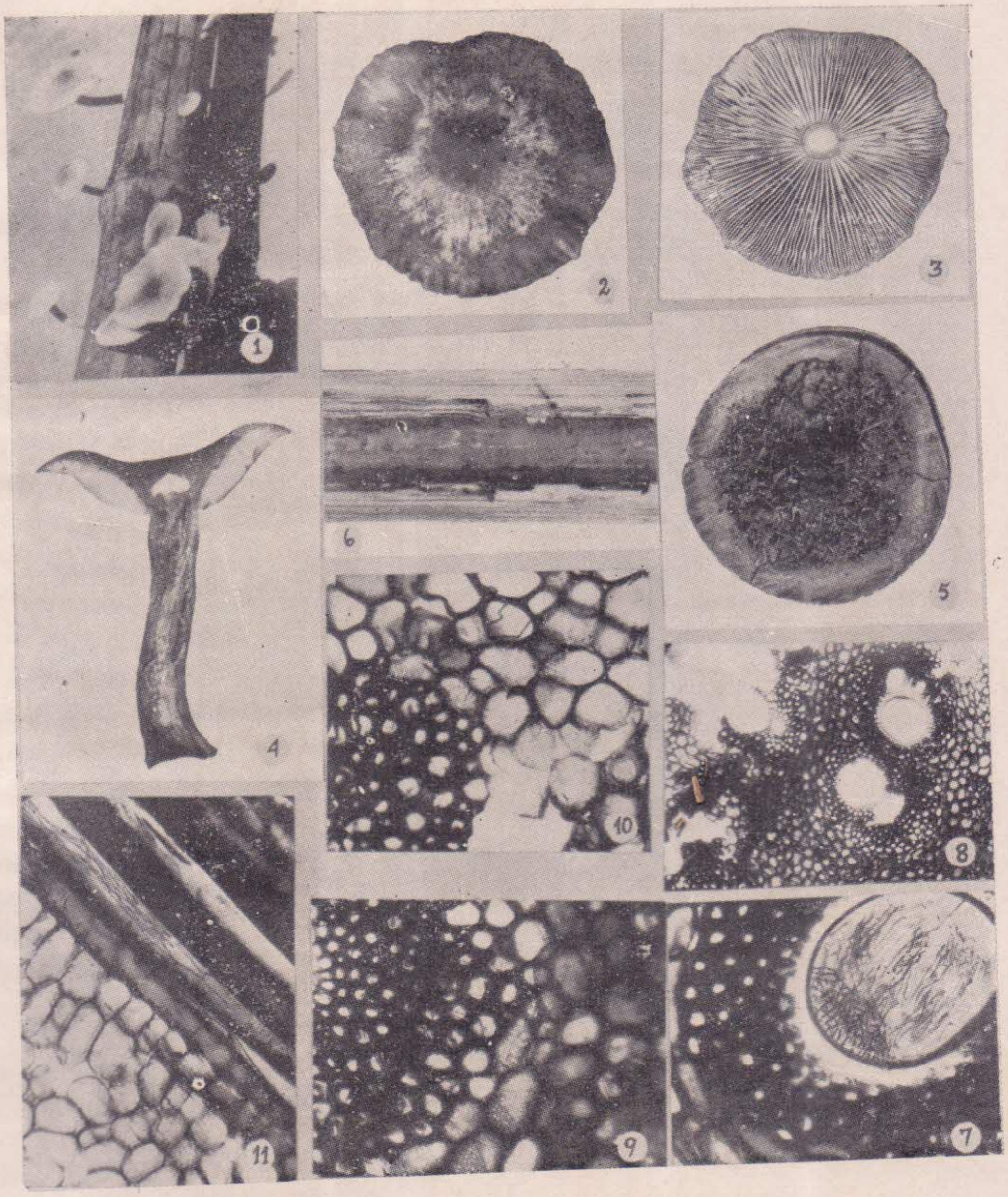
Berkeley, M. J. and Broome, C. E. (1871). Enumeration of the fungi of Ceylon. *Jour. Linn. Soc. Lond.* **11** : 542.

Bose, S. R. (1920). Records of Agaricacese of Bengal. *J. Asia. Soc. Bengal.* **16** : 347-351.

Cartwright, K. S. G. (1929). A satisfactory method of staining fungul mycelium in wood sections. *Ann. Bot. Lond.*, **43** : 412-413.

——— and Findley. W. P. K. (1946). *Decay of timbers and its prevention.* Lond.

Chidester, M. S. (1937). Temperature necessary to kill fungi in wood. *Proc. Amer. Wood Pres. Assoc.*, **35** : 316.



On *Flammula*

B. Nandi

Chidester, M. S. (1937). Further studies on temperature necessary to kill fungi in wood. *Prcc. Amer. Wood Pres. Assoc.*, 35 : 319.
Findlay, W. P. K. (1938). The natural resistance to decay of Empire timbers. *Emp. Forest J.*, 7 : 249-259.
Nobles, M. K. (1958). A rapid test for extracellular oxidase in cultures of wood inhabiting Hymenomycetes. *Canad. J. Res.*, 36 : 91-99.
Proctor, (1941). Penetrations of the walls of wood cells by the hyphæ of wood destroying fungi. *Yule Univ. Sch. For. Bull.*, 47 : 1-11.
Savory, J. G. (1954). Deterioration of timber by fungi and its preservation. *Brit. Eng.*
Singer, R. (1962). *The Agaricales in modern taxonomy*. J. Cramer, N. Y.

(Accepted for publication 20th September 1969)

PLATE I

Fig. 1. Fructifications of *Flammula dilepis* B. & Br. growing on bamboo.
Fig. 2. Fructifications of *F. dilepis* showing the upper surface with characteristic scales.
Fig. 3. Fructifications of *F. dilepis* showing the hymenial surface with gills.
Fig. 4. Longitudinal section of the fructification *F. dilepis* showing adnate gill and solid stipe.
Fig. 5. Cross section of a pole of bamboo showing fibrous rot towards centre.
Fig. 6. Part of a longitudinal section of bamboo showing fibrous rot towards centre.
Fig. 7. Part of a transverse section of bamboo in an advanced stage of decay with lumina of vessles field with mycelia of *F. dilepis*.
Fig. 8. Part of transverse section of bamboo in an advanced stage of decay showing partial disintegration of parenchyma tissue and vessels.
Fig. 9. Part of transverse section of bamboo showing ramification of mycelium through parenchyma tissue.
Fig. 10. Part of transverse section of bamboo showing cracked sclerenchyma tissue due to decay.
Fig 11. Part of radial longitudinal section of bamboo showing ramification of mycelium within the vessel.