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STUDIES ON *IRPEX FLAVUS KL.* –I

ANTAGONISM BETWEEN DIFFERENT STRAINS AND THEIR HOST-RELATIONS

By

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(with Plates I-II and 2 Text-Figures)

The strains of *Irpex flavus* obtained from *Shorea robusta* Gaertn.f., *Swietenia mahagoni* Linn. and *Albizzia lebbek* Benth., have been paired in all possible combinations with a view to find out the types of reaction existing between the interacting mycelia. Incidentally, the host-relations of the strains have also been studied on the basis of their relative variation in decay.

The fruit-bodies collected from the aforesaid hosts are morphologically more or less similar. The antagonistic effect between the interacting strains, irrespective of the types of mycelia (monosporous and polysporous mycelia or the isolates), has been noticed in all the pairings excepting between the subcultures of the same mycelium.

It appears from the cross-inoculation experiments that the variation in the average percentage of loss in dry weights of each host-species is dependent on the types of mycelia and also on the different strains of the same type of mycelium. But the strains under consideration show their maximum ability to decay in case of *Albizzia*, minimum in *Shorea* and moderate in *Swietenia* and hence *Albizzia* may be considered as 'least resistant' among the three hosts while *Shorea* is 'most resistant' and the remaining one may be placed in the intermediate category.

Introduction

Irpex flavus Kl., a common wood-rotting fungus in India, attacks our structural timbers, such as *Shorea robusta* Gaertn.f., *Swietenia mahagoni* Linn., *Albizzia lebbek* Benth. and several other hard-wood species and monocotyledons causing decay of large quantities of wood annually. Besides India, it also occurs in the warmer countries of the world. Its effuso-reflexed, coriaceous, yellowish fruit-bodies with dentate hymenial surface, so common on logs, stumps and dead trunks of trees, provide characteristic features by which it can be readily recognized in the field. Owing to its frequency of occurrence, many citations are made to this fungus, but strangely enough no intensive studies have so far been undertaken on this species. Since the host-species of *Irpex flavus* are of great economic importance, it is felt that any information concerning the fungus is desirable. With this end in view, investigations have been undertaken and the present paper forms the first contribution of the series.

This investigation comprises two main lines of inquiry. Firstly, pairing experiments have been carried out in order to study the interactions between the strains obtained from *S. robusta*, *S. mahagoni and A. lebbek*. Secondly, cross-inoculation-tests of the sapwood of the aforesaid hosts with the different isolates and monosporous mycelia have been performed under a set of controlled conditions in order to establish host-relations of the available strains under consideration.

Material and Methods

For this investigation three economic timbers, *viz.*, *Shorea robusta*, *Swietenia mahagoni* and *Albizzia lebbek* were selected and obtained from different timber-yards of Calcutta in the months of August to November, 1957. Short cylindrical cross-sections of the poles of the three types of infected logs were brought to the laboratory, examined and studied in a fresh condition. Plenty of fructifications of *Irpex flavus* were also collected separately from the different hosts. From each type of fruit-body copious spore-deposits were obtained on 2 per cent sterile agar plates within few hours after collections and from these spores polysporous cultures were made on 2.5 per cent *malt-agar* slants. Under ordinary conditions of temperature (30°-32°C.) and diffused light of the laboratory, the spores germinated and the resulting mycelia covered the surface of the slants within a week. In this way polysporous cultures of different strains were obtained. Similarly, monosporous cultures from the spores obtained from the different fruit-bodies were prepared by the usual dilution method (Smith, 1954). The mycelia from three different hosts were also isolated on 2.5 per cent *malt-agar* following the method adopted by Banerjee (1955). Out of thirty-six isolations from the different regions of sapwood and heartwood blocks of the three hosts, pure cultures were obtained in twenty-four cases. In some cultures fructifications began to develop within a fortnight. These cultures were then compared with monosporous and polysporous cultures of the respective strains and found to be identical as regards their macroscopic appearance and microscopic details. It may be worthwhile to note that clamp-connexions were absent in all cases.

Antagonism

With a view to determine the types of reaction between the strains of *Irpex flavus* obtained from different hosts, pairing experiments have been undertaken. Several workers like Schmitz (1925), Verrall (1934) and others have made notable contributions on other fungi in this line. It has been found that antagonism exists between the strains obtained from different host-species. Though similar antagonistic behaviour has been noticed in certain cases of heterothallic species yet it is not uncommon in other cases also. For the present investigation both monosporous and polysporous mycelia have been obtained from fruit-bodies on *Shorea robusta*, *Swietenia mahagoni* and *Albizzia lebbek* and different isolates have also been taken from the afore-said hosts. These have then been paired with the presumption, that if there be any variation between the strains, there is every possibility of the existence of antagonistic reaction among themselves irrespective of the types of mycelia and the isolates.

Schmitz (1925) while working with *Fomes pinicola* paired the cultures from four different hosts, namely, Douglas fir, white fir, western hemlock and western white pine and found no intermingling among themselves. However, according to him the line of demarcation found between two monosporous and polysporous mycelia of *Fomes pinicola* was probably due to physiological variation existing in the mycelia and that caused an upset in the metabolism when the two cultures met. The maximum metabolic change was indicated by the presence of a dense line of demarcation between them.

Similarly, Verrall (1934) worked with *Fomes igniarius*. He also paired the cultures from different hosts and came to the conclusion that when the isolates from the different hosts were paired, irrespective of their similarity or difference, there would be antagonism. In case of haploid cultures, he showed two types of interactions, one with 'no sexual fusion' and other with 'sexual fusion'. Under the type 'no sexual fusion' he pointed out that all haploid lines from different hosts and all haploid lines of the same sex would show this type of reaction. In such cases demarcation line would be formed between the paired cultures and the subculture from the line of demarcation might represent the original cultures by separation.

In case of *Irpex flavus*, aforesaid types of mycelia were paired in possible combination in Petri dishes (100 mm.) containing 2% cleared *malt-agar* and kept at ordinary temperature (27°–32°C.), humidity (66%–96%) and diffused light of the laboratory. After four days of inoculation the line of contact of the pairings of different monosporous and polysporous mycelia and the isolates were examined directly under the microscope. The hyphae of the advancing mycelia were found to overlap each other but so far as the observations go, no hyphal fusion was found in any case (PL.I., FIGS. 1-3). Gradually, a dense white demarcation line appeared in all cases between the interacting mats within a week (PL. II., FIGS. 5-7). These white lines ultimately became dark coloured in the majority of the cases (PL. II., FIGS. 8-10). Furthermore, subcultures from the line of demarcation also produced two separate mats within a fortnight (PL. I., FIG. 4.)

The results of pairing experiments are given in Tables 1-3.

Table 1. *Pairings of three different monosporous mycelia of* Irpex flavus *obtained from the fruit-bodies from three different hosts.*

	Sm	Mm	Am
Sm	C	A	A
Mm	A	C	A
Am	A	A	C

Sm = Monosporous mycelium from *Shorea robusta*
Mm = Monosporous mycelium from *Swietenia mahagoni*
Am = Monosporous mycelium from *Albizzia lebbek*
A = Antagonistic
C = Compatible

Table 2. *Pairings of three different polysporous mycelia of* Irpex flavus *from three different hosts.*

	Sp	Mp	Ap
Sp	C	A	A
Mp	A	C	A
Ap	A	A	C

Sp = Polysporous mycelia from *Shorea robusta*
Mp = Polysporous mycelia from *Swietenia mahagoni*
Ap = Polysporous mycelia from *Albizzia lebbek*
A = Antagonistic
C = Compatible

Table 3. *Pairings of three different isolates of* Irpex flavus *from three different hosts.*

	Si	Mi	Ai
Si	C	A	A
Mi	A	C	A
Ai	A	A	C

Si = Isolate from *Shorea robusta*
Mi = Isolate from *Swietenia mahagoni*
Ai = Isolate from *Albizzia lebbek*
A = Antagonistic
C = Compatible

From the foregoing tables, it will be evident that antagonism occurs in every case except when a mycelium is paired with its own subculture. In the latter case, the interacting mycelia intermingle freely showing clear hyphal fusions between them and there is no line of aversion. These interacting subcultures have been expressed as compatible to each other. On the other hand, monosporous mycelia obtained from the fruit-bodies on different hosts exhibit complete antagonism between them. Similar interactions have been observed in case of polysporous mycelia and the isolates. The antagonistic behaviour between the mycelia obtained from different host species directly indicate the presence of strain variation within the species. Such intersterility between forms, strains and varieties of the same species has also been reported by Verrall (1934), Arnold (1935), Biggs (1937) and others in different fungi.

Host-relations

It has been found by several workers that aggressiveness and virulence of different strains of a species are not always uniform so far as their host-

relations are concerned. To establish this relationship, experiments have been carried out in order to evaluate the activities of the different strains of *Irpex flavus* on certain selected host species. Cross-inoculation experiments have been made and the method adopted by Banerjee (1955) for the decay-resistance-tests has been followed but with some modifications.

Sound and rectangular wood blocks ($2'' \times \frac{1}{2}'' \times \frac{1}{2}''$) were cut from the sapwood portions of the logs of *Shorea robusta*, *Swietenia mahagoni* and *Albizzia lebbek*. These were examined both macroscopically and microscopically for their soundness and compared with the decayed wood blocks for confirmation. They were serially numbered and dried to constant weights in an oven at 60°C. They were then soaked in distilled water under reduced pressure in order to raise quickly their moisture-content much above the 'fibre-saturation-point'.

The test-blocks were sterilized in an autoclave for 15 minutes at 15 lbs. pressure. About a fortnight before exposing the test-blocks to the strains of the test fungus, several 2% *malt-agar* slants were prepared in Kolle flasks, each containing about 90 c.cm. of the medium. Different sets of Kolle flasks were inoculated either with the monosporous mycelium or the isolate of the different strains under consideration and incubated at 20°C. In about ten days the mycelia covered the surfaces of all the slants. At this stage, the sterilized sapwood blocks of *Shorea*, *Swietenia* and *Albizzia* were separately introduced into the flasks and placed serially over the growing mycelia. The test-blocks of each host were exposed to six different cultures (monosporous mycelia and isolates of three strains) and incubated for a period of four months at 20°C. and diffused light of a temperature controlled culture room. In order to obtain significant result, this period was allowed following the recommendation of Cartwright and Findlay (1946). Another set of Kolle flasks with test-blocks, but without the test fungus, were kept under identical conditions and these served as controls.

The average moisture-contents of the test-blocks of *Shorea*, *Swietenia* and *Albizzia* before the experiment were determined to be 44%, 54% and 70% respectively. In all cases they were obviously much above the 'fibre-saturation-point' and as such favourable for the fungal activity.

The final average moisture-contents of the test-blocks of *Shorea*, *Swietenia* and *Albizzia* at the end of the experimental period were also determined. It was found that the moisture-contents increased to 65%, 102% and 140% respectively. This may possibly be due to further absorption of water from the medium.

At the end of the test period, the wood blocks were taken out from the Kolle flasks, the superficial mycelia were carefully removed and then dried to constant weights. The difference between the initial and final oven dry weights gave the amount of loss in four months in each case. The results are given in Table 4.

Table 4. *Comparison of the average loss in dry weights of sapwood blocks of* Shorea robusta, Swietenia mahagoni *and* Albizzia lebbek *due to different monosporous mycelia and isolates of three strains of* Irpex flavus *after 4 months' test.*

Types and sources of mycelia	Average loss in % with S.E.		
	Shorea	*Swietenia*	*Albizzia*
Monosporous (*Shorea*)	10.857±0.429	11.965±0.517	19.225±2.024
Monosporous (*Swietenia*)	4.673±0.672	13.402±0.408	14.248±2.013
Monosporous (*Albizzia*)	3.600±0.251	5.835±0.235	10.841±0.811
Isolate (*Shorea*)	6.272±0.358	6.797±0.485	8.313±0.466
Isolate (*Swietenia*)	5.752±0.466	16.905±0.514	17.801±0.690
Isolate (*Albizzia*)	4.560±0.365	7.928±0.543	15.128±1.064

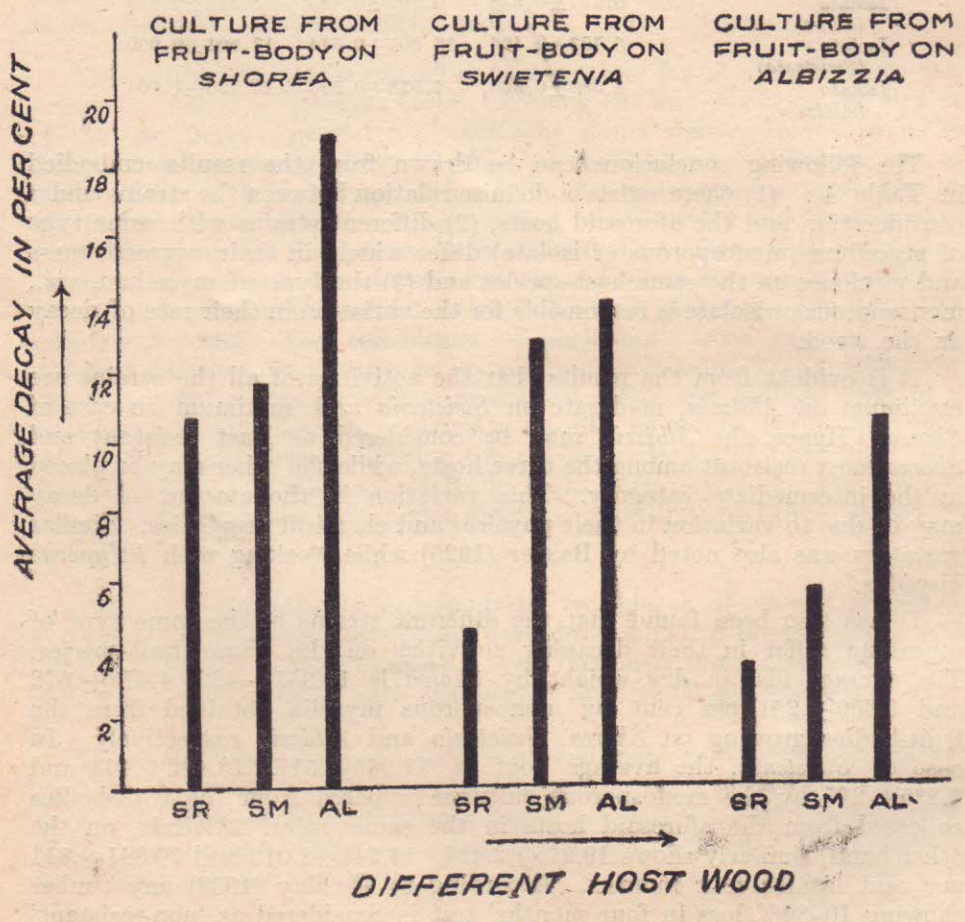
The following conclusions can be drawn from the results embodied in Table 4 : (1) there exists a definite relation between the strains under consideration and the aforesaid hosts, (2) different strains with same type of mycelium (monosporous or isolate) differ widely in their aggressiveness and virulence on the same host-species and (3) the type of mycelium, viz., monosporous or isolate is responsible for the variation in their rate of decay on the wood.

It is evident from the results that the activities of all the strains are maximum on *Albizzia*, moderate on *Swietenia* and minimum in case of *Shorea*. Hence, the *Albizzia* may be considered as least resistant and *Shorea* most resistant among the three hosts, while the other may be placed in the intermediate category. This variation in the amount of decay may be due to variation in their physical and chemical properties. Similar variation was also noted by Baxter (1925) while working with *Polyporus hispidus*.

It has also been found that the different strains of the same type of mycelium differ in their decaying activities on the same host-species. The average loss in dry weight by *Shorea* is 10.857±.429, 4.673±.672 and 3.600±.251 per cent by monosporous mycelia obtained from the fruit-bodies growing on *Shorea*, *Swietenia* and *Albizzia* respectively. In case of *Swietenia*, the average loss is 11.965±.517, 13.402±.408 and 5.835±.235 by the monosporous cultures prepared from the fruit-bodies collected from the aforesaid hosts in the same order. *Albizzia*, on the other hand, similarly shows 19.225±2.024, 14.248±2.013 and 10.841±.811 per cent loss in four months. According to Findlay (1938) any timber showing 10–30% loss in four months' test is considered as 'non-resistant' and the average losses in weight up to about 5% is called 'resistant'. So in this case same host may be even 'non-resistant' to one strain and 'resistant' to the other. This variation in the amount of decay is possibly to be due to the different physiological condition of the strains obtained from different host species (TEXT-FIGS. 1 & 2).

GRAPH SHOWING THE RELATIVE VARIATION IN DECAY OF THE SAPWOOD OF *SHOREA ROBUSTA* *SWIETENIA MAHAGONI* & *ALBIZZIA LEBBEK* CAUSED BY DIFFERENT MONOSPOROUS MYCELIA OF *IRPEX FLAVUS* KL.

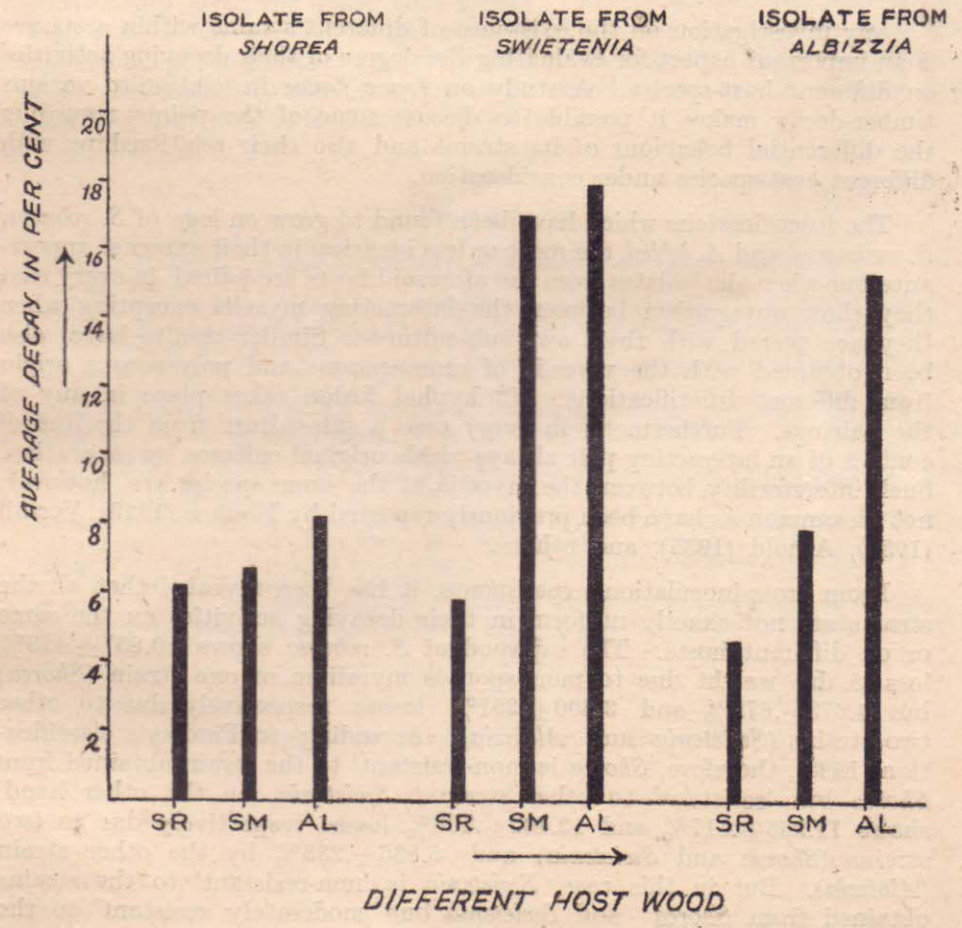
SR = *SHOREA* SAPWOOD
SM = *SWIETENIA* SAPWOOD
AL = *ALBIZZIA* SAPWOOD



TEXT-FIG. 1.

GRAPH SHOWING THE RELATIVE VARIATION
IN DECAY OF THE SAPWOOD OF *SHOREA ROBUSTA*,
SWIETENIA MAHAGONI & *ALBIZZIA LEBBEK* CAUSED
BY DIFFERENT ISOLATES OF *IRPEX FLAVUS* KL.

SR = *SHOREA* SAPWOOD
SM = *SWIETENIA* SAPWOOD
AL = *ALBIZZIA* SAPWOOD



TEXT-FIG. 2.

It is also obvious from the results that the isolates from *Swietenia* and *Albizzia* are rapid decayers than the corresponding monosporous mycelia. But in case of *Shorea*, monosporous mycelium is rapid decayer than its corresponding isolate. The explanation of this variation in the amount of decay caused by the different types of mycelia on the same host species is still awaiting investigation. However, from the above state-ment it can be said that the natural resistance of timbers not only depends on the kind of wood or types of mycelia or in some cases moisture-content of the wood blocks but also on the nature of the strains of the same type of mycelium of a species.

Discussion

Any investigation on the existence of different strains within a species is an important aspect for evaluating the degree of their decaying activities on different host-species. A study on *Irpex flavus* in relation to various timber-decay makes it possible to discuss some of the points regarding the differential behaviour of its strains and also their relationships with different host-species under consideration.

The fructifications which have been found to grow on logs of *S. robusta*, *S. mahagoni* and *A. lebbek* are more or less identical in their external appearance but when the isolates from the aforesaid hosts are paired, in every case they show antagonism between the interacting mycelia excepting when they are paired with their own sub-cultures. Similar results have also been obtained with the mycelia of monosporous and polysporous origin from different fructifications. No hyphal fusion takes place in any of the pairings. Furthermore, in every case a sub-culture from the line of contact of an interacting pair always yields original cultures by separation. Such intersterility between the mycelia of the same species are, however, not uncommon as have been previously reported by Mounce (1929), Verrall (1934), Arnold (1935), and others.

From cross-inoculation experiments, it has been revealed that all the strains are not exactly uniform in their decaying activities on the same or on different hosts. The sapwood of *S. robusta* shows $10.857 \pm .429\%$ loss in dry weight due to monosporous mycelium of one strain (*Shorea*) but $4.673 \pm .672\%$ and $3.600 \pm .251\%$ losses respectively due to other two strains (*Swietenia* and *Albizzia*). According to Findlay's classification (1938), therefore, *Shorea* is 'non-resistant' to the strain obtained from *Shorea* but 'resistant' to other strains. *Swietenia*, on the other hand, shows $11.965 \pm .517\%$ and $13.402 \pm .408\%$ losses respectively due to two strains (*Shorea* and *Swietenia*) and $5.835 \pm .235\%$ by the other strain (*Albizzia*). But in this case, *Swietenia* is 'non-resistant' to the strains obtained from *Shorea* and *Swietenia* but 'moderately resistant' to the remaining one. *Albizzia* undergoes $19.225 \pm 2.024\%$, $14.248 \pm 2.013\%$ and $10.841 \pm .811\%$ losses by all the three strains (*Shorea*, *Swietenia* and *Albizzia*) respectively and hence this wood is considered to be 'non-resistant' to all of them. Similar differential behaviour is also evident in cases of the isolates obtained from three different hosts. The decaying ability

of the isolates are, however, not similar to that by the monosporous mycelia. It has been found that the isolates from *Albizzia* and *Swietenia* are rapid decayers than that from *Shorea* and their corresponding monosporous mycelia. But the isolates from *Shorea* show less ability to decay than the other two isolates, and its corresponding monosporous mycelium. Thus, on the basis of the average percentage of losses in dry weights it can be said that the sapwood of *Albizzia* is 'least resistant' in comparison with the sapwood of other two hosts as the available strains irrespective of the types of mycelia exhibit maximum decay in this case. Of the three, *Shorea* appears to be the most 'resistant' while the *Swietenia* may be placed in the intermediate category. It is not desirable to generalize from the above statement that any strain of *Irpex flavus* will show the same effect on the aforesaid hosts or the available strains will maintain their same relationship with the other host species. The reason for the differential behaviour of the strains on the same wood may be due to their different physiological conditions. Of course, it is often suggested that the physiogical characteristics may possibly be governed by the genetic factors, but this fact still awaits further investigation.

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Explanation of Plates

Plate I

Fig. 1. Photomicrograph of 4-days-old paired cultures of *Irpex flavus* on 2% *malt-agar* showing overlapping without hyphal fusion between the monosporous mycelia obtained from the fruit-bodies on *Shorea* and *Swietenia*. (×240).

Fig. 2. Photomicrograph of 4-days-old paired cultures of *Irpex flavus* on 2% *malt-agar* showing overlapping without hyphal fusion between the monosporous mycelia obtained from the fruit-bodies on *Albizzia* and *Shorea*. (×240).

Fig. 3. Photomicrograph of 4-days-old paired cultures of *Irpex flavus* on 2% *malt-agar* showing overlapping without hyphal fusion between the monosporous mycelia obtained from the fruit-bodies on *Swietenia* and *Albizzia*. (×240).

Fig. 4. Photograph showing separation of original cultures from the inoculum taken from the line of contact of the two monosporous mycelia of *Irpex flavus* obtained from the fruit-bodies on *Albizzia* and *Swietenia*.

Plate II

Fig. 5. Photograph of 7-days-old paired cultures of *Irpex flavus* on 2% *malt-agar* showing the white line of demarcation between the isolates from *Swietenia* and *Shorea*.

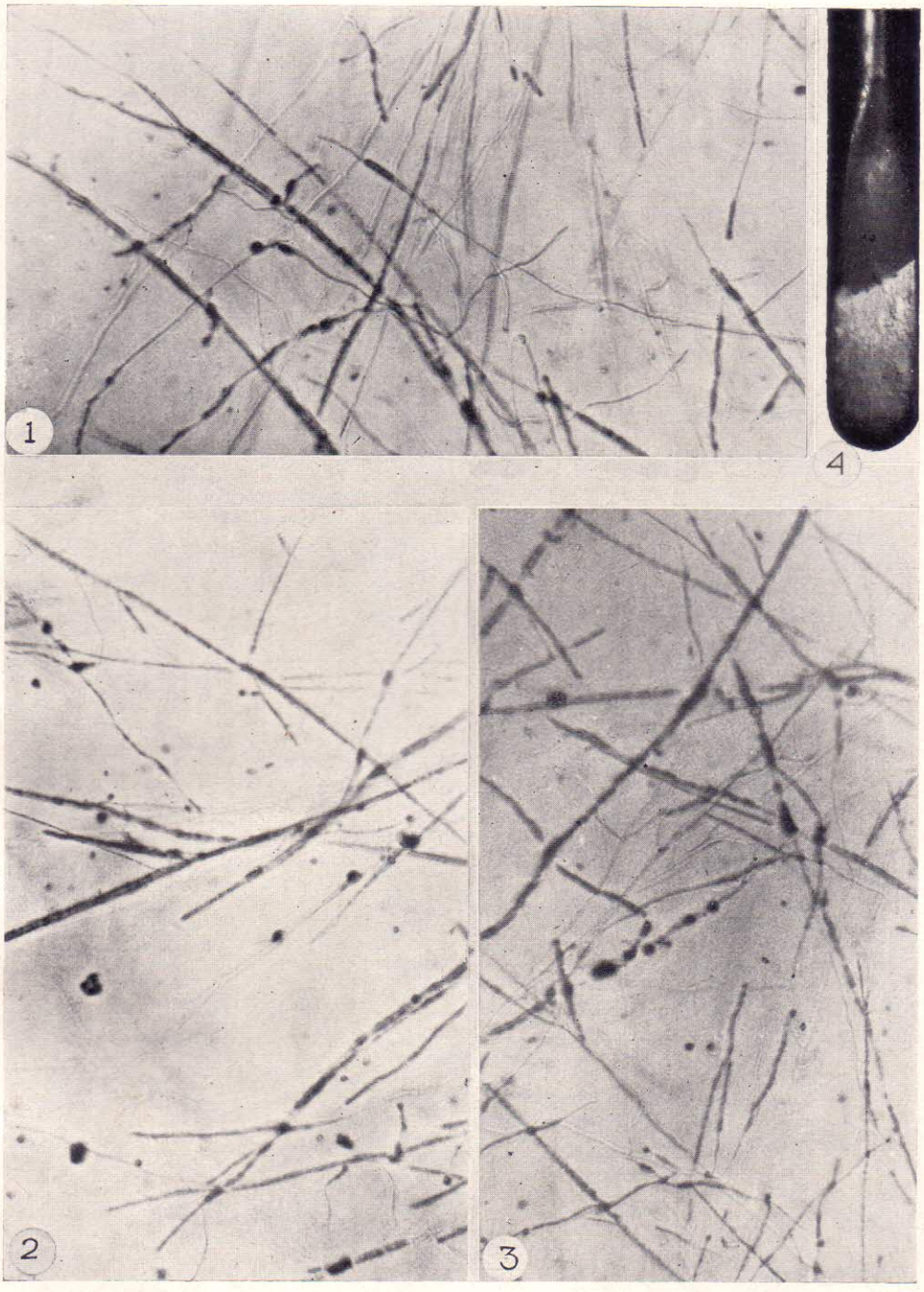
Fig. 6. Photograph of 7-days-old paired cultures of *Irpex flavus* on 2% *malt-agar* showing white line of demarcation between the isolates from *Albizzia* and *Shorea*.

Fig. 7. Photograph of 7-days-old paired cultures of *Irpex flavus* on 2% *malt-agar* showing the white line of demarcation between the isolates from *Albizzia* and *Swietenia*.

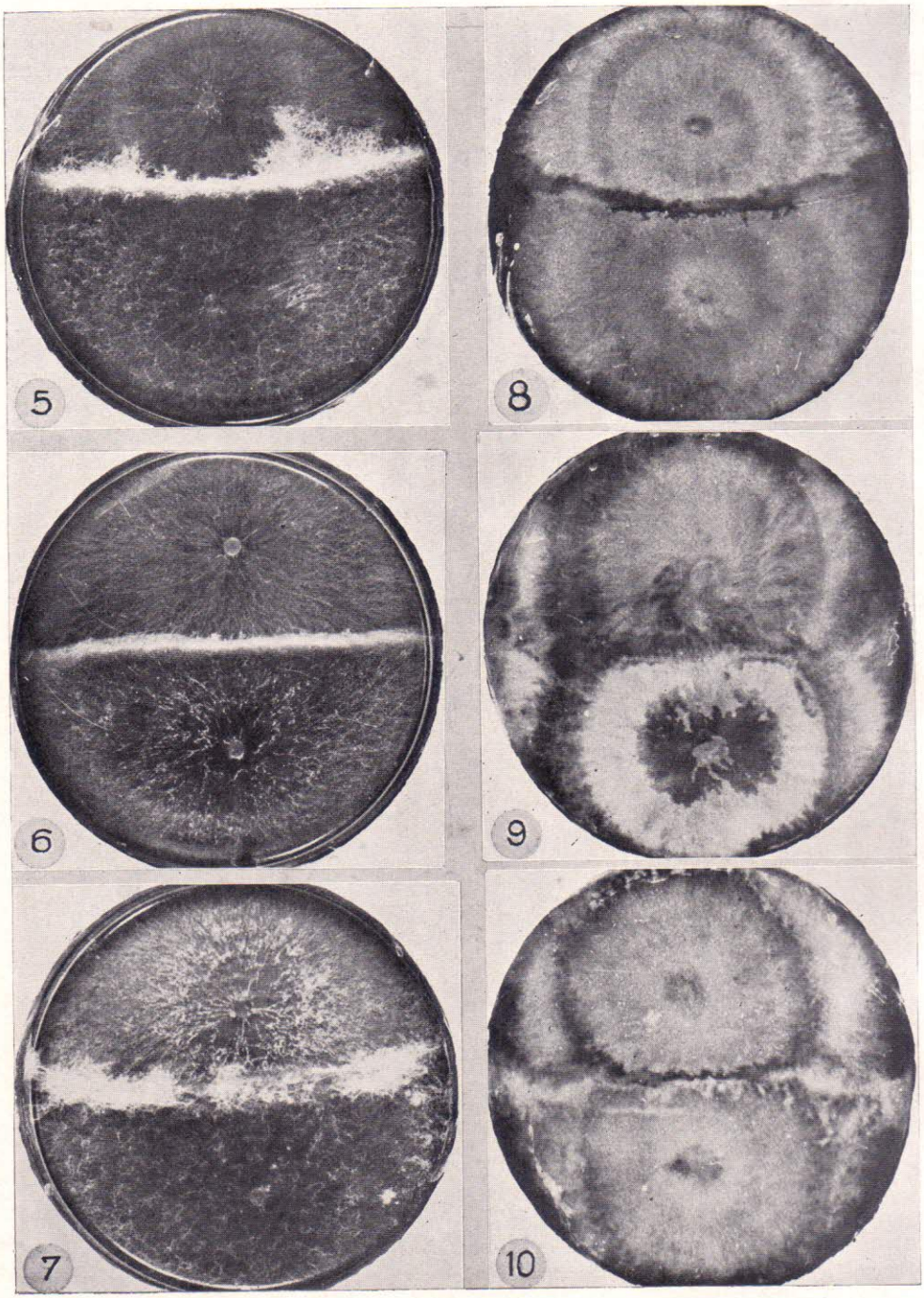
Fig. 8. Photograph showing 14-days-old paired cultures of *Irpex flavus* on 2% *malt-agar* showing the dark coloured area between the monosporous mycelia obtained from the fruit-bodies on *Shorea* and *Swietenia*.

Fig. 9. Photograph showing 14-days-old paired cultures of *Irpex flavus* on 2% *malt-agar* showing the dark coloured area between the monosporous mycelia obtained from the fruit-bodies on *Albizzia* and *Shorea*.

Fig. 10. Photograph showing 14-days-old paired cultures of *Irpex flavus* on 2% *malt-agar* showing the dark coloured area between the monosporous mycelia obtained from the fruit-bodies on *Albizzia* and *Swietenia*.



Irpex flavus S. N. Banerjee & R. P. Purkayastha



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