Discussion

Shorea robusta Gaertn. f. (Sal), the timber of which is well known for its natural durability, is a naturally growing as well as cultivated wood-species of Assam and is of much economic importance. Because of its high durability, the timber of Sal is used in various fields from the time immemorable. Although the living tree seem to be highly resistant, Bakshi, et al. (1963) reported loss of as much as 10 per cent of the wood due to fungal infection. The proportion of decay of wood in service due to fungal infection is recognizable and may account for a loss of economic significance. In Sal, the development of the outer heartwood and inner heartwood is attended by darkening in colour due to formation and deposition of various chemicals called extractives in the cell-cavities, add resistance to the wood. The greater quantity of resistance produced per unit of newly formed heartwood is not stable, subsequently deteriorating the effectiveness with age (Scheffer and Hopp, 1949). And as such, in spite of its high resistance to decay, like all other wood (Baxter, 1925; Shigo, 1962; Wilcox, 1970), the timber of Shorea robusta is subject to decay by fungi. The wood-rotting fungi such as Fomes lignosus, F. lividus, Trametes cubensis, Polystictus steinheilianus, Stereum hirsutum, Polyporus ostreoides,
Irpex destruens etc. that occur in Sal, very often associated with their associates namely Terminallia alata, T. ballarica, T. chebula, Lagerstroemia parviflora, L. lanceolata, Madhuca longifolia, Cordia dichotoma, Bridalia retusa, Cleistanthus collinus etc. and continue their activity on the stumps and continue perpetuation. The fungi may also remain active on logs and converted timber (Bakshi, 1972).

With the diminishing supply and increasing demand of the national resources, particularly more important conventional timbers, a rational use of timbers has become necessary and important. Decay of wood is a natural process caused mainly by the members of the Polyporaceae (Bakshi, 1971), but infection court is first established by fungi or microorganisms other than Basidiomycetes in general (Blanchette and Shaw, 1978; Shigo and Sharon, 1968 and 1970). Although decay of Shorea robusta timber by Irpex destruens is frequently observed in the eastern part of our country, much less is known of the infection and of the ways and means of controlling the same.

Histological studies of the naturally infected wood of Shorea robusta by Irpex destruens have shown a conspicuous ramifications of the hyphae of the fungus inside the vessels
of the host tissue. (Cartwright, 1929) following a general way - the long axis of the cell, probably in search of nutrients out of the cell-wall constituents. The way, that is followed, by the mycelia of the fungus to pass from cell to cell by making bore holes and to ramify along the longitudinal axis lying adpressed to the cell-wall, may signify the process to be a chemical one to suck food materials from wood cell-wall. The fact is also revealed by the histological changes as evidenced by the micro-chemical tests. The hyphae in the infected wood could clearly be seen in both radial and tangential sections and were more abundant in vessels of vascular tissue.

Carbohydrates are the most common and important sources of carbon for the fungi. The pathogen infecting the wood of Shorea robusta possibly is influenced by the nutritive composition of the host as it contains carbohydrates, proteins, trace elements, minerals etc. (Singh, 1970). The growth of Irpex destruens indicates that it is influenced by concentrations and limits of different nutritive factors in vitro in a way that may be parallel to the growth of the fungus in vivo.

It has been observed, in vitro, that growth of
Irpex destruens is influenced by the carbohydrates, nitrogen salts, trace elements, vitamins, and pH and temperature. Significant loss in weight of J. robusta wood, in vitro, also pointed out to the possibility of significant correlation between host-substrate and its utilization by the pathogen. The possible correlation was further manifested by the best growth of the pathogen in the nutrition medium containing cellulose. Growth was further accelerated when carbon source was replaced with carboxymethyl cellulose (a derivative of cellulose). The growth is better adapted to carboxymethyl cellulose than other carbon sources like dextrose, sucrose etc. although Nord and Vitucci (1947) found Lentinus lepideus to utilize xylose. The failure of the fungus to utilize an oligosaccharide as against a polysaccharide may be due to the lack of necessary hydrolytic enzyme or due to its inability to utilize the component sugars. The poor growth of the fungus on other carbohydrates may be due to inhibitory effects of sugar breakdown (Barner and Cantino, 1952) possibly during autoclaving. The best growth of I. destruens in the nutrition media containing carboxymethyl-cellulose was in conformity to the results of Herrick (1940) and Jensen (1971) for Stereum gausapatum. Dry weight production (in mg.) of I. destruens was at par with the linear growth (in mm) when grown in liquid media with either celllobiose,
cellulose or carboxymethyl cellulose maintaining the concentrations used earlier.

It has not, however, been possible to correlate the growth of the fungal pathogen inside the tissues of the host, with the nutritive composition of the host itself as there has been no appropriate method, unlike that in some fruits or soft tissues characterized by soft rot, for measuring the rate of advance of the hyphae into the tissues.

Carbon compounds are utilized by fungi as a source of energy or as a source of chief structural element but as the concentration in a medium is increased, the economic coefficient declines. Carbohydrates and other compounds having the same structure, but with mirror-image configuration, may differ physiologically. Further, the two processes may be same until a number of chemical transformations have taken place, but may then diverge after a certain intermediate products are formed. The little growth that occurred in the control was probably due to contamination of sugar source through inoculum.

It was interesting that for many fungi, increase in carbohydrate beyond optimum point results in an absolute as well as relative decrease in growth but in any case, the
demand for nutrient requirements must be taken into account considering the effect of metallic, non-metallic elements and also other factors affecting growth.

Burkholder and McVeigh (1940) opined, within limits growth of a fungus is increased by higher carbohydrate concentration in a nutrition medium provided adequate nitrogen is supplied. Wood is a substrate deficient in nitrogen (rarely greater than 0.3% by weight and more commonly may be only 0.03-0.10%) but the fungi which decay wood, possess some characteristics enabling them to use wood as a source of required nutrients. In the present experiment *Irpex destruens*, which was found to thrive well in the wood of *Shorea robusta* had its highest growth in nutrition medium provided with as much as 0.3% ammonium phosphate followed by ammonium nitrate. The preference of ammonium nitrogen to other sources was at par with Pettersson et al. (1963) and Jensen (1971). Haskins and Weston (1930) reported - similar preference of ammonium nitrogen to nitrate by *Karlingia rosea*, which could grow equally well on either, when the other was absent. The fair growth of *I. destruens* in nitrogen free medium indicated its ability to utilize nitrogen from sources other than available in the substrate.
The carbon-nitrogen (C:N) relation in the nutrition medium in relation to dry weight production of *Irpex destruens* was critical as the utilization of the nitrogen source solely depended upon the carbon supply. The growth was best favoured by C:N ratio of 1.6 : 1 (0.5% : 0.3%), where the proportion of nitrogen was very high in comparison to the ratio of 1250 : 1 in heartwood as studied by Levi and Cowling, 1968. The gain of nitrogen fraction in decayed wood (Singh, 1970), reduction in synthesis amino-acids, peptides and protein at higher C:N ratio and also unique capacity to produce cellulyolytic enzymes in nitrogen starvation by *Polyporus versicolor* (Levi and Cowling, 1969) have added complexity to C:N relationship of *I. destruens*. It is, therefore, a problem of more intensive investigation for the determination of a proper nitrogen source and also its utilization, in combination with other growth factors as may be found in the nature. One such investigation might prove to be beneficial in finding out the mode of wood-decay of *S. robusta* by *I. destruens*.

Shigo (1970) found *Polyporus glomeratus*, *Poria obliqua*, *Fomes igniarius* and *Pholiota squarossa-adiposa* to grow better in media amended with zinc, iron, manganese and
calcium. 100 per cent increase in the yield of *Phycomyces blakesleeanus* was possible, when nutrition media were amended with zinc and iron (Leonian and Lilly, 1940). Growth of *Irpex destruens* in nutrition medium was retarded in absence of either zinc, iron, manganese, cobalt and calcium. The need of iron was pronounced requiring a higher concentration than those of the others. The lowest concentration of 1 mg. ferric citrate per litre reduced the growth while highest concentration of 13 mg. per litre could be toxic having inhibitory effect. Growth at the zero concentration of cobalt and manganese was fair as compared to their optimum requirement. The effect of calcium did not seem to be specific.

*I. destruens* preferred potassium hydrogen phosphate as the source of phosphorus for its optimum growth. Growth sharply declined at higher than the optimum concentration which might have been due to inhibitory action or some other factor becoming limiting under such condition.

Although it is generally assumed that a fungus which is independent of an externally supplied vitamin is so by virtue of its ability to synthesize the compound, Pettersson *et al.* (1963) rightly included thiamine in the medium for
wood-rotting fungi. The need of thiamine hydrochloride for the growth of *Polyporus versicolor* was estimated at 100 mcg. (Pettersson et al., 1963), while the optimum dose of the same which was estimated (Jensen, 1971) at 6 mg. for *Stereum gausapatum*, enhanced the dry weight production as well as cellulyolytic activity of the fungus. Growth of *Irpex destruens*, although occurred in the control was poor, and higher concentration lead to hamper growth in the present investigation.

The wood-rotting fungi are known to remain dormant for a longer period and to start its activity as soon as the climatic conditions including temperature become favourable. In this respect, temperature play a vital role in its development, specially in the tropical countries, where the fungi are likely to encounter a well-spread range of temperature. Cartwright and Findlay (1934) opined that temperature not only affect the rate of growth of wood-rotting fungi, but also the rate of decay of the timber. In the present study *I. destruens*, which failed to grow at 0°C, started its activity from 15°C having its optimum growth at 30°C. With further rise in temperature, the pathogen failed to grow. Growth, although controlled by a certain range of temperature, continued for prolonged period in the optimum temperature.
Growth of a fungus (wood-rotting) which has also direct correlation between utilization of the substrate (wood in the nature) and temperature, cause stimulation of enzyme-induction as well. It was, therefore, be of vital significance to study the physiology of *Irpex destruens* in terms of enzymology for its better interpretation.

Fungi produce acids from non-acidic nutrients such as carbohydrates which ultimately come in the way of development of a pathogen in the substrate. Early works of Banerjee and Purkayastha (1967) revealed that two isolates of *Irpex flavus* differed in their optimum $P^H$ requirements. *I. destruens* also had a wider range of $P^H$ requirement in the present study, varying from 4.0 to 9.5, which had its highest dry weight production at $P^H$ 5.5 with an incubation period of 10 to 20 days but optimum $P^H$ was 6.0, when incubated for 30 days. $P^H$ of the filtrate at different stages of incubation varied and in that respect an initial $P^H$ 5.5 was found to be the optimum, where overall change of $P^H$ was around 6.0. $P^H$ of the culture media for fungi which influenced growth, also differed enzyme induction (Damle, 1952; Elegersma, 1976).

Wood-decay is a process by which the invading organism, gets its nourishment from wood and in the process
of utilization of the wood constituents affect ultimate reduction in weight of the wood. Rates of decay of Shorea robusta wood varied with the substrates used for the growth of the pathogen. Growth rates did not correlate to the rate of decay in the corresponding substrate, in which wood-blocks were incorporated. The inconsistence probably referred to the degree of enzyme induction by the fungus in the way of disintegration of the cell-wall constituents.

Effect of temperature on the rate of decay of Shorea robusta wood by Irpex destruens in the method of Bakshi et al (1967) was studied. The method was nearly natural and in all probability offered accurate findings. 30°C which was the optimum, was also most favourable for mycelial growth of the pathogen. The range of temperature which was beneficial for growth of the pathogen and decay (weight loss) as well must play key role in the ultimate decay of wood, although other factors at sight must also be considered.

Nitrogen source in the nutrition media had positive effect on the rate of decay of wood by fungi (Darbyshire et al 1969). Similar findings of definite relationship between nitrogen content of the wood of Populus grandidentata and its decay by Lenzites trabea and Polyporus versicolor were
reported by Merrill and Cowling, 1965. However, there was no major influence of nitrogen source in the medium on the rate of decay of Shorea robusta wood by Irpex destruens. The pathogen, which had very sluggish growth in absence of carbon and also nitrogen source could produce weight loss of as much as 8.00% at the end of three months whereas the same was 9.00% when the blocks were incorporated in media containing 0.1% of ammonium phosphate. It was possible that time factor was as much essential as those of growth factors, because the highest rate of decay (weight loss) occurred in absence of nitrogen source.

Histological changes associated with infection of the wood, when investigated by micro-chemical test and microscopic examination, results were in support of better utilization of cellulose than the lignin fraction. Infected-wood sections when treated with Chlor-zine-iodine, stained very faint blue, while secondary walls of healthy wood stained light blue. Infected wood stained light pink when treated with phloroglucin and hydrochloric acid. The former test was in support of degradation of cellulose and the later for a modified state of lignin. The Maule test further signified that a little alteration, probably not full utilization, of lignin was possible. Similar micro-chemical and microscopic
observation (Husain and Kelman, 1958) revealed utilization of cellullosic and pectic materials by Pseudomonas solanacearum.

The principle, that the disease in plants or parts thereof, is affected by one or many enzymes at different conditions and levels of the life process, differs markedly from the various types of wood-tissue disintegration. The life processes of organisms were controlled and directed by a complicated and interrelated series of enzymes or enzyme system (Dixon, 1949). Gilbertson (1980) rightly opined that a true wood-rotting fungus is the one which can draw its nutrients from the wood-tissues by the action of a series of enzymes.

Cellulose, which figure as the most important component of the cell-wall, is relatively resistant to microbial deterioration, but wood-rotting fungi produce enzymes responsible for its decomposition (Das et al, 1979). The unienzymatic theory for hydrolysis of cellulose (Whitaker 1953, 1957) postulates that only one enzyme - cellulase can convert native cellulose into glucose. Certain wood-rotting fungi such as Collibia vellutipes and Polyporus anonus require an enzyme glucosidase in addition to cellulase to convert cellulose into glucose (Markans 1957
A and B). The multienzymatic theory (Reese, 1956) postulates that an enzyme \( (C_1) \) acts upon crystalline cellulose to loosen the bonds and in the next phase another cellulase \( (C_x) \) acts upon the produce the soluble sugars. Production of two types of glucosidase by Macrophomia phaseolina has been reported by Saha et al (1960).

Reduction in viscosity of the Carboxymethylcellulose solution by the culture filtrate of *Irpex destruens* was due to the production of exocellular enzyme, referred to as "cellulase" and was responsible for degradation of native cellulose, in one or other step of enzyme reaction. The degradation presumably occurred in the following steps.

Native cellulose \( \text{cellulase} \) \( \text{cellobiose} \) \( \text{glucosidase} \) glucose.

The corresponding poor rise in the loss of viscosity of the enzyme-substrate reaction-mixture at the end of 180 minutes was primarily due to instability of the enzyme systems for the long duration. A downward loss in viscosity of 1.0% carboxymethyl cellulose solution might indicate weak and slow action of the enzyme system. The period of incubation played major role on the enzyme system and 30 days of incubation produced the highest enzyme-substrate reaction. The system which got its peak on 30 days but tends to fall at 40 days was an indication that the degradation of the
cellulosic components in vivo is accomplished at an early stage of mycelial development and at a later stage, rise in the pH come in the way.

The cellulolytic activity of *Irpex destruens* was found to be governed by the source of carbon. The increase in activity of the system was little when carboxymethyl-cellulose was replaced with either of cellulose, cellobiose or sucrose. The velocity of enzyme reaction was corresponding to the growth activity in similar carbon sources. *Botryosphaeria ribis*, *Glomerella cingulata* and *Physalospora obtusa* had similar selectivity of carbon source (Husain and Dimond, 1958). Source of nitrogen influenced cellulase activity as much as the carbon sources. Preference of ammonium phosphate to other sources was at par with mycelial growth.

Increase in activity of the cellulase system with the increase in temperature was due primarily to the influence of temperature on the velocity of enzyme reaction, because the enzyme system is stable over this range of temperature. With further increase in temperature, cellulase activity was reduced. *I. destruens*, which had its highest mycelial growth around 30°C, developed its maximum cellulase activity at 32°C and the system which retained its activity up to 35°C, must exert a role that might be responsible for the degradation of cellulose.
Cellulase system had higher $P^H$ optimum as against a lower for growth.

The function of pectinolytic enzymes among wood-rotting fungi still remains a question as wood contain a negligible amount of pectin. However, reports are there in support of production of protopectinase (Das et al., 1979). Irpex destruens is found to produce protopectinase as is evident from the macerating effect of the purified culture filtrate. The activity was found to differ with the source of carbon and was highest when it was pectin. However, the system was not active as much as that of Fusarium vasinfectum (Kahadevan, 1965). 30 to 40 days of incubation along with carbon source further enhanced the protopectinase activity of I. destruens Polygalacturonase and pectin-methyl-esterase activity (relative enzyme activity) was poor irrespective of carbon source and incubation period of the pathogen as well as of the reaction mixture. Unlike that of cellulase activity, $P^H$ was found to be of no effect on polygalacturonase activity of I. destruens.

Production of faint brown colour in media containing phenol indicated a poor phenol-oxidase activity of I. destruens. The activity, supposed to be responsible for the degradation of
lignin, was poor and was responsible for the slow degradation of the lignin fraction of wood-substrate. However, Kirk and Kelman (1965) reported *Polyporus dichorus, Poria taxicola* and *Stereum frutulatum* to degrade lignin, but produced no colour reactions in the phenol-containing media.

The exact mechanism by which the biochemical reaction takes place in browning of tissues, which is a characteristic feature of hypersensitivity in the host, is not clearly known. Horsefall and Dimond (1960) after histological observation of necrosis (browning and drying) in the injured tissues of infected plants, draw the general assumption that "(i) polyphenolic compounds are oxidised by polyphenol oxidase which may exist in latent state in the intact plant tissues and become activated on exposure to pathogenic infection and (ii) the oxidised poly-phenols, now quinones, are condensed to form poly-quinoid structures or sometimes react with amino-acids or proteins to form melanin like substances. The net effect of these reactions may constitute defence mechanism to the host by forming barrier."

In the present investigation cellulase and polygalacturonase activity as was manifested by the culture
filtrates, were also encountered in the wood extracts. A high degree cellulase activity in naturally infected wood than those in the artificially infected wood was probably because of incidence of greater mycelial ramification. A much higher rate of enzymatic activity in the artificially infected wood than in the healthy wood further justified the fact that mycelial growth was the cause of enzyme production and ultimate degradation of wood tissue.

The lower rate of polygalacturonase activity in the infected wood extracts than in the culture filtrates indicated the system to be of no major importance. Proteinase activity which was found to be stronger than phosphatidase refer to the efficacy of the system in relation to the amount of protein in the wood tissue.

Induction of such a great variety of enzymes by a single pathogen and the occurrence of which might be simultaneous in a chain, might lead to the disintegration of cell-wall fraction as well as other deposits and to the ultimate decay of wood-tissue.

Although no hard and fast rule could be applied to the selection of resistant host or the control of wastage of wood in use as well as in store stock due to the infection of Irpex destruens, the correlation between percentage of
superficial infection, phenolic concentrations, and the
degree of resistance to attack is significant from a
practical point of view. The general picture of the vast
changes, even in the few substrates studied in this work,
shows that the variations in host-parasite relationship
and disease reactions are brought about by the changes in
a small group of chemical systems in the host and the
parasite, and not due to an over-all change. The phenomenon
of the concentration of faint phenolic compounds in the host
tissue and the production of a faint phenol-oxidase system by
the pathogen, Irpex destruens, is also a clear indication of
the mechanisms of attack and defence of the diseased systems.

It would be of interest to carry out further
researches into the qualitative and quantitative changes in
the host tissue system and also in the primary metabolic
compounds such as amino-acids, carbohydrates, organic acids
and secondary components such as phenols and alkaloids and a
graphic representation of these quantitative changes during
incubation of a parasite should help in the better
understanding of resistance or susceptibility of the host
tissue to a specific disease or pathogen.