Review Article

Studies on the Wood Decay by a Soft Rot Fungus, *Chaetomium globosum* KUNZE

Munezoh TAKAHASHI*

Contents

Introduction

The correlation between fungi and decay in wood had been noticed from antiquity, but it was not until the second half of the last century that a group of the

* Division of Wood Biology.

Basidiomycotina and a small group of the Ascomycotina were generally recognized as the causal agents of decay in wood[1]. These organisms were divided by CARTwright et al.[2] in 1931 into two groups, the brown rot fungi and the white rot fungi, according to the way in which the cell wall materials of wood were broken down. The idea was prevalent for the first half of the twentieth century that decay of wood was normally due to one or the other of these two groups of organisms. A particular type of fungal decay commonly called "soft rot" afterwards was not generally recognized in spite of a series of observations made between 1850～1950. FINDLAY and SAVORY[3] firstly demonstrated that the deterioration of timbers in cooling towers, which had been explained as only chemical phenomena, was in fact caused by a number of microfungi, i e. members of the Ascomycotina and some Deuteromycotina. The term "soft rot" was coined by SAVORY[4] on account of the softening produced in the surface layers of wood by the action of these fungi. Following their work it became currently accepted that this type of decay occurred in vastly varying situations, particularly in wood with high contents of water such as that in cooling towers, greenhouses, quays, poles in moist ground, etc. Stored pulpwood chips have also been proven to be seriously attacked by this type of organisms[5].

Microscopically, soft rot is generally quite different from either a white rot or a brown rot. Soft rot has been characterized by the presence of distinct elongated cavities within the middle layer of the secondary wall of fibres, tracheids and vessels[6]. Comparisons of the properties of soft rot with the brown- and white rots have also been made on the chemical- and mechanical properties of wood undergoing fungal attack. Chemical analysis of soft-rotted beech wood by SAVORY and PINION[7] showed carbohydrate depletion with little lignin attack. That is corresponding to the characteristic chemical situation of brown-rotted wood. The gradual increase in alkali solubility of soft-rotted wood is more analogous to white rot than to brown rot[7]. The effect of a soft rot fungus, *Chaetomium globosum*, on the impact resistance of beech wood was compared by ARMSTRONG and SAVORY[8] with that of white rot- and brown rot fungi. These results suggest that soft rot of beech is more closely comparable to a white rot than to a brown rot.

So far as the past evidences indicate, softwoods do not seem to be as readily attacked by soft rot fungi as are hardwoods. Although SAVORY[4] and DUNCAN[9] suggested that the higher content of lignin in softwoods was the main agent for the higher resistance of softwoods to soft rot fungi, none has yet proved entirely enough. An apparent wood preference has also been found for many of the brown rot- and white rot fungi. Brown rot fungi are associated most frequently with decay of softwoods and white rot fungi with decay of hardwoods. This dominant tendency has also not been fully explained yet.

Another series of investigations have proven that soft rot fungi are often more tolerant to fungicides than are wood-decaying Basidiomycotina[9], and that it is difficult to obtain the required fixation of the preservatives in the secondary cell wall[10], where the soft rot fungi are able to grow. However, ecological studies on soft rot fungi[6] revealed that they are of economic importance only when lack and excess of moisture or low dosage of treating preservatives inhibits the growth of the more vigorous Basidiomycotina. Soft rot fungi may also act as a precursor of the wood-decaying Basidiomycotina in the deterioration of wood in ground contact[6].

Much remains to be done to achieve a better understanding of the nature of soft rot fungi. Particularly, the possible preference of soft rot fungi for hardwoods has aroused a considerable interest. Work along these lines could well throw light on the chemistry and biochemistry of wood decay, and on new ways of rapid disposal of wood waste by fungi.

Numerous species of fungi have been recorded as having the ability to induce soft rot in wood in pure culture. However, there have been conflicting reports as to the ability of fungal species to produce soft rot other than *Chaetomium globosum*. Furthermore, inferring from the past data, *C. globosum* seems to have the highest ability to degrade wood among soft rot fungi recorded. Therefore, *C. globosum* was used as a sole species of soft rotter in the present investigation.

The objects of the present study are three:
1) to obtain informations on the effect of *C. globosum* on the physical- and chemical properties of wood, for accomplishing a better understanding of the mechanisms of wood degradation by soft rot fungi, 2) to examine whether softwoods and hardwoods differ in their susceptibility to *C. globosum*, and if the difference is found, 3) to demonstrate which factors are more responsible for the difference in susceptibility.

1. Analyses of testing conditions providing a higher wood-decaying capacity of *Chaetomium globosum*

The wood-decaying capacity of fungi, which is expressed mostly as the weight loss of wood specimen and occasionally as the retaining strength of wood in accelerated laboratory tests, often varies greatly with testing conditions. Many investigators[11~20] have indicated several factors that are responsible for the great variation of the data; wood species, fungal species, uniformity of test blocks, size of test blocks, temperature, test period, composition of medium, etc. Although a great deal of experiment has been attempted to make the decay tests more reproducible and more reliable, no completely satisfactory test has been devised. However, as a means of understanding how wood preservatives function and how woods resist against fungal attack, several techniques have been standardized by an official agency of

country. In Japanese Standard, established as JIS Z 2119 in 1963, the sand-block test using Peptone-Malt Extract Medium is adopted.

The aim of this study is two: 1) to examine whether the Japanese Standard is applicable to test for a soft rot fungus, *C. globosum,* and 2) to find an appropriate composition of culture medium and an adequete size of test blocks which give a higher wood-decaying capacity of the fungus.

Effect of several factors which affect on the rate of wood decay is usually estimated by a classical combination method. In this method, the effect of a given factor could be estimated only when the other factors are equally fixed to a certain level. Although the results obtained by the method seem to be plausible, it is uncertain whether they never fluctuate under the different experimental conditions. Hence, reproducibility and reliability of the results are not always guaranteed by other laboratories. Long-term and expensive experiments are required to make the preciser estimate.

Design of experiment has been often introduced to investigate the reliable estimation of factorial effect more efficiently[17,22]. Characteristics of the method are[23]: randomization of order of experiment, orthogonal lay out of experiment, quantitative evaluation of factorial effect, and improved data-analysis.

1.1 Experiment I using Peptone-Malt Extract Medium

Experiment I was carried out on four experimental factors with three levels using two wood species. These factors are: size of test block, fungal species, amount of peptone and amount of malt extract. Test blocks were prepared from the sapwoods of *Fagus crenata* Blume and *Cryptomeria japonica* D. Don. The sizes of test blocks are: 1.5 (*tangential*) × 1.5 (*radial*) × 3.0 (*longitudinal*), 1.0 (*t*) × 1.0 (*r*) × 2.0 (*l*), and 0.5 (*t*) × 0.5 (*r*) × 1.0 (*l*) (cm). *Chaetomium globosum* Kunze (IAM* 8059) was used as a test fungus. For comparison, a white rot fungus, *Coriolus versicolor* Quél. (FES** 1030), and a brown rot fungus, *Tyromyces palustris* Murr. (FES 0507), were used. Both fungi are designated as test fungi by JIS Z 2110-1963. Concentrations of peptone (Kyokuto Seiyaku, Japan) are: 0.1, 0.3 and 0.3 (%). Concentrations of malt

Table 1. Elemental analyses of peptone and malt extract used in Peptone-Malt Extract Medium.

	C (%)	H (%)	N (%)
Peptone	39.96	6.32	11.56
Malt extract	40.25	6.68	0.78

* Institute of Applied Microbiology, University of Tokyo, Japan.
** Government Forest Experiment Station, Tokyo, Japan.

Table 2. Lay out of Experiment I using Peptone-Malt Extract Medium.

Factor / No.	A	B	C	D
	Peptone (%)	Malt extract (%)	Fungus*	Test block**
1	0.1	none	P	small
2	0.1	0.75	V	middle
3	0.1	1.5	G	large
4	0.3	none	V	large
5	0.3	0.75	G	small
6	0.3	1.5	P	middle
7	0.5	none	G	middle
8	0.5	0.75	P	large
9	0.5	1.5	V	small

* P: *Tyromyces palustris*, V: *Coriolus versicolor*, G: *Chaetomium globosum*.
** small: $0.5\times0.5\times1.0$, middle: $1.0\times1.0\times2.0$, large: $1.5\times1.5\times3.0$ (cm).

extract (Difco Laboratories, USA) are: none, 0.75 and 1.5 (%). Elemental analyses of these materials are shown in Table 1. The nine experiments were laid out according to the orthogonal table $L_9(3^4)$ as shown in Table 2. The decay test was made by the sand-block method. Cylindrical glass bottles (9 cm in diameter and 16 cm in height), containing 350 g of quartz sand (ca. 30 mesh, Nakarai Chemicals, Japan) and 120 ml of nutrient solution, were screwed with metal caps. The bottles were autoclaved and inoculated with test fungi which had been previously allowed to cover the surface of the medium before the test blocks were inserted. Blocks were put in contact with the mycelial mat at radial surface. Two bottles which contained three blocks in each were used in each series of experiment. The composition of the nutrient solution is as follows:

KH_2PO_4 3.0 g, $MgSO_4\cdot7H_2O$ 2.0 g, glucose 25.0 g, peptone, malt extract and distilled water 1000 ml.

The weighed test blocks were sterilized by fumigation with propylene oxide, and then exposed to fungi. The temperature was maintained at 28°C throughout five different incubation periods of 12-, 24-, 36-, 48- and 60-days. The decayed blocks were cleaned of mycelium and dried to constant weight in an oven at 65°C. After calculation of the loss of weight by decay, the decayed blocks were put to the test of compressive strength parallel to the grain. The test was made by a Shimadzu REH-10 multi-purpose testing machine following the JIS Z 2111.

Analyses of variances were calculated from the data obtained according to the procedure of *F*-distribution. Tables 3 and 4 show the contribution rate of each factor, and Figs. 1~4 show the effect of each factor graphically. All of the four factors were significant in most cases of experiments including two species of wood,

Table 3. Contribution rate of each factor in Experiment I (*Fagus crenata*).

Factor*	Incubation period (day)									
	12		24		36		48		60	
	ρ_1 %	ρ_2 %	ρ_1 %	ρ_2 %	ρ_1 %	ρ_2 %	ρ_1 %	ρ_2 %	ρ_1 %	ρ_2 %
A	21.3	6.6	31.8	24.4	15.3	20.0	14.2	19.3	20.5	32.8
B	4.9	10.9	5.6	6.1	1.0	0.4	1.3	1.2	8.1	5.7
C	38.1	6.6	28.6	16.3	38.7	40.7	53.2	59.4	28.2	23.0
D	1.1	26.9	2.1	13.8	37.3	12.2	20.5	6.5	25.8	3.0
e	34.7	39.0	31.9	39.4	7.7	26.7	10.8	13.6	17.4	35.5

* A: Amount of peptone, B: Amount of malt extract, C: Fungal species, D: Size of test block, *e*: Error. ρ_1: Based on the loss of weight in wood after decay. ρ_2: Based on the compressive strength of wood after decay.

Table 4. Contribution rate of each factor in Experiment I (*Cryptomeria japonica*).

Factor*	Incubation period (day)									
	12		24		36		48		60	
	ρ_1 %	ρ_2 %	ρ_1 %	ρ_2 %	ρ_1 %	ρ_2 %	ρ_1 %	ρ_2 %	ρ_1 %	ρ_2 %
A	33.7	23.0	14.3	12.4	10.5	20.1	9.0	8.7	26.9	26.1
B	8.5	23.1	4.2	1.4	6.3	0.0	0.0	7.5	14.3	11.0
C	29.1	23.1	34.4	31.6	51.9	64.8	38.6	52.6	18.3	37.9
D	20.2	23.0	11.0	1.3	11.3	6.5	2.2	1.4	27.3	13.7
e	8.5	7.8	36.1	53.3	20.0	8.6	54.2	29.8	13.2	11.3

* A: Amount of peptone, B: Amount of malt extract, C: Fungal species, D: Size of test block, *e*: Error. ρ_1: Based on the loss of weight in wood after decay. ρ_2: Based on the compressive strength of wood after decay.

five different incubation periods, and two measurements of weight loss and compressive strength. Factor C (fungal species) showed the highest contribution, since *C. globosum* could not degrade both woods at all in all experimental conditions. This apparently evidenced the non-availability of Peptone-Malt Extract Medium to test for *C. globosum*, although fungal growth on the medium was abundant at every experimental condition. The result supports previous proposals[15,24] that higher growth rate of a fungus is not necessarily accompanied with higher growth rate of wood decay. Consequently, significant effects of other factors must be attributed mostly to the activities of the two Basidiomycotina fungi used, and contribution rates of these factors might be greater unless *C. globosum* was used as a test fungus. Factor A (amount of peptone) was the next to Factor C for the contribution rate. Wood-decaying capacity increased with the amount of peptone. As shown in Table 1, peptone was substantially the sole source of nitrogen in the medium used. This suggests that decrease of the carbon to nitrogen (C:N) ratio brought the

increase of the rate of decay by the two Basidiomycotina fungi used. A similar result was obtained by LEVI and COWLING[25], who investigated the effect of added nitrogen (as casein hydrolysate) on the rate of decay of aspen sapwood by *Coriolus versicolor*.

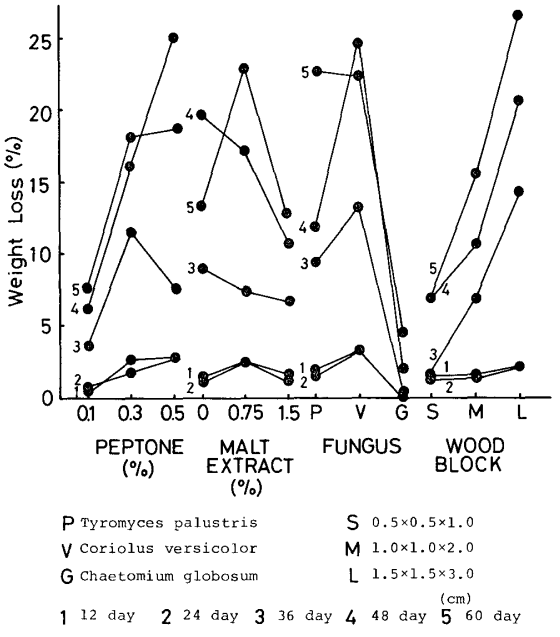

Fig. 1. Effect of factors in Experiment I using *Fagus crenata* (Based on the loss of weight in wood after decay).

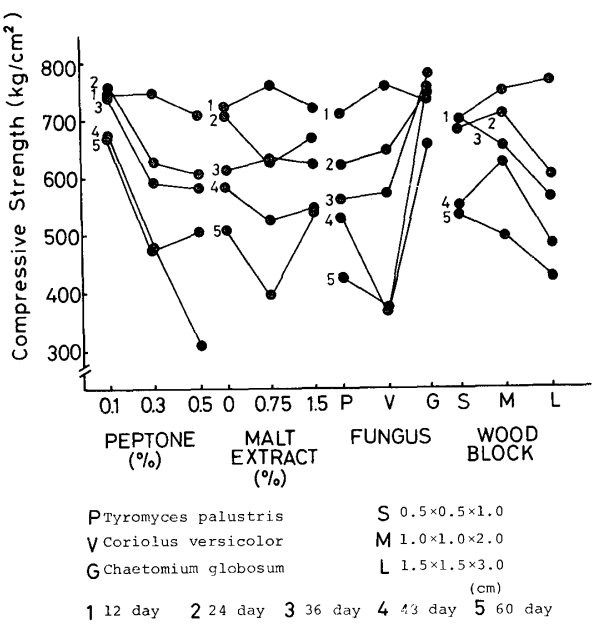

Fig. 2. Effect of factors in Experiment I using *Fagus crenata* (Based on the compressive strength of wood after decay).

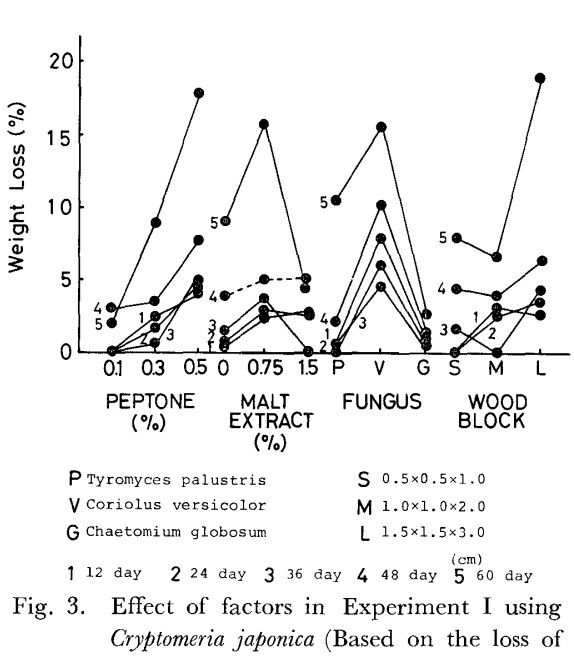

Fig. 3. Effect of factors in Experiment I using *Cryptomeria japonica* (Based on the loss of weight in wood after decay). Dotted line represents the non-significance at 1 and 5% levels.

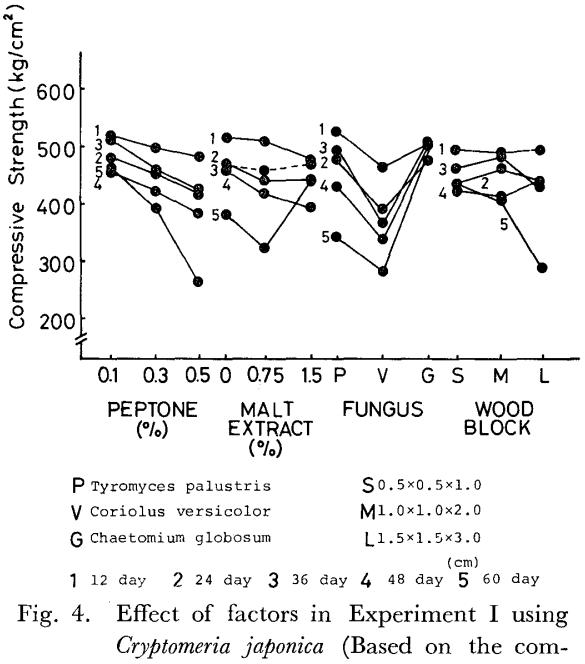

Fig. 4. Effect of factors in Experiment I using *Cryptomeria japonica* (Based on the compressive strength of wood after decay). Dotted line represents the non-significance at 1 and 5% levels.

In the JIS Z 2119, the amounts of peptone, malt extract and glucose are designated to 0.3, 1.5 and 4.0 (%), respectively. However, from the results obtained here, it is concluded that modification of such a composition to decrease C:N ratio is required for obtaining the larger amount of decay. Factor D (size of test block) was the third in the contribution rate. Higher wood-decaying capacity was observed as the dimentions increased. This was more pronounced in *F. crenata* than in *C. japonica*. Factor B (amount of malt extract) was the last in order of the contribution. The highest rate of decay was observed at 0.75% of malt extract. *C. japonica* was not readily attacked than was *F. crenata*. Poor overall decay was evidenced in *C. japonica* even at 0.3% level of peptone which permitted the considerable amount of decay of *F. crenata*. This suggests that higher amount of nitrogen should be provided for the incipience of decay of resistant wood.

As to the incubation period, the longer, for example 48- or 60-days, may be required to investigate the effect of each factor more precisely. Difference among the three levels in each factor was not much evidenced for the shorter periods of incubation. Significance of factor determined by the compressive strength was approximately the same to that by the loss of weight. In *F. crenata*, however, contribution rate of error by the former was always slightly greater than that by the latter. This means that some factors other than the four factors may contribute more actively at the determination of the compressive strength. They seem to be involved in the condition of measurement, the uniformity of test blocks and the location of destruction in decayed blocks.

1.2. Experiment II using enriched Abrams's Medium

Four experimental factors with three levels each were dealt with in the Experiment II using enriched Abrams's Medium. These factors are: size of test block, wood species, amount of ammonium nitrate and kind of sugar. Ammonium nitrate and sugar are the sole sources of nitrogen and carbon, respectively. Original Abrams's Medium[26] contains 3 g of NH_4NO_3, 2 g of K_2HPO_4, 2.5 g of KH_2PO_4 and 2 g of $MgSO_4 \cdot 7H_2O$ per litre of water. This synthetic medium was used by SAVORY[4] together with several media for the isolation of fungi from cooling towers. He showed that *C. globosum* caused the highest weight loss of beech blocks on Abrams's agar. Nitrates are known to be excellent sources of nitrogen for many fungi, although inability to utilize nitrates is especially common in some groups. e.g., the higher Basidiomycotina, the Saplolegniales and Blastocladiales of Mastigomycotina, etc[27]. However, the effect of nitrate concentration in the medium on the rate of decay has been little studied.

Mycelial growth on the whole surface of medium prior to subjecting test blocks to fungal attack has been recommended for the successful performance of decay

Table 5. Lay out of Experiment II using Enriched Abrams's Medium.

Factor / No.	A	B	C	D
	NH_4NO_3 (%)	Carbon source	Wood*	Test block**
1	0.1	glucose	P	small
2	0.1	xylose	F	middle
3	0.1	mannose	L	large
4	0.3	glucose	F	large
5	0.3	xylose	L	small
6	0.3	mannose	P	middle
7	0.5	glucose	L	middle
8	0.5	xylose	P	large
9	0.5	mannose	F	small

* P: *Pinus densiflora*, F: *Fagus crenata*, L: *Shorea* sp.
** small: $0.5\times0.5\times1.0$, middle: $1.0\times1.0\times2.0$, large: $1.5\times1.5\times3.0$ (cm).

tests. Addition of sugar as carbon source is essential for desirable abundant mycelial growth. Test blocks were prepared from the sapwoods of *Fagus crenata* BLUME, *Shorea* sp. commonly called "red lauan", and *Pinus densiflora* SIEB. et ZUCC. The sizes of test block are the same as those in Experiment I. Concentrations of ammonium nitrate are: 0.1, 0.3 and 0.5 (%). Kinds of sugar are: D-glucose, D-xylose and D-mannose. Concentration of each sugar is equally 2.0%. The nine experiments were laid out according to the orthogonal table $L_9(3^4)$ as shown in Table 5. All the procedures involved in the decay test and the determination of compressive strength are the same as those of Experiment I.

Contribution rate and effect of each factor are shown in Table 6 and in Figs. 5 and 6, respectively. All of the four factors were significant at every incubation period in either weight loss or compressive strength. Factor C (wood species) showed the highest contribution rate, since *C. globosum* could not attack substantially the

Table 6. Contribution rate of each factor in Experiment II.

Factor*	Incubation period (day)									
	12		24		36		48		60	
	ρ_1 %	ρ_2 %	ρ_1 %	ρ_2 %	ρ_1 %	ρ_2 %	ρ_1 %	ρ_2 %	ρ_1 %	ρ_2 %
A	0.8	1.4	1.8	1.8	0.8	0.4	7.3	0.1	6.2	3.3
B	3.1	10.7	0.0	15.5	0.0	7.3	0.7	0.4	0.1	5.8
C	52.7	6.7	70.1	29.8	64.9	64.6	74.3	63.5	82.1	67.0
D	17.4	2.3	12.6	5.8	22.5	12.7	12.1	7.82	6.2	10.5
e	26.0	78.9	15.5	47.1	13.8	15.0	5.6	8.2	5.4	13.4

* A: Amount of ammonium nitrate, B: Kind of sugar, C: Wood species, D: Size of test block, *e*: Error. ρ_1: Based on the loss of weight in wood after decay. ρ_2: Based on the compressive strength of wood after decay.

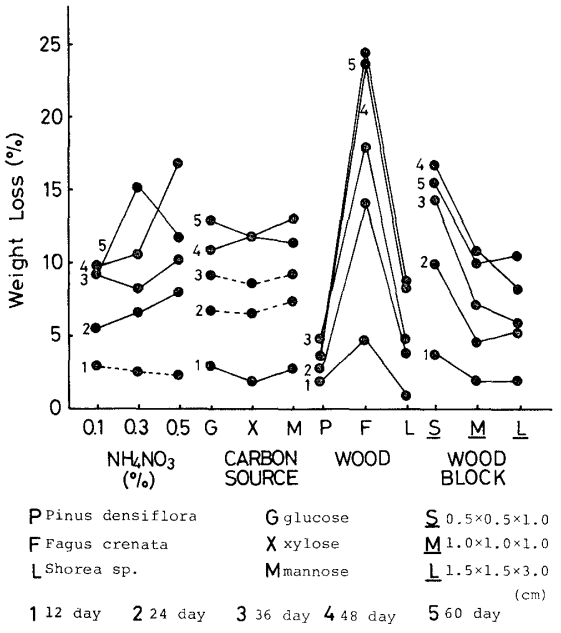

Fig. 5. Effect of factors in Experiment II (Based on the loss of weight in wood after decay). Dotted line represents the non-significance at 1 and 5% levels.

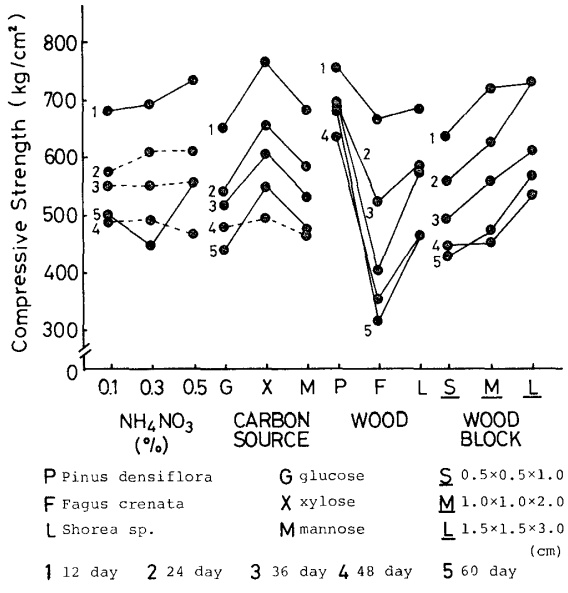

Fig. 6. Effect of factors in Experiment II (Based on the compressive strength of wood after decay). Dotted line represents the non-significance at 1 and 5% levels.

test blocks of *P. densiflora* and *Shorea* sp. The contribution rates of other factors might be greater if more susceptible species are used. The effects of Factor A (amount of ammonium nitrate) and Factor B (kind of sugar) were not so much obvious. However, higher amount of wood decay was obtained at 0.3 or 0.5 (%) of ammonium nitrate and on the medium containing glucose or mannose. The compressive strength of test blocks remained at higher level on xylose-containing medium. Factor D (size of test block) was the next to Factor C for the order of the contribution rate. Small blocks were attacked more severely than others. The effect of size was remarkably pronounced in the compressive strength. Similar results have been published recently by KERNER-GANG[28], who investigated several factors influencing soft rot testing in the vermiculite burial method. Decomposition rates of preservatives-treated beech blocks were much higher for the smaller ones.

The contribution rate of error in the compressive strength was always greater than that in the loss of weight. The results obtained here showed that forty-eight or sixty day's incubation was required to investigate the preciser effect of each factor and to yield more than 20% of weight loss of beech blocks by *C. globosum*.

2. Effect of *Chaetomium globosum* on the physical- and chemical properties of wood

Detailed study on physical and chemical effects of soft rot fungi has not appeared

yet in contrast with other wood-decaying fungi. LEVI and PRESTON[29] observed that DP (degree of polymerization) of the holocellulose during the attack of beech wood by *C. globosum* initially increased and then decreased gradually. This mode of attack seems to differ from that of either brown rot fungi or white rot fungi, which cause a rapid loss in DP of the total cellulose[30], or only a gradual loss in DP[30]. CARTWRIGHT et al.[2] found long ago that brown rot fungi cause a more rapid drop in strength properties than do white rot fungi. Although the reduction in impact bending strength was determined for soft-rotted wood by a few investigators[8,31], degrees of reduction in other strengths have not been studied yet, and no comparison with those for brown-rotted- and white-rotted woods has been made. To obtain information on the characteristics of wood decay by soft rot fungi, 1) ability of *C. globosum* to reduce the strengths of wood has been compared with that of brown rot- and white rot fungi, and 2) infrared spectral changes in wood constituents during the attack by *C. globosum* and 3) carbon nutrition of this fungus are investigated.

2.1 Changes in strength properties of wood on decay[32]

Compressive strength parallel to the grain

When *Fagus crenata* was exposed to *Tyromyces palustris*, reduction of the strength was rapid during the decrease of specific gravity to the value of 0.36, and then gradual. The strength for the brown-rotted beech was expressed by the equation ($P=2250d-650$, $d\geqq0.36$), where P and d are the strength and the specific gravity, respectively. In the soft-rotted beech, decay was concentrated largely at the surface part of block and did not proceed into the inner portion. However, similar equation ($P=2250d-670$) was obtained within the limited range of specific gravity over 0.40. On the other hand, the strength for the white-rotted beech exposed to *Coriolus versicolor* did not reduce rapidly and the equation ($P=2000d-670$, $d\geqq0.40$) was applicable. The reduction in strength of attacked wood of *Cryptomeria japonica* was evidently rapider for *T. palustris* ($P=4100d-980$, $d\geqq0.24$) than for *C. versicolor*.

Compressive strength perpendicular to the grain

In *F. crenata* exposed to *C. globosum*, the strength reduced more rapidly than that parallel to the grain. The reduction in the strength for the brown-rotted beech preceded similarly that for the white-rotted one. The former was expressed by the equation ($P=550d-170$, $d\geqq0.30$), and the latter by the equation ($P=550d-125$, $d\geqq0.30$).

Static bending strength

The reductions in the strength for both the brown-rotted woods were rapider than those for the white-rotted and the soft-rotted ones in both wood species. The reduction for the soft-rotted wood of *F. crenata* preceded that for the white-rotted when the specific gravity reduced to ca 0.45, although approximately the same

pattern at the greater specific gravity was observed for both types of decay. The modulus of elasticity decreased in all the cases as in the reduction in strength. The reductions in strength for the brown-rotted and soft-rotted beech blocks were especially rapid within the range between 40 and 50 of the modulus of elasticity. The rate of reduction in the strength was higher for the wood of *C. japonica* than that for *F. crenata*.

Tensile strength parallel to the grain

Clear patterns were not obtained due to the greater variations among the data and to the lower rate of decay. However, the reductions in the strength for the brown-rotted woods seemed to still precede those for other woods. The reduction was evidently detected even in the wood of *C. japonica* exposed to *C. globosum*.

Changes in strength properties of wood caused by decay have been actively studied since CARTWRIGHT et al's 1931 publication[2]. In all early studies, strengths versus incubation periods or losses of weight in whole wood blocks were plotted on graphs. As described in Chapter 1, size of wood blocks usually influences the rate of decay. Size of blocks should be varied with kind of strength determination. In the determination of bending- and tensile strengths, the rate of decay of the portion where loading and stress are concentrated greatly influences the results. On the long and slender blocks used for the test of bending strength, a uniform rate of decay is never expected throughout the whole length of block. Furthermore, on the blocks for tensile strength, a precise determination is not made when the ends of blocks are degraded by fungal attack. In these cases, the reduction in strength can not be compared exactly with the loss of weight in whole blocks. To enhance the decay at the central portion of block, blocks for the bending- and tensile strengths were sealed with paraffin and paraffin films leaving the central portion unsealed. In addition, the specific gravity in the central portion of block was calculated to compare the rate of reduction in each determined strength on the same basis.

Results obtained are summarized as follows:

(1) With the exception of tensile strength, the reduction in strength was not apparent in *C. japonica* exposed to *C. globosum*,

(2) The order of the reduction in strength caused by decay was tensile parallel to the grain>bending>compression perpendicular to the grain>compression parallel to the grain,

(3) The order of the ability to reduce the strengths of wood of *F. crenata* was *T. palustris* (brown rot)>*C. globosum* (soft rot)>*C. versicolor* (white rot).

In agreement with earlier findings[2,23], brown rot fungus caused a rapid drop in strength properties than did white rot fungus. The order of the reduction in strength also coincided approximately with the list by CARTWRIGHT and FINDLAY[30].

The reduction in strength for soft-rotted wood has been studied by a few investigators[8,31,34]. Impact bending strength of wood exposed to several soft rot fungi was determined and compared with each other.

On the micro-morphological aspect, the following characteristics have been known for the different types of wood decay[35]:

(1) soft rot; (a) the formation of chains of cavities in the S_2 layers of tracheids and fibres,

(2) white rot; (a) the progressive thinning of the secondary cell walls from lumen outwards,
(b) the decomposition occurring uniformly in the regions attacked,

(3) brown rot; (a) no thinning of the secondary cell walls until the very late stages of decomposition,
(b) the irregular patchy attack of the tissues.

The walls of wood cells are composed of three groups of structural substances which are classed as framework, matrix and encrusting materials by WARDROP[36]. The framework substance is cellulose which occurs in the form of microfibrils. Cellulose is closely associated with the matrix and encrusting substances in the paracrystalline regions of the microfibrils. The microcapillaries surrounding the cell wall framework are also filled with these amorphous substances. Hemicelluloses are incorporated into the cell wall as the matrix substances. The lignin skeleton that remains after the enzymatic removal of carbohydrates from wood provides clear evidence of the microcapillary system in the cell wall.

There does not exist any generally accepted theory on the fracture in wood which is induced by stress and strain. However, the fracture is accepted as progressive damage, which has three stages[37]: (1) initiation of submicroscopic slips, (2) propagation of microscopic deformations and cracks, and (3) occurrence of macroscopic failures. There are many weak spots in the anatomical structure of wood which create favorable conditions for crack initiation. In the case of decayed wood, additional weak spots which are caused by fungal attack may give a facilitating effect in such a crack initiation. It is reasonably concluded that the rapid depolymerization of cellulose molecules and the irregular patchy attack of the tissues may cause the rapid drop in strength properties of brown-rotted woods, although there is no experimental evidence for these in the literature and in the present experiment. *C. globosum*, a soft rot fungus, has been ranked in the second order of the ability to reduce the strength of wood of *F. crenata*. However, it is difficult to explain the mode of strength reduction in relation to the cavity formation in the secondary wall and the different pattern of attack on cellulose polymer by this fungus. Remaining of the sound core which is caused by the progressive destruction from the surface

inwards is a macroscopic characteristic of soft-rotted wood[31]. This seems to be responsible at some extent for the rank of *C. globosum*. The distribution of cavity may also influence the reduction in strength. LIESE and AMMER[31], who investigated the reduction in impact bending strength of beech wood exposed to soft rot fungi, reported a close correlation between the number of cells attacked and the strength loss. On the contrary, ZYCHA[34] could not find such a correlation when he made a similar investigation. Cavity formation creates undoubtedly more or less favorable conditions for crack initiation. However, it is difficult to predict statistically the effect of cavity formation on the strength properties of wood, since it has not been found any generally accepted theory on the fracture.

2.2 Changes in infrared spectra of wood on decay[38]

Changes in infrared spectra are described separately in five regions of wave number.

In the 1800～1500 cm^{-1} region

(a) The absorption at 1730 cm^{-1} in *F. crenata* decreased markedly during the degradation by *C. globosum*, whereas only slightly by *T. palustris* and *C. versicolor*.

(b) The same absorption in *C. japonica* decayed by the two Basidiomycotina fungi decreased constantly. The decrease was observed also in the wood scarcely decayed by *C. globosum*. The occurrence of absorption at about 1715 cm^{-1} was realized in the woods of both species, but strongly in *C. japonica*.

(c) The absorption band centered at 1640 cm^{-1} was broadened in *C. japonica* decayed by *T. palustris*.

(d) The absorptions at 1595 and 1510 cm^{-1} were sharpened in *F. crenata* decayed by *T. palustris*. The latter absorption in *C. japonica* decayed by the same fungus also increased significantly. These absorptions, however, decreased in *F. crenata* decayed by *C. versicolor* and *C. globosum*. The absorption at 1510 cm^{-1} in *C. japonica* decayed by *C. versicolor* also decreased.

In the 1500～1300 cm^{-1} region

(a) The absorption at 1460 cm^{-1} increased with the progress of decay in both woods exposed to *T. palustris*. This was more noticeable for *F. crenata* than for *C. japonica*. Thus, the absorption in *F. crenata* became greater than that at 1420 cm^{-1} as decay proceeds.

(b) The absorption at about 1405 cm^{-1} which was not present in original sound wood was visualized after decay, especially in *F. crenata* decayed by *C. globosum* and in *C. japonica* by *C. versicolor*.

(c) The absorption at 1330～1310 cm^{-1} increased slightly in *F. crenata* exposed to *T. palustris*, while it decreased in *C. japonica* exposed to the same fungus.

In the 1300～1100 cm^{-1} region

(a) The broad absorption band at 1300～1200 cm^{-1} in *F. crenata* exhibited the different shape with progress of decay by *T. palustris*, and that was characterized by the occurrence of two distinct absorptions at 1270 and 1230 cm^{-1}. These absorptions were present originally in sound wood of *C. japonica* and increased similarly with the progress of decay by the same fungus.

(b) The broader band at 1200～1000 cm^{-1} weakened progressively, as a whole, as decay proceeds. Details in the change, however, varied with the wood and fungal species. The absorption at 1160 cm^{-1} decreased in *F. crenata* exposed to *T. palustris* and *C. globosum*, and in *C. japonica* to *T. palustris*. The absorption at 1120 cm^{-1} increased in *F. crenata* decayed by *T. palustris* and *C. globosum*, but not in *C. japonica* by *T. palustris* and *C. versicolor*.

In the 1100～900 cm^{-1} region

(a) The absorption band at 1050～1030 cm^{-1} in *F. crenata* was divided into two weak absorptions at 1050 and 1030 cm^{-1} after decay by *T. palustris*.

(b) These absorptions were present in *C. japonica* before decay. The absorption at 1030 cm^{-1} became greater than that at 1050 cm^{-1} with the progress of decay by *T. palustris* and *C. versicolor*.

In the 900～650 cm^{-1} region

(a) The absorption at 895 cm^{-1} decreased progressively in *F. crenata* decayed by *T. palustris* and *C. globosum*. Such a decrease was also observed in *C. japonica* decayed by *T. palustris*.

(b) The decrease in the same absorption was not found in *F. crenata* exposed to *C. versicolor* but slightly in *C. japonica*.

The absorption at 1730 cm^{-1} decreased rapidly in *F. crenata* exposed to *C. globosum*. This was not noticeable in *C. japonica* exposed to the same fungus and in *F. crenata* to *T. palustris* and *C. versicolor*. This absorption is assigned to the C=O stretching vibration of carboxyl and acetyl groups in xylan, precisely the *O*-acetyl (4-*O*-methyl glucurono) xylan in hardwoods and the arabino-(4-*O*-methyl glucurono) xylan in soft woods[39]. The absorption at 1460 and 1230 cm^{-1} are due to the CH_2 deformation vibration in lignin and xylan with benzene ring vibration in lignin, and to the acetyl and carboxyl vibration in xylan with C=O stretching vibration in lignin, respectively[39]. These absorptions did not change in *F. crenata* exposed to *C. globosum* and *C. versicolor* but increased in the same wood decayed by *T. palustris*. Among several absorptions refered to cellulose and hemicelluloses, absorptions at 1380, 1160 and 895 cm^{-1} decreased in *F. crenata* exposed to *C. globosum* and *T. palustris*. These results suggested that *C. globosum* was characterized by the specific action on carboxyl and acetyl groups in hemicelluloses and that *C. globosum* and *T. palustris* attacked actively cellulose and hemicelluloses in the decay of hard-

woods. The absorptions at 1595 and 1510 cm^{-1} are due to the well known stretching vibrations of the benzene ring in lignin. These absorptions increased in both woods decayed by *T. palustris* but decreased in those by *C. versicolor* and in *F. crenata* by *C. globosum*. The five absorptions at 1460, 1420, 1270, 1230 and 1030 cm^{-1} are also refered to lignin. Among these, the absorption at 1460 cm^{-1} is assigned to the CH_2 deformation with benzene ring vibration and that at 1420 cm^{-1} is to the CH_3 bending vibration[39]. The other absorptions are due to the C=O stretching vibrations, but they also associated with cellulose or hemicelluloses[39]. These five absorptions did not decrease in the brown-rotted woods but showed little change in the white-rotted and soft-rotted ones. Changes in absorptions due to lignin in the soft-rotted wood were thus quite different from those in the brown-rotted one, while but similar to those in the white-rotted one.

C. globosum has been shown to cause depletion of the lignin in beech wood[7,29,40]. The lignin remaining in the decayed wood was deficient in methoxyl and more acid-soluble than that in the sound wood[29]. Thus, this soft rot fungus can bring about at least some degradation of lignin, but it is not known what specific sequential changes are involved[41]. In comparison of the characteristics of soft rot with other types of wood decay[7], carbohydrate depletion with little lignin attack has been emphasized as the similarity to brown rot, and the gradual increase in alkali solubility as that to white rot. However, the results obtained here suggest that soft rot fungi are more active against hardwood lignin than are brown rot fungi.

Infrared spectra of wood exposed to *C. versicolor* were not very different from those of sound wood, with the exception of few absorptions at 1595, 1510 and 1380 cm^{-1}. Earlier investigations[30,42] revealed that the solubility of wood in water, 1% NaOH, and in organic solvents did not change considerably during decay by white rot fungi. This indicates that the rates of production and utilization of degradation products are approximately equal. In accord with this, the polysaccharides[30] and lignin[43] remaining in white-rotted wood at various stages of decay are not very different in properties from these substances in sound wood. Therefore, only small parts of the polymers are attacked at any given time, and the affected portions are completely degraded and assimilated before other parts of the polymers are significantly affected.

2.3 Utilization of carbohydrates[44]

The following carbohydrates were added as the sole carbon source to the basal medium:

L-arabinose, D-glucose, D-mannose, D-galactose, D-glucuronic acid, D-galacturonic acid, D-maltose, CMC (carboxymethyl cellulose sodium salt, 0.5 to 0.7 in degree of substitution, and 300 to 450 in degree of polymerization), and crude xylan

extracted from wood of *Fagus crenata*.

Abram's solution[26] was used as the basal nutrient medium. Amounts of carbon source were 10 g per litre for xylan and 20 g for other carbohydrates. When using D-glucuronic acid and D-galacturonic acid as carbon source, pH values of these uronic acid media were adjusted to 6.5 with sterilized sodium hydroxide. Growth of *Chaetomium globosum* on various carbon sources other than crude xylan and con-

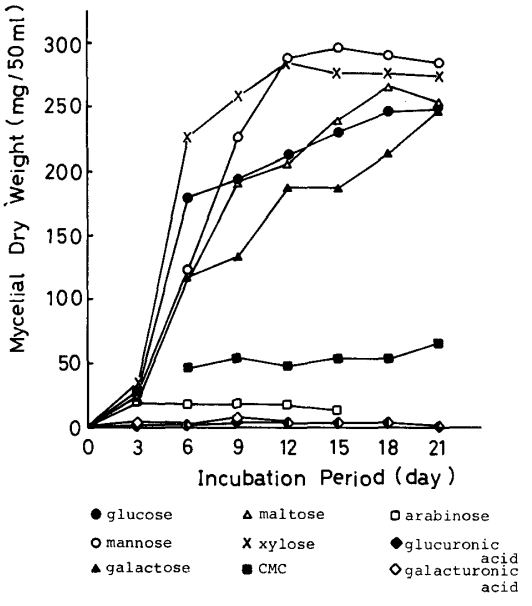

Fig. 7. Mycelial dry weight of *Chaetomium globosum* grown in Abrams's medium with various carbon sources.

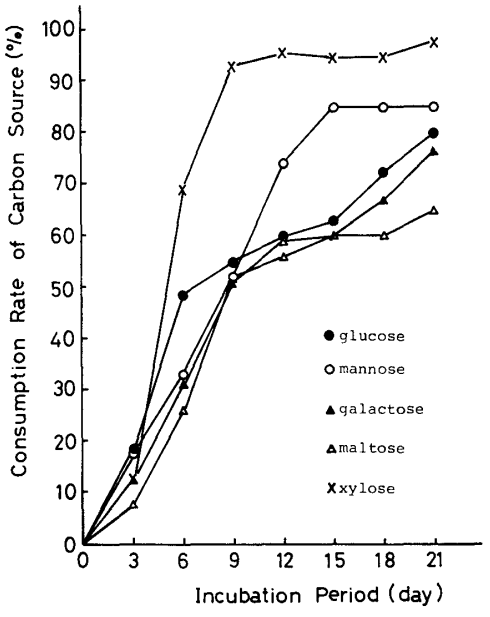

Fig. 8. Consumption rate of five carbon sources by *Chaetomium globosum* grown in Abrams's medium.

sumption rate of some carbon sources are shown in Figs. 7 and 8. Xylose, mannose, glucose, galactose and maltose were available to *C. globosum*. Of these sources, however, galactose apparently gave slower fungal growth, although the final mycelial yield was nearly equal to other sources. COCHRANE[45] reviewed the carbon nutrition of fungi and concluded that galactose was utilized by most fungi but was not usually so good sources as glucose. Results obtained here suggest that *C. globosum* is adapted to metabolize galactose during incubation, and that the metabolic activity to galactose may not be constitutive for the fungus. There are many reports showing that glucose is a good carbon source for many fungi including wood-decaying fungi and that xylose and mannose are equivalent to glucose[45,46]. In the present investigation, however, glucose was slightly inferior to xylose and mannose in daily growth rate. In the metabolism of glucose by fungi, three different pathways have been established, namely EMP (Embden-Meyerhof-Parnas) pathway, HMP (hexose monophosphate) pathway and ED (Enter-Douroroff) pathway. Mannose and galactose are converted into fructose-6-phosphate via glucose and/or phosphorylated

derivatives of these sugars, and derived into the EMP and HMP pathways[45], whereas xylose is firstly converted into xylulose-5-phosphate via xylulose and connected to HMP pathway to produce fructose-6-phosphate[47]. Rapid growth and consumption in xylose medium seem to reflect active isomerization, phosphorylation and other reactions, leading the major participation of HMP pathway in *C. globosum*. From the present results it can be seen that mannose is a good carbon source with xylose for *C. globosum*. KEILICH et al.[48], in agreement with this result, reported that *C. globosum* was able to degrade both glucomannans and glucuronoxylan. As to the enzyme activity, however, they found that xylanase activity of this fungus was higher than mannanase activity.

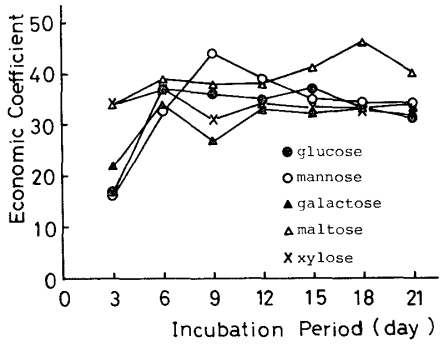

Fig. 9. Efficiency of conversion of five carbon sources to mycelium of *Chaetomium globosum*.

Changes of economic coefficient in five carbon sources are shown in Fig. 9. Economic coefficient is one of the methods to determine the efficiency of conversion of carbon source to mycelium. It is defined by the formula;

$$\frac{\text{mycelium dry weight (g)}}{\text{amount of carbon compound consumed (g)}} \times 100$$

Economic coefficient is maximal when respiratory carbon dioxide and soluble metabolic products are minimal in quantity. Very low values reflect the production of significant amounts of soluble compounds in high-carbohydrate media[46]. Economic coefficient of xylose maintained the same moderate level throughout the incubation period, whereas that of mannose increased rapidly in early period and then decreased gradually. Although consumption rate of maltose was slower than that of other sources, economic coefficient of the source was throughly kept at a higher level, and mycelial yield was equivalent to other sources. This indicates the presence of maltase and the effective use of maltose for mycelial formation.

L-Arabinose occurs as a hemicellulose component, though the content is very low. In contrast to D-xylose, this sugar was not utilized at all by *C. globosum*. The fact probably indicates the lack of metabolic pathway—arabinose→ribulose→ribulose

5-phosphate→xylulose 5-phosphate[47]. Inability to utilize both L and D isomers of arabinose has been reported on many fungi[49]. Glucuronic acid and galacturonic acid which occur as components of glucuronoxylan and galactoglucomannan were not utilized at all, even when the pH values in these uronic acid media were adjusted to 6.5 with sodium hydroxide solution. On the solid agar medium which contained glucuronic acid as the sole carbon source and was adjusted to pH 6.5 with sodium hydroxide solution, poor growth of *C. globosum* was observed better than on no carbon medium, but it was apparently lesser than of *Coriolus versicolor* and *Tyromyces palusris*. This indicates that the ability of *C. globosum* to utilize the uronic acid in wood hemicellulose is very low. Growth on CMC medium was also very poor. As a cellulolytic activity of *C. globosum* was not estimated enzymatically, the reason for such poor growth was uncertain.

Growth, consumption rate and economic coefficient of three fungal species in xylan-containing medium are shown in Figs. 10~12. The contents of Klason lignin and uronic anhydride of this source were 6.25% and 5.56%, respectively. Determination of pentosan was made with the neutral fraction from hydrolyzed culture filtrates. Correction to the fulfural yield from uronic acids was not made. Maximum mycelial yields of test fungi were almost the same. However, *C. globosum* grew more rapidly and kept the same level of growth for longer period than did *C. versicolor* and *T. palustris*. Economic coefficient for *C. globosum* kept a higher level throughout

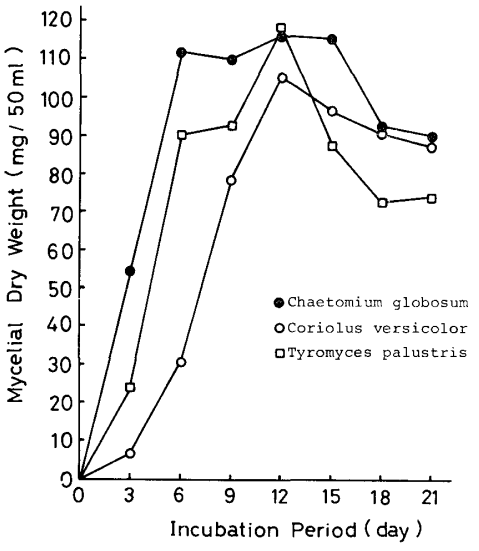

Fig. 10. Mycelial dry weight of three fungal species grown in Abrams's medium with crude xylan as sole carbon source.

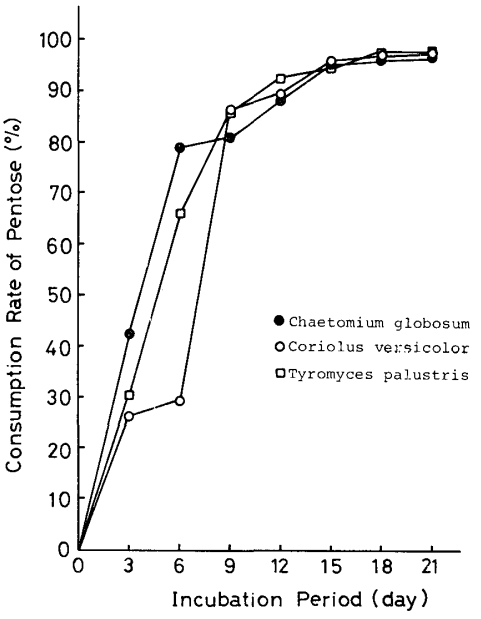

Fig. 11. Consumption rate of pentose by three fungal species grown in Abrams's medium with crude xylan as sole carbon source.

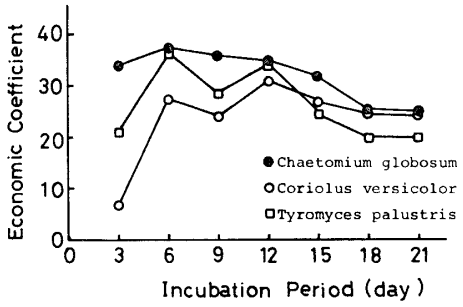

Fig. 12. Efficiency of conversion of crude xylan to mycelium of three fungal species.

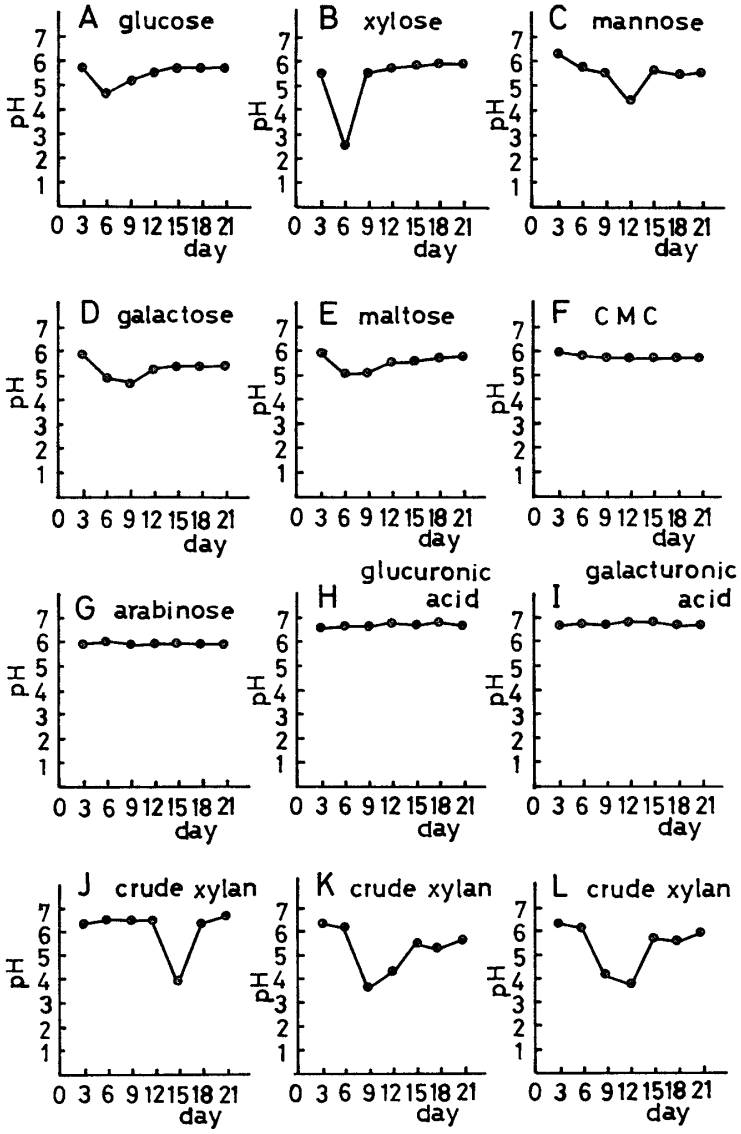

Fig. 13. Change in pH value of Abrams's medium with various carbon sources during incubation (A-J: *Chaetomium globosum*, K: *Coriolus versicolor*, L: *Tyromyces palustris*).

the incubation period, although the progressive increase of comsumption rate showed a fairly similar pattern to those of other fungi.

As shown in Fig. 13, the pH value of each nutrient medium from which carbon source was available to test fungi exhibited a similar pattern of transition, that is, a considerably rapid fall and recovery of the value during the course of incubation. Such a pattern has been usually observed for many fungi and seems to reflect the accumlation of some acidic intermediate products.

From the present results that *C. globosum* is able to utilize xylose and xylan, it can be seen that this soft rot fungus has an active pathway to metabolize these mono- and polysaccharides, indicating the possible connection to the greater susceptibility of hardwoods to soft rot fungi.

3. Natural decay resistance of wood against *Chaetomium globosum*

Although soft rot fungi seem to attack hardwoods more readily than softwoods, they have been isolated frequently even from softwoods serviced in various environments[9,50]. GREAVES and LEVY[51] investigated on the durability of wood which was subjected to a long-term exposure (3300 to 5 years) in widely differing environments. The most intensive destruction, according to them, was found to occur in mining timber in which bacteria and soft rot fungi had combined to erode completely the secondary cell wall of fibres and tracheids. Thus, the higher resistance of softwoods against soft rot fungi which is evidently exhibited in the laboratory tests does not mean necessarily the predominant occurrence of soft rot on hardwoods, and might be overcome at some extent with some fungal/bacterial associations, or any of unknown biological combinations and non-biological effects. In this chapter, decay resistance of various wood species (138 spp. of temperate hardwoods, 64 spp. of tropical hardwoods and 45 spp. of softwoods) against *Chaetomium globosum* is described with reference to methanol extractives in wood. For comparison, a white rot fungus, *Coriolus versicolor*, was used in tests for tropical hardwoods and softwoods. Considering the surface action of soft rot fungi to the wood block, the size of test block was decided as 2.0 (t) $\times$ 2.0 (r) $\times$ 0.5 (l) (cm).

3.1 Temperate hardwoods[52]

One hundred and thirty-eight species covered 89 genera over 41 families. The resistance of hardwood species was divided into five classes as follows:

I Very resistant, loss of weight 0 to 5 (%)
II Resistant, loss of weight 5 to 15 (%)
III Moderately resistant, loss of weight 15 to 25 (%)
IV Non-resistant, loss of weight 25 to 40 (%)
V Perishable, loss of weight over 40 (%)

Thirty-two species were classified into class I and class II. Of these species, *Castanea crenata*[53,54], *Zelkova serrata*[53,54], and *Morus bombycis*[53~55] are well known for their higher decay resistance. It is conceivable that presence of tannins and related compounds in *C. crenata*[56], keyakinin and keyakinol in *Z. serrata*[49], and oxyresveratol and resveratol in *M. bombycis*[57] is mainly responsible for the higher resistance of these species. *Ternstroemia japonica* is known as a very resistant species against termite attack[58]. It is recognized that the cause of the resistance is associated with the presence of saponins in the wood extractives[55,58,59], though the mechanism of the antitermitic action has not been elucidated satisfactorily. If saponins are also associated with the higher resistance of *T. japonica* against *C. globosum*, surface and hemolytic activities of saponins may play an important role in their antifungal action[60]. *Magnolia obovata*[53], *Maackia amurensis* var. *buergeri*[54], *Robinia pseudoacacia*[53,54], *Rhus succedanea*[59] and *Camellia japonica*[58] have been also designated as resistant species. The correlation between resistance and extractives has not yet been established on these species, though various extractives have been identified, for example, alkaloids of *Magnolia*[61,62], fisetin and fustin of *Rhus*[63] and saponins of *Camellia*[64]. Information on the resistance of other species classified in class I and class II could not be obtained. However, oxyresveratol and resveratol have been isolated from *Cudrania tricuspidata*[64] and *Morus alba*[64]. Furansesquiterpene and paulownin may be partly responsible for the higher resistance in *Neolitsea aciculata*[65] and *Paulownia tomentosa*[66], respectively. All Ericaceous species tested, i.e. *Rhododendron tashiroi*, *Pieris japonica*, *Lyonia elliptica*, and *Vaccinium bracteatum*, were very resistant against *C. globosum*. Grayanotoxin, which normally occurs in this family[67], may be partly associated with the higher resistance of these species. In addition, occurrence of pieristoxin in *P. japonica*[68], and of lyoniatoxin and lyoniol in *L. elliptica*[67,70] has been reported.

Cercidiphyllum japonicum[53], *Cinnamomum camphora*[54], *Distylium racemosum*[54][70] and *Prunus sargenti* subsp. *jamasakura*[54] were not so resistant in this experiment as expected from their reputed resistance. On the contrary, *Juglans mandschurica* subsp. *sieboldiana*[54], *Alnus hirsuta* var. *sibirica*[53], *Quercus serrata*[33], *Passania edulis*[53], *Aesculus turbinata*[54] and *Elaeocarpus japonicus*[58] were not so susceptible as expected.

Among species designated as non-resistant (class IV) and perishable (class V), *Populus maximowiczii*[54], *Populus nigra* var. *italica*[58], *Pterocarya rhoifolia*[53,54], *Betula platyphylla* var. *japonica*[58], *Fagus crenata*[54], *Castanopsis cuspidata*[54,58], *Ulmus davidiana* var. *japonica*[54], *Cercidiphyllum japonicum*[54], *Albizzia julibrissin*[54], *Phellodendron amurense*[54], *Tilia japonica*[54], *Idesia polycarpa*[53,58] and *Kalopanax septemlobus*[53,58] have long been known as non-resistant or perishable species.

From the results obtained here, decay resistance of heartwood against *C. globosum*

is classified on the family-base as follows:

Moraceae (excepting *Broussonetia*) and Ericaceae; very resistant,

Lauraceae and Leguminosae; resistant,

Fagaceae, Rosaceae and Aceraceae; moderately resistant to non resistant,

Betulaceae, Araliaceae and Oleaceae; non-resistant,

Salicaceae and Ulmaceae (excepting *Zelkova*); perishable,

Magnoliaceae and Theaceae; variable in resistance.

Such variances of decay resistance among families and species do not seem to be much different from those for Basidiomycotina found in the literature.

Among 32 species of class I and class II, 25 species were tested for the effect of methanol extraction on decay resistance of heartwood samples. In addition, 4 species of class III, 1 species each of class IV and class V were tested for comparison. Unexpectedly, a positive effect (higher percent of weight loss in extracted blocks than that in non-extracted blocks) was significant in only five species (*Castanea crenata, Morus bombycis, Crataegus chlorosarca, Picrasma quessioides* and *Paulownia tomentosa*) of class I and class II. Such an effect was most pronounced in *P. tomentosa*. A negative effect (lower percent of weight loss in extracted blocks) was found in neary a half of species tested. A positive effect is possibly due to removal of toxic materials from wood. A non-significant effect found in resistant species may be attributed to; (1) insolubility of toxic materials in methanol, (2) insufficient removal of toxic materials soluble in methanol, and (3) presence of factors other than toxic materials. A negative effect in resistant species may be due to; (1) inhibiting action of methanol remaining in wood after extraction treatment, and (2) change in pH value caused by methanol extraction. A negative effect in less resistant species probably resulted mainly from a removal of nutrient substances during the extraction treatment.

3.2 Tropical (Southeast Asian) hardwoods[71]

Sixty-four species covered 22 families, including 25 genera. Based on the results described in Chapter 1, the composition of the nutrient solution for *C. versicolor* was modified as follows:

KH_2PO_4 3.0 g, $MgSO_4 \cdot 7H_2O$ 2.0 g, peptone 5.0 g, malt extract 10.0 g, glucose 25.0 g and distilled water 1000 ml.

Weight loss in percent was not over 40% for all the species used excepting *Ilex* sp. and *Alstonia* sp. To facilitate the discussion, decay resistance was divided into four classes. Class I, II and III are based on the same scheme described in 3.1 but class IV represents the case in which the loss of weight is over 25%. Tables 7 and 8 show the summarized results with the decay resistance of 64 species with specific gravity and content of methanol extractives. From Table 7 it can be seen that the higher average specific gravity and the more amount of methanol extractives

Table 7. Summarized data for the decay resistance, specific gravity and content of methanol extractives of 64 tropical hardwood timber species.

Fungus	*Chaetomium globosum*				*Coriolus versicolor*			
Class in decay resistance*	I	II	III	IV	I	II	III	IV
Average specific gravity	0.75	0.64	0.56	0.53	0.80	0.64	0.60	0.48
Average content of methanol extractives (%)	5.11	2.76	2.32	2.07	5.54	3.22	2.78	2.11
Number of species	24	18	19	3	20	15	18	11

* Weight loss due to decay, I: 0~5%, II: 5~15%, III: 15~25%, IV: over 25%.

Table 8. Statistical significance among four classes in decay resistance of 64 tropical hardwood timber species in relation to the specific gravity and the content of methanol extractives.

Fungus		*Chaetomium globosum*				*Coriolus versicolor*			
	Class*	I	II	III	IV	I	II	III	IV
Specific gravity	I	—	2.381**	4.153***	2.150**	—	5.177***	5.674***	7.336***
	II	2.381**	—	2.077**	1.420	5.177***	—	0.878	3.592***
	III	4.153***	2.077**	—	0.347	5.674***	0.878	—	2.637**
	IV	2.150***	1.420	0.347	—	7.336***	3.592**	2.637**	—
	Class*	I	II	III	IV	I	II	III	IV
Content of methanol extractives	I	—	2.484**	3.317***	1.468	—	2.338**	3.153***	3.103***
	II	2.484***	—	1.158	0.977	2.338**	—	0.542	0.702
	III	3.317***	1.158	—	0.369	3.153***	0.542	—	1.684
	IV	1.468	0.977	0.369	—	3.103***	0.702	1.684	—

* Weight loss due to decay, I: 0~5%, II: 5~15%, III: 15~25%, IV: over 25%.
** Significant at 5% level by *t*-distribution.
*** Significant at 1% level by *t*-distribution.

of the wood species result in the more resistant class in both cases of test fungi. As shown in Table 8, this tendency was statistically significant, and the relationship between specific gravity and decay resistance was most pronounced for *C. versicolor*. When using *C. globosum*, there are more wood species belonging to class I and fewer in class IV than when using *C. versicolor*. It has been reported that decay by soft rot fungi, even under favorable conditions, occurs more gradually than that by Basidiomycotina does[72]. From the data obtained here, it may be possible to generalize that dense or extractive-rich species are more resistant to decay, though the more resistant species do not always fit into the rule. The specific gravity and the amount of extractives are frequently associated with each other reciprocally. High densities in *Balanocarpus heimii*, *Shorea laevis* and *Vatica micrantha* may be associated with their high content of methanol extractives, but dense and resistant species called "Selangan

batu" do not contain so great a quantity of extractives, with the exception of *Shorea laevis*.

Shorea spp. have been usually divided into four groups, namely red meranti, white meranti, yellow meranti and Selangan batu, according to their specific gravity, hardness and color. Fig. 14 shows the relationship between the percent of weight loss due to decay by *C. versicolor* and original specific gravity of 20 *Shorea* species. It suggests that the correlation between decay resistance and specific gravity is highly significant in this case.

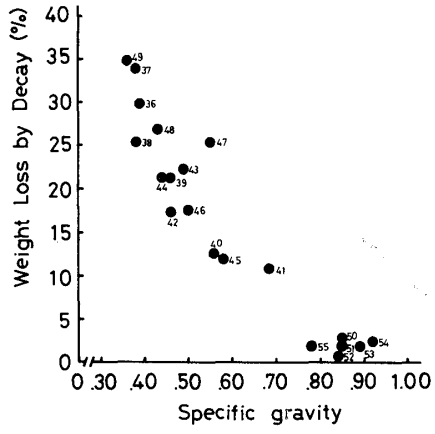

Fig. 14. Relationship between weight loss of *Shorea* spp. due to decay by *Coriors versicolor* and their specific gravity (36; *Shorea fallax*, 37; *S. gysbertsiana*, 38; *S. leprosulla*, 39; *S. macroptera*, 40; *S. ovalis*, 41: *S. parvifolia*, 42: *S. platycarpa*, 43; *S.* sp., 44; *S.* sp., 45; *S. agami*, 46; *S. symingtonii*, 47; *S.* sp., 48; *S. gibbosa*, 49; *S. hopeifolia*, 50; *S. exelliptica*, 51; *S. hypoleuca*, 52; *S. laevis*, 53; *S. leptoderma*, 54; *S.* sp., 55; *S.* sp.) (36～44; red meranti, 45～47; white meranti, 48～49; yellow meranti, 50～55; Selangan batu).

The majority of the very resistant species were common to both two test fungi; all species (with only one exception of *Metrosideros* sp.) assigned to class I by their decay resistance to *C. versicolor* were classified also in the same class in the case of *C. globosum*. In contrast with the case of temperate hardwoods, a positive effect of methanol extraction was found in as many as 17 species and a negative effect only in 4 species among the 42 in class I and class II when tested with *C. globosum*. In the case of *C. versicolor*, a positive effect was found in 11 species and a negative effect in 3 species among the 35 in these classes. A positive effect common to both fungi occurred in only 10 species. This suggests that the two test fungi have different sensitivities to wood extractives.

3.3 Softwoods[52)]

Forty-five species of softwoods covered 9 families, including 25 genera. Very low decaying ability in *C. globosum* was confirmed here for various softwood species.

Also *C. versicolor* could not cause severe weight loss of softwoods, as expected from its hardwood-preference. For the convenience of discussion, decay resistance of softwoods was divided into four classes as follows:

A; loss of weight 0 to 5 (%),
B; loss of weight 5 to 0 (%),
C; loss of weight 10 to 15 (%),
D; loss of weight over 15 (%).

On the decay resistance of softwoods, it is generally accepted that most species in Pinaceae are more susceptible to fungal attack than those in Taxodiaceae and Cupressaceae. Such a tendency was apparent in both cases of *C. globosum* and *C. versicolor*. Tables 9 and 10 show the summarized data for decay resistance and effect of methanol extraction related to content of methanol extractives in wood.

Table 9. Summarized data for the decay resistance of 45 softwood timber species against *Chaetomium globosum* and *Coriolus versicolor*.

Fungus	*C. globosum*				*C. versicolor*			
Class in decay resisrance*	A	B	C	D	A	B	C	D
Number of species	37	5	3	0	12	13	10	10
Pinaceae	16	5	3	0	4	5	6	9
(Abietoideae)	(10)	(0)	(2)	(0)	(1)	(4)	(3)	(4)
(Pinoideae)	(6)	(5)	(1)	(0)	(3)	(1)	(3)	(5)
Taxodiaceae	7	0	0	0	2	2	2	1
Cupressaceae	6	0	0	0	1	4	1	0
Others	8	0	0	0	5	2	1	0
Average content of methanol extractives (%)	4.40	1.36	1.85	—	7.15	3.62	2.59	1.65

* Weight loss due to decay, A: 0~5%, B: 5~10%, C: 10~15%, D: over 15%.

Table 10. Summarized data for the effect of methanol extraction on the decay resistance of 45 softwood timber species against *Chaetomium globosum* and *Coriolus versicolor*.

Fungus	*C. globosum*			*C. versicolor*		
Effect of extraction on decay*	+	±	−	+	±	−
Number of species	13	28	4	14	30	1
Pinaceae	10	12	2	5	18	1
(Abietoideae)	(4)	(7)	(1)	(2)	(10)	(0)
(Pinoideae)	(6)	(5)	(1)	(3)	(8)	(1)
Taxodiaceae	0	7	0	2	5	0
Cupressaceae	0	5	1	3	3	0
Others	3	4	1	4	4	0
Average content of methanol extractives (%)	2.89	4.10	5.43	7.22	2.31	1.52

* Examined at 1% level of significance by *t*-distribution.

A positive effect was significant in *Abies sachalinensis*, *Pinus densiflora* and *Pinus thunbergii* in both cases of *C. globosum* and *C. versicolor*. In *P. densiflora*, a positive effect was observed again, when tested with other wood-decaying fungi as shown below:

Fungus	Loss of weight (%)	
	Non-treated	Methanol-treated
Coniophora puteana	3.02	26.33
Serpula lacrymans	9.81	40.55
Ganoderma lucidum	2.62	8.06
Lenzites betulina	2.82	17.67

As is evident from Table 10, frequencies of positive effect were equal in both cases of *C. globosum* and *C. versicolor*. However, positive effect common to both fungi occurred in above-described 3 species only. In the case of *C. globosum*, a positive effect was found mainly in the species of Pinaceae (10 species among the 13), and was not found in those of Taxodiaceae and Cupressaceae. In the case of *C. versicolor*, a positive effect was found scattering in several families. Different effects of extraction in a species were found in a few species (*Pinus nigra, Sciadopitys verticillata* and *Thuja standishii*). Different sensitivities to wood extractives for the two test fungi were found in tropical woods, too.

Average weight loss values of non-treated and treated blocks were 1.43 and 1.87 (%) in the case of *C. globosum*, and 1.84 and 10.45 (%) in *C. versicolor*, respectively. This suggests that when decayed by *C. versicolor* methanol extraction was more significant for species in class A than decayed by *C. globosum*. Average content of methanol extractives in the species in class A was higher than that of other classes in the case of *C. versicolor*. Such a trend was verified statistically. Furthermore, average content of methanol extractives in the species which showed a significant positive effect was higher than others. This was also confirmed statistically. Consequently, in the case of softwoods as well as tropical hardwoods, it may be concluded that extractive-rich species are more resistant against *C. versicolor* and that the greater part of such species become less resistant after methanol extraction. However, such tendency did not hold exactly for *C. globosum* in both cases of hardwoods and softwoods. From the results obtained here, it can be concluded that the role of wood extractives is generally insignificant for softwoods which show the higher resistance against soft rot fungi.

4. Effects of pre-treatments of wood on the wood-decaying capacity of *Chaetomium globosum*

Very low ability of *C. globosum* to degrade softwoods was demonstrated with

various wood species. Thus, *C. globosum*, as well as white rot fungi, has been characterized by the preference for hardwoods. However, higher resistance of woods against white rot fungi was often depressed significantly by removal of extractives, whereas that against *C. globosum* was not so much. This has been shown more obviously in softwoods than in hardwoods.

A higher xylanase activity than the mannanase activity in culture filtrates from *C. globosum*[48], rapid growth and consumption of carbon source in xylose- and xylan media inoculated with this fungus[44] may partly explain the greater susceptibility of hardwoods to soft rot fungi. However, these evidences are still insufficient to enable us to establish a conclusive correlation between the hemicellulase activities of soft rot fungi and the hemicellulose compositions of the prefered wood, since enzyme activities for glucuronoxylan, the main hemicellulose of hardwoods, and glucomannan, the main hemicellulose of softwoods, and also amount of growth and consumption rate for xylose medium and mannose medium, were not so different. In this chapter, the effects of some biological and chemical treatments of softwoods on the wood-decaying capacity of *C. globosum* are described. For the purpose of further comparison, data are included on a hardwood *Fagus crenata* and on white rot- and brown rot fungi.

4.1 Biological treatment

The main components of woody cell walls are degraded by various groups of organism to different extents. However, the intimate association of the lignin with the polysaccharides apparently makes wood resistant to decomposition by both enzymes and whole organisms that can decompose the isolated wood polysaccharides[41]. In the white rot, polysaccharides and lignin are attacked more or less simultaneously; in brown rot and soft rot, on the other hand, the polysaccharides are principally utilized. Although lignin can be degraded by certain microorganisms, it can not be regarded as a favorable or a good energy-yielding substrate[73]. The ability of white rot fungi to decompose lignin might therefore be regarded as effective means to gain access to the polysaccharides in lignified cell wall[74,75]. Brown rot fungi, however, are able to utilize the polysaccharides in lignified cell walls without causing any significant degradation of the lignin. Although a soft rot fungus, *C. globosum*, has been shown to attack the polysaccharides similarly in beech wood[7,29,40], untreated softwoods are virtually unattacked by any of soft rot fungi. BAILEY et al.[76] proposed the existence of "pre-cellulolytic system" in organisms capable of utilizing the cellulose in wood. This term was used by them simply to describe all the steps that are necessary for the initiation of cellulose degradation. In this section, the effect of pre-exposure to certain wood-decaying fungi on the susceptibility of softwoods to *C. globosum* is investigated. Wood blocks, 2.0 (t) $\times$ 2.0 (r) $\times$ 0.5 (l) (cm),

Table 11. Fungal species used in the pre-exposure treatment of wood.

Name	Type	Strain*
Coniophora puteana KARST.	brown rot	IFO 6275
Serpula lacrymans S. F. GRAY	brown rot	IFO 8697
Tyromyces palustris MURR.	brown rot	FES 0507
Coriolus versicolor QUÉL.	white rot	FES 1030
Ganoderma lucidum KARST.	white rot	M-1
Lenzites betulina FR.	white rot	FES L5b
Cryptoderma pini IMAZEKI	white rot	FES F15c
Pycnoporus coccineus BOND. et SING.	white rot	FES Ps1h
Schizophyllum commune FR.	white rot	FES Sch2a
Trichoderma viride PERS. ex FR.	mould	IFO 4847

* IFO: Institute for Fermentation, Osaka, Japan.
FES: Government Forest Experiment Station, Tokyo, Japan.
M-1: Morimoto Yokin-en, Kyoto, Japan.

were prepared from the heartwoods of two softwoods (*Pinus densiflora* SIEB. et ZUCC. and *Cryptomeria japonica* D. DON) and one hardwood (*Fagus crenata* BLUME). Blocks were extracted with ethanol-benzene (1 : 1) for 8 hours and soaked in warm water (50°C) for 4 hours before pre-exposure to fungal attack. Fungal species used for the pre-exposure treatment are listed in Table 11. The composition of the nutrient solution for the treatment with 9 Basidiomycotina fungi is the same as that for the decay test using *C. versicolor* described in Chapter 3. The solution for the treatment with *Trichoderma viride* is the same as that for the test using *C. globosum*. Wood blocks for the pre-exposure to *T. viride* were soaked in sterilized distilled water after the sterilization with propylene oxide. The temperature was maintained at 20°C for *S. lacrymans* and at 28°C for other fungi throughout three different incubation periods of 2-, 4- and 8 weeks. Three blocks each in two bottles were used in each fungal- and wood species or each incubation period. The treated blocks were sterilized by fumigation with propylene oxide, cleaned of mycelium, soaked in running water for three days and then dried to constant weight in an oven at 105°C. The weighed blocks were sterilized again by fumigation with prpylene oxide, and soaked in sterilized distilled water before exposure to *C. globosum*. The temperature was maintained at 28°C during the 8 week-incubation period. The decayed blocks were cleaned of mycelium and then dried to constant weight in an oven at 105°C. The percent of weight loss was calculated from initial and final weights. Table 12 shows the weight loss in wood blocks pre-exposured to several fungi. Among the three wood species, *C. japonica* was the most resistant against fungal attack. In cases of *S. commune* and *T. viride*, wood blocks were attacked only at a small extent. For facilitating the discussion on the relation between the weight losses by the pre-

Table 12. The loss of weight in wood pre-exposured to several fungi.

Fungus	Wood	Loss of weight (%)		
		Incubation period (week)		
		2	4	8
Coniophora puteana	*Pinus densiflora*	8.51	21.48	26.33
	Cryptomeria japonica	1.75	1.54	4.63
	Fagus crenata	0.29	3.56	19.56
Serpula lacrymans	*Pinus densiflora*	4.22	16.39	40.55
	Cryptomeria japonica	1.86	10.89	27.54
	Fagus crenata	0.57	1.18	21.71
Tyromyces palustris	*Pinus densiflora*	4.96	6.35	9.03
	Cryptomeria japonica	5.60	7.26	11.87
	Fagus crenata	0.56	2.07	3.28
Coriolus versicolor	*Pinus densiflora*	0.71	3.34	13.78
	Cryptomeria japonica	1.11	4.11	12.87
	Fagus crenata	6.48	17.51	45.52
Ganoderma lucidum	*Pinus densiflora*	0.66	1.07	8.06
	Cryptomeria japonica	2.40	2.68	2.59
	Fagus crenata	0.86	7.09	33.85
Lenzites betulina	*Pinus densiflora*	3.06	10.59	17.67
	Cryptomeria japonica	1.69	6.97	14.07
	Fagus crenata	7.08	21.73	44.57
Cryptoderma pini	*Pinus densiflora*	2.01	1.96	1.83
	Cryptomeria japonica	0.38	1.32	1.18
	Fagus crenata	1.03	2.54	4.40
Pycnoporus coccineus	*Pinus densiflora*	2.08	2.63	4.92
	Cryptomeria japonica	0.76	1.53	1.86
	Fagus crenata	5.22	19.50	41.04
Schizophyllum commune	*Pinus densiflora*	2.18	2.42	2.25
	Cryptomeria japonica	1.90	1.79	1.60
	Fagus crenata	0.67	0.73	1.54
Trichoderma viride	*Pinus densiflora*	1.45	1.95	3.18
	Cryptomeria japonica	2.20	2.49	2.57
	Fagus crenata	0.88	1.32	1.54

exposure treatment and those by direct exposure to *C. globosum*, the wood blocks exposed to *C. globosum* were rated into four groups according to weight losses caused by the pre-exposure. The effect of pre-exposure was examined by comparing the average value of weight loss caused by *C. globosum* in each group with that in wood

blocks directly exposed to this fungus. Such an effect was examined by *t*-distribution at 5, 2 and 1% level of significance. Table 13 shows the results with the effect of pre-exposure treatment on the wood-attacking capacity of *C. globosum*. A positive (negative) effect means the case in which weight loss in treated blocks was larger (smaller) than that in untreated blocks. Unexpectedly, a positive effect was found significant only in a few combinations of fungal and wood species. Although the occurrence of positive effect was oftener in the case of *P. densiflora* than were in those of the other wood species, the extent of acceleration of attack was not still so large even in the case. However, it is clear that so far as *P. densiflora* is concerned this wood became apparently less resistant after pre-exposure to certain white rot fungi, especially to *Ganoderma lucidum*.

Table 13. The effect of pre-exposure to several fungi on the wood-attacking capacity of *Chaetomium globosum*.

Wood / Fungus	*Pinus densiflora*			*Cryptomeria japonica*			*Fagus crenata*		
	A	B	C	A	B	C	A	B	C
Coniophora puteana	6.90	3.72	++	0.30	2.83		0.46	37.03	
	14.90	3.01		1.80	3.07	++	4.86	45.08	+
	24.24	2.45	−	3.56	1.88		17.48	32.74	
	32.98	2.31		5.49	2.24		23.75	30.47	
Serpula lacrymans	4.22	3.15		1.86	1.09	−−−	0.57	44.08	
	16.90	2.98		7.87	1.99		1.18	57.30	+++
	35.75	3.85	+++	15.14	2.23		14.34	39.05	
	50.16	2.52		31.80	1.84		25.39	39.05	
Tyromyces palustris	1.10	1.74	−−	2.62	1.49		0.43	45.42	+++
	5.09	1.70	−−−	6.19	1.50		1.34	40.63	
	7.55	2.38		9.36	1.85		2.68	39.10	
	9.03	3.02		12.56	1.71		7.05	37.51	
Coriolus versicolor	0.71	3.39		1.11	1.59	−	3.94	35.39	
	3.34	6.15	+++	4.11	2.51	+	11.83	36.43	
	12.88	2.71	+++	12.01	0.99	−−−	32.56	33.23	
	15.88	4.97		14.61	0.24	−−−	50.73	24.07	
Ganoderma lucidum	0.27	3.67	++	0.57	1.48	−	0.73	32.09	−
	1.68	3.62	+++	2.04	2.25		6.35	26.93	−−−
	4.36	4.10		2.88	1.70		23.49	36.43	
	12.05	7.46	+++	5.11	1.62		37.45	28.22	−−
Lenzites betulina	1.17	1.44	−−−	1.86	2.04		7.08	28.00	−−
	6.42	3.85		6.81	1.81		20.15	33.38	
	17.09	5.73	+++	10.52	2.12		38.92	20.92	−−−
	27.52	5.22		16.73	2.23		51.25	24.75	−−−

Table 13. The effect of pre-exposure to several fungi on the wood-attacking capacity of *Chaetomium globosum* (Continued).

Wood / Fungus	*Pinus densiflora*			*Cryptomeria japonica*			*Fagus crenata*		
	A	B	C	A	B	C	A	B	C
Cryptoderma pini	0.73	1.60	− − −	0.22	1.45		0.90	46.18	+ +
	1.95	1.07	− − −	0.54	1.05	− −	2.23	36.63	
	2.46	1.86		1.13	1.37	− − −	3.39	34.17	
	5.74	3.75		1.54	0.56	− − −	5.84	24.56	− − −
Pycnoporus coccineus	1.93	1.88	− − −	0.64	2.01		5.22	26.13	−
	2.87	1.07	− − −	1.07	1.78		16.37	41.67	
	5.28	4.17		1.65	0.60	− − −	25.24	31.50	
	7.70	8.59	+ + +	2.05	0.18	− − −	43.55	36.12	
Shizophyllum commune	1.73	1.16	− − −	0.48	1.30		0.43	34.81	
	2.23	1.36	− − −	1.56	0.81	− − −	0.76	42.33	
	2.45	1.23	− − −	2.17	1.11	− −	1.24	38.70	
	2.61	0.92	− − −	2.75	1.49		1.69	45.31	+ + +
Trichoderma viride	1.39	1.22	− − −	2.07	1.01	−	0.84	42.84	
	1.92	0.92	− − −	2.27	1.60		1.18	46.36	
	3.00	0.95	− − −	2.55	1.16	− −	1.37	48.68	+ + +
	3.54	0.67	− − −	3.16	0.95	− − −	1.76	45.06	
None	—	2.86	—	—	2.09	—	—	37.28	—

A: Percent of weight loss by pre-exposure treatment.
B: Percent of weight loss by exposure to *C. globosum*.
C: Effect of pre-exposure treatment.
+, + + and + + + mean positive effect significant at 5, 2 and 1% level, respectively.
−, − − and − − − mean negative effect significant at 5, 2 and 1% level, respectively.
All values are expressed on the basis of the weight of extractive-free wood before pre-exposure treatment.

White rot fungi derive nourishment from all the major constituents of the wood cell walls. However, they differ in relative rates to remove the major components; some, such as *Polyporus berkeleyi*, remove the lignin faster than either the cellulose or the hemicelluloses, especially in early stages of decay[77]. Others, such as *Coriolus versicolor*, remove the three major components approximately simultaneously[77]. Apparently only a few fungi remove the carbohydrates somewhat more rapidly than the lignin[77]. On the other hand, brown rot fungi mainly decompose the polysaccharides in wood and usually cause only a small loss in the lignin. The cellulose and hemicelluloses are removed at about the same relative rates by various species of brown rot fungi[30,78].

A positive effect may be due to; (1) removal of some hindrances which are present in unknown associations of the three major components to enzyme activity

of *C. globosum*, (2) depolymerization and structural modification of these components, (3) supplement of nitrogen source derived from dead fungal cells, and (4) facilitation of penetration of *C. globosum* into cell lumen via bore hole previously produced by a test fungus. A negative effect may be attributed to; (1) insufficient removal of toxic materials produced by a test fungus, (2) exhaustion of food reserves in parenchyma cells, and (3) depletion of polysaccharides available to *C. globosum*. It can be seen from these results that a lower attacking capacity of *C. globosum* to softwoods is not accelerated so much by biological treatment, even by the action of white rot fungi which cause to degrade and modify the lignin and its association with polysaccharides.

4.2 Chemical treatment

Alkali- and acid treatments

Cold soda treatments were found to give little effect on the lignin and pentosan content of pulps[79]. BLAND and WATSON[80] demonstrated that most of the lignin was retained in cold soda semichemical pulps and that its properties showed no difference from those of original lignin. Cold soda treatments therefore would be expected to cause some effect on the lignin with little delignification. BLAND and MENSHUN[81] investigated the effect of alkali pre-treatment of the wood on the yield and carbohydrate retention of the lignin by extraction with acetone of the treated milled wood. They found that a mild alkali pre-treatment resulted in a large yield of lignin and suggested that this pre-treatment appeared to hydrolyse the bonds between lignin and carbohydrates. They later concluded[82] that a large part of the lignin was bound to polysaccharides by alkali resistant bonds and these could be ether bonds as deduced by BROWNELL[83]. On the other hand, from the results with treatments of lignin-carbohydrate complexes with alkali and acid, BOLKER and WANG[84] concluded that mild treatment with acid was effective in separating the lignin from the carbohydrate while alkali treatment was not. They considered that these results support previous proposals[85~89] that lignin and carbohydrate in plants may be joined by acid-labile bonds, or that the breaking of such bonds is at least necessary before effective separation of the two materials. In the first part of this section, the effect of mild alkali- and acid treatments on the susceptibility of softwoods to *C. globosum* is investigated.

Size of wood blocks and wood species used in the experiment are the same as described in 4.1. Blocks were extracted with ethanol-benzene (1 : 1) for 8 hours and soaked in warm water (50°C) for 4 hours before alkali- and acid treatments. Wood blocks were soaked in various concentrations of sodium hydroxide (30 ml/g wood) or hydrochloric acid (30 ml/g wood) for 20 hours at 20°C, and subsequently well washed with water, air dried and then dried again in an oven at 105°C. Con-

centrations of sodium hydroxide are as follows:

for the treatment of *P. densiflora* and *F. crenata*

0.05, 0.1, 0.2, 0.5, 1.0 and 2.0 *M*,

for the treatment of *C. japonica*

0.1, 0.2, 0.4, 1.0, 2.0 and 4.0 *M*.

Concentrations of hydrochloric acid are: 0.1, 0.2, 0.4, 1.0, 2.0 and 4.0 *M* for all wood species.

To define the relation between the weight losses and the decrease in lignin amounts by treatments, wood shavings (0.3 mm thick in longitudinal direction, cut to about 4 mm in the fibre direction) were prepared from the same wood specimens and treated with alkali or acid under the same condition as mentioned above. Concentrations of sodium hydroxide are: 0.1, 0.5 and 2.0 *M*, and those of hydrochloric acid are: 0.2, 1.0 and 4.0 *M*. The Klason lignin content in treated wood shavings was determined by the JIS P 8008-1961.

Table 14. The loss of weight by alkali- and acid treatments and the Klason lignin content in treated wood shavings.

Wood / Treatment	M	*Pinus densiflora*				*Cryptomeria japonica*				*Fagus crenata*			
		A	B	C	D	A	B	C	D	A	B	C	D
Sodium hydroxide	0.1	5.2	26.8	6.3	4.8	4.7	33.1	4.5	4.6	5.2	25.1	6.0	4.9
	0.5	7.5	26.1	8.7	7.0	5.0	32.4	6.9	4.0	9.8	24.1	9.7	9.8
	2.0	8.9	23.7	17.1	5.6	6.0	31.4	9.8	4.0	12.1	23.9	10.5	12.7
Hydrochloric acid	0.2	2.4	27.4	4.2	1.7	2.7	34.0	2.3	2.9	1.2	26.2	1.9	1.0
	1.0	2.8	27.4	4.2	2.2	2.9	33.8	2.9	2.9	1.6	26.2	1.9	1.5
	4.0	4.1	27.0	5.6	3.5	3.7	33.7	3.2	4.0	2.8	25.8	3.4	2.6
None	—	0	28.6	0	0	0	34.8	0	0	0	26.7	0	0

M: Concentration (*M*).
A : Percent of weight loss by treatment.
B : Klason lignin content.
C : Percent of loss of Klason lignin.
D : Percent of loss of materials other than Klason lignin.

Table 14 shows the loss of weight by alkali- and acid treatments and the Klason lignin content in treated wood shavings. Each value is an average of data from three separate samples. From the difference between the loss of weight and the decrease in the Klason lignin content during the treatment, the decrease in materials other than Klason lignin was roughly estimated as follows:

$$\text{Loss of materials other than Klason lignin in } \% = \frac{L-(K_0-K_1)}{100-K_0} \times 100$$

where L is the percent of weight loss in wood by treatment, K_0 is the percent of the Klason lignin content in untreated wood, and K_1 is the percent of the Klason lignin content in treated wood. As shown in Table 14, both treatments caused a certain extent of delignification but they are not selective in removing lignin only from all of the three wood species. *C. japonica* was more resistant against the alkali treatment than others. In the case of *F. crenata*, lignin and other substances were removed at about the same relative rates by the alkali treatment. In the cases of two softwoods, lignin was removed more rapidly than were other substances. It might be hemicelluloses that was removed together with the lignin by alkali treatment.

The wood blocks exposed to *C. globosum* were rated into four groups according to weight losses caused by alkali or acid treatment. The effect of treatment was examined by comparing the average value of weight loss caused by *C. globosum* in each group with that in untreated blocks, and the data was examined by *t*-distribution at 5, 2 and 1% level of significance. Table 15 shows the results with the effect of alkali-and acid treatments on the wood-attacking capacity of *C. globosum*. Positive- and negative effects were found significant only in a few cases. However, the occurrence of positive effect was oftener in the case of *P. densiflora*. This is similar to the case of the wood subjected to the pre-exposure treatment described in 4.1. As shown in Table 14, delignifying effect was larger in the alkali treatment than was in the acid treatment. However, in cases of the two softwoods, the extent of

Table 15. The effect of alkali- and acid treatments of wood on the wood-attacking capacity of *Chaetomium globosum*.

Wood / Treatment	*Pinus densiflora*			*Cryptomeria japonica*			*Fagus crenata*		
	A	B	C	A	B	C	A	B	C
Sodium hydroxide	2.94	1.51		2.76	0.42	−	2.14	44.34	
	5.70	3.49		3.21	1.08		4.27	56.52	+++
	8.47	4.78	+	3.52	1.72		6.89	44.21	
	10.67	3.17	+	3.80	1.89		8.09	38.76	
Hydrochloric acid	1.67	4.70	+++	1.32	1.06		1.01	26.24	−−−
	1.98	4.67	+++	1.57	1.03		1.33	30.96	
	2.58	4.12	+++	2.09	0.71		1.74	30.09	
	3.49	3.00		3.21	0.74		2.41	35.36	
None	0	1.82	—	0	1.06	—	0	37.28	—

A: Percent of weight loss by treatment.
B: Percent of weight loss by exposure to *C. globosum*.
C: Effect of treatment.
+ and +++ mean positive effect significant at 5 and 1% level, respectively.
− and −−− mean negative effect significant at 5 and 1% level, respectively.
All values are expressed on the basis of the weight of extractive-free wood before treatment.

acceleration of decay by *C. globosum* was not different between the two treatments. In the case of *P. densiflora*, small extent of the acceleration was found in both treatments, whereas it was not in the case of *C. japonica*. Both treatments apparently caused not only the delignification of wood but also some extent of modification of lignin itself or its association with the polysaccharides. Alhougth it is not clear at this stage which agent is main in the acceleration of decay, mild treatment with alkali or acid is not sufficient to make softwoods less resistant as well as beech wood.

Partial delignification with acidified sodium chlorite[90]

As mentioned above, all of the four treatments—removal of extractives, pre-exposure to fungal attack, alkali treatment and acid treatment—were not effective substantially for the acceleration of attack on softwoods by *C. globosum*. The greater resistance of softwoods to soft rot attack has often been attributed to a physical blocking of the enzymes by the higher lignin content[4,7]. Brown rot fungi, however, are able to utilize the carbohydrates of softwood cell walls without causing any significant depletion of the lignin. Significance of the higher lignin content therefore seems to vary with the type of decay. Effect of delignification should be investigated on the susceptibility of softwoods to wood-decaying fungi. BAILEY et al.[76] reported that chlorite treatment of *Picea abies* bringing about 2% of weight loss made this wood nearly as susceptible as beech to three species of soft rot fungi, but that such a treatment did not influence on the susceptibility of this wood to brown rot fungi.

Many physicochemical studies have been made on the important commercial pulping processes. AHLGREN and GORING[91] found that the chlorite process was selective in removing lignin from spruce wood (*Picea mariana*) during the first 60% of delignification, and concluded, by comparing that with earlier data[92~95], that the selectivity of the chlorite procedure was much greater than conventional pulping processes.

In the second part of this section, the effect of the chlorite delignification of softwoods on the wood-decaying capacity of *C. globosum* is studied at various stages of delignification. Forty-four softwood- and 1 hardwood species were employed in the experiment. Wood specimens were used in two forms; (1) 2.0 (t) $\times$ 2.0 (r) $\times$ 0.5 (l) (cm) blocks, and (2) 0.3 mm thick longitudinal shavings cut to about 4 mm in the fibre direction. Wood blocks were prepared from all of the species and subjected to the decay test. Shavings were used for the lignin determination and taken from the three species only (*Pinus densiflora*, *Cryptomeria japonica* and *Fagus crenata*). *C. globosum* was mostly used as a test fungus. For comparison, two species of white rot fungi, *Coriolus versicolor* (FES 1030) and *Pycnoporus coccineus* (FES Pslh), and two species of brown rot fungi, *Coniophora puteana* (IFO 6275) and *Serpula lacrymans* (IFO

8697), were occasionally used.

The weight losses after the chlorite treatment for 6 hours considerably varied with species. The lowest weight loss was 1.58% for *Chamaecyparis obtusa* and the highest was 21.92% for *Larix gmelini*. The average value of 44 softwoods was about 8%. The resistance of softwoods to acidified sodium chlorite was classified into four classes:

I ; weight loss was less than 4% for 4 species,
II ; weight loss was 4~8% for 19 species
III; weight loss was 8~12% for 16 species,
IV; weight loss was over 12% for 5 species.

Fig. 15 shows the acceleration of decay which is given by the difference in weight losses between treated and untreated samples. Close relation is demonstrated between the weight losses by the treatment and those by decay. The highest acceleration was 54.06% for *Picea glehnii* and the lowest was 5.93% for *C. obtusa*. *L. gmelini*

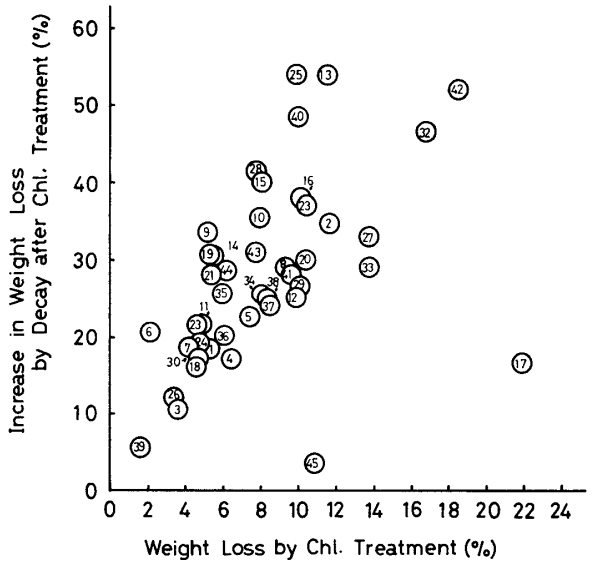

Fig. 15. Acceleration of wood-attacking capacity of *Chaetomium globosum* on 44 softwood timber species aftetpartial chlorite delignification for 6 hours at 40°C. 1; *Ginkgo biloba*, 2; *Taxus cuspidata*, 3; *Torreya nucifera*, 4; *Podocarpus macrophylla*, 5; *Podocarpus nagi*, 6; *Cephalotaxus harringtonia*, 7; *Abies firma*, 8; *Abies mariesii*, 9; *Abies sachalinensis*, 10; *Abies sachalinensis* var. *mayriana*, 11; *Pseudotsuga japonica*, 12; *Tsuga sieboldii*, 13; *Picea glehnii*, 14; *Picea jezoensis*, 15; *Picea abies*, 16; *Larix leptolepsis*, 17; *Larix gmelini*, 18; *Keteleeria davidiana*, 19; *Pinus densiflora*, 20; *Pinus pentaphylla*, 21; *Pinus thunbergii*, 22; *Pinus tabulaeformis*, 23; *Pinus radiata*, 24; *Pinus rigida*, 25; *Pinus taeda*, 26; *Pinus sylvestris*, 27; *Pinus nigra*, 28; *Pinus strobus* 29; *Pinus virginiana*, 30; *Pinus elliottii*, 31; *Sciadopitys verticillata*, 32; *Sequoia sempervirens*, 33; *Metasequoia glyptostroboides*, 34; *Glyptostrobus pensilis*, 35; *Taxodium distichum*, 36; *Cryptomeria japonica*, 37; *Cunninghamia konisii*, 38; *Taiwania cryptomerioides*, 39; *Chamaecyparis obtusa*, 40; *Chamaecyparis pisifera*, 41; *Chamaecyparis formosensis*, 42; *Thuja standishii*, 43; *Thujopsis dolabrata*, 44; *Juniperus viginiana*, 45; *Fagus crenata*).

exceptionally showed lower acceleration in spite of the lowest resistance to chemical attack. *F. crenata* was almost the same as *L. gmelini* in respect of its lower acceleration.

There are several indications that the amount of acid-soluble lignin from untreated softwoods is very small. In fact, the acid-soluble lignin was only 0.26% for *P. densiflora* and 0.37% for *C. japonica*. *F. crenata* showed relatively higher content (2.14%). This value was lower than that of American beech (*Fagus grandiflora*) obtained by MUSHA and GORING[96] but was within the range from 1 to 4%, as reported by SCHÖNING and JOHANSSON[96] for hardwoods and straws. A high level of the soluble lignin during the chlorite process agreed well with some previous reports[98~100]. The maximum was reached at about 9% of Klason lignin content for *P. densiflora*, at about 14% for *C. japonica*, and at about 10% for *F. crenata*. BROWNING[101] pointed out that a considerable portion of the total lignin is soluble after the acid treatment and the amount of insoluble residue is not a realistic measure of lignin content. According to him, the total lignin content was determined as insoluble Klason lignin plus the ultraviolet-estimated acid-soluble lignin.

The patterns of lignin removal from the three species of wood during the chlorite treatment are shown in Figs. 16~18. Difference between the losses in Klason and total lignins is evident for each species. For facilitating the discussion, smoothed values from the curves in Figs. 16~18 are compiled in Table 16. In addition, the estimated lignin losses which were calculated on the assumption that the chlorite process is selective in removing lignin only are shown in this table. In the case of *C. japonica*, the smoothed values nearly agreed with the estimated values throughout the process. On the other hand, in the cases of *P. densiflora* and *F. crenata*, the two values did not agree during the whole stage. Such disagreement was more conspicuous for *F. crenata*. In both woods, considering from the material balances,

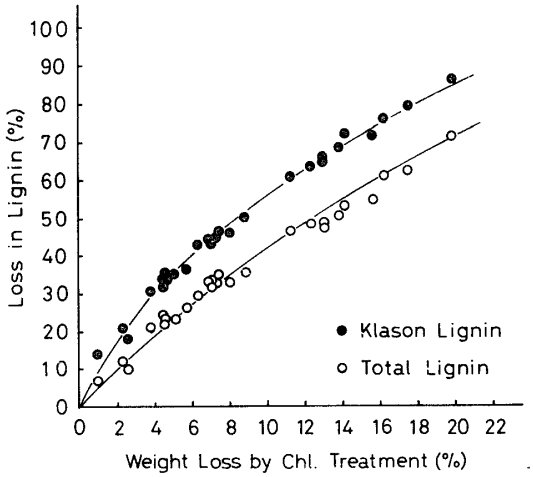

Fig. 16. Decrease of lignin in *Pinus densiflora* during chlorite treatment.

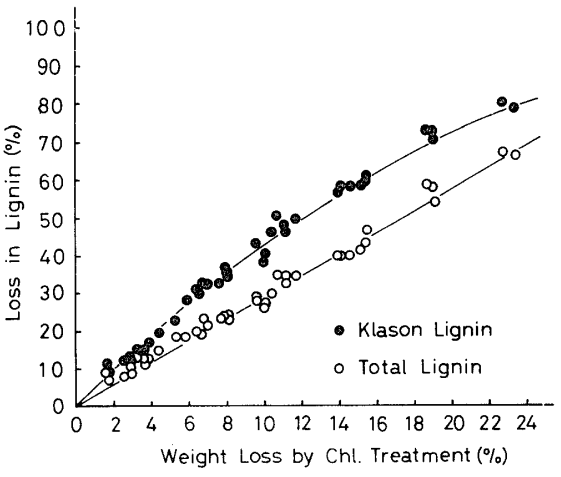

Fig. 17. Decrease of lign in *Cryptomeria japonica* during chlorite treatment.

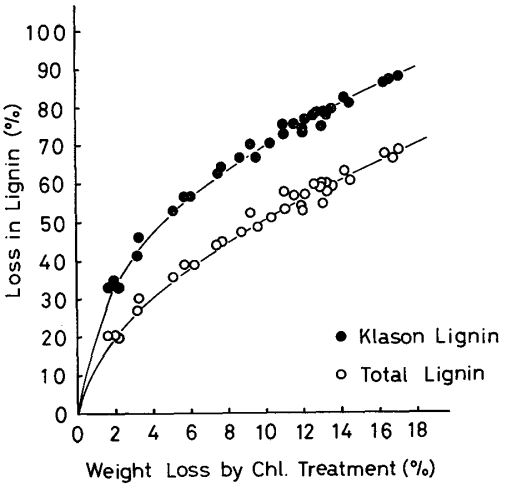

Fig. 18. Decrease of lignin in *Fagus crenata* during chlorite treatment.

Table 16. The losses in total lignin content at different stages of chlorite treatment.

Weight loss in wood (%)*	Loss in total lignin (%)					
	Smmothed			Estimated		
	PD	CJ	FC	PD	CJ	FC
0	0	0	0	0	0	0
2	11	7	20	7	6	7
4	20	12	31	14	12	14
6	29	18	39	21	18	21
8	36	24	46	28	23	28
10	43	30	53	35	29	35
12	49	35	57	42	35	42
14	55	41	62	49	41	49
16	60	47	66	56	47	56
18	67	53	70	63	53	63
20	72	59	—	70	59	70
22	—	65	—	77	65	77

* $\frac{\text{Weight of wood extracted with ethanol-benzene}}{\text{Weight of chlorite treated wood}} \times 100$

PD: *Pinus densiflora*, CJ: *Cryptomeria japonica*, FC: *Fagus crenata*.

some substances other than lignin must be removed during the process. Therefore, the chlorite process proved to be selective in removing lignin only from *C. japonica* as was from *Picea mariana*. However, it can be considered that the process was less selective in the cases of *P. densiflora* and *F. crenata*.

The decayed blocks were rated into eight groups according to weight losses caused by chlorite treatment before decay. Average values of weight losses after treatment and exposure to fungal attack were calculated for each group. Figs. 19~23

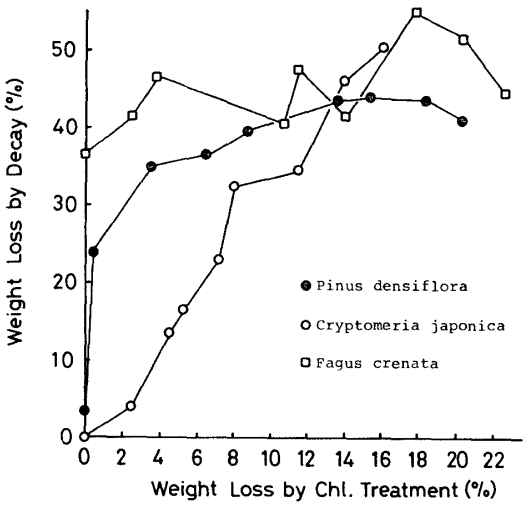

Fig. 19. Weight loss in wood exposed to *Chaetomium globosum* after different degrees of delignification.

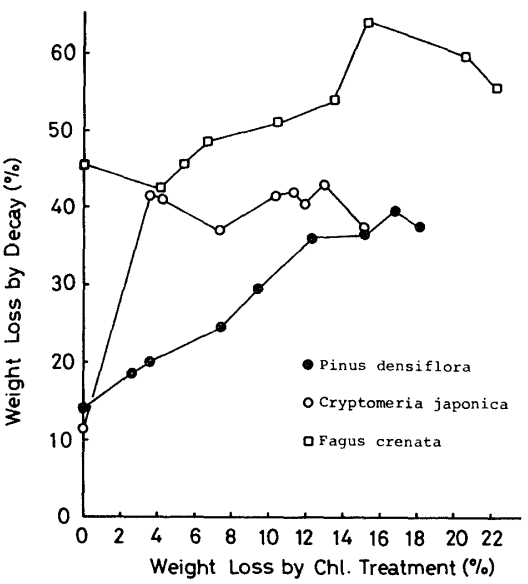

Fig. 20. Weight loss in wood exposed to *Coriolus versicolor* after different degrees of delignification.

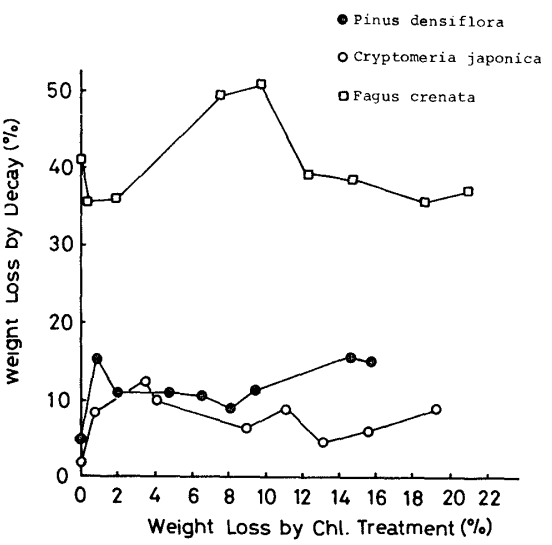

Fig. 21. Weight loss in wood exposed to *Pycnoporus coccineus* after different degrees of delignification.

show the results with the average weight losses in chlorite treated wood for each group after exposure to fungal attack. In the case of *P. densiflora* attacked by *C. globosum*, the acceleration curve was steep during the first 4% of weight loss by chlorite treatment, which was equivalent to 20% of delignification, and reached the maximum during 10 to 14% of weight loss (43 to 55% of delignification). However, in the case of *P. densiflora* attacked by *C. versicolor*, the curve was gentle but reached the maximum at greater stage of delignification. In the case of *C. japonica*, the acceleration of decay was nearly reverse for the two fungi. At the first

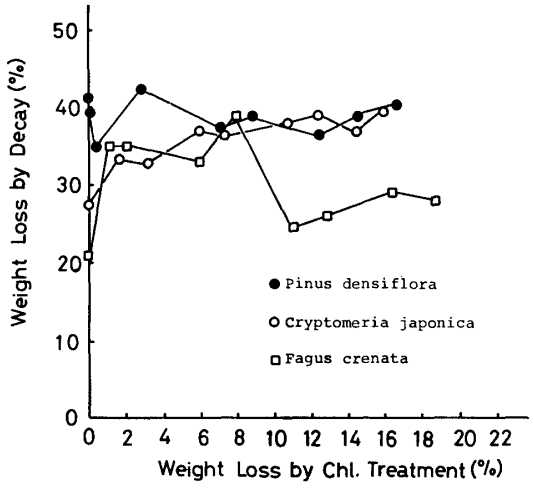

Fig. 22. Weight loss in wood exposed to *Serpula lacrymans* after different degrees of delignification.

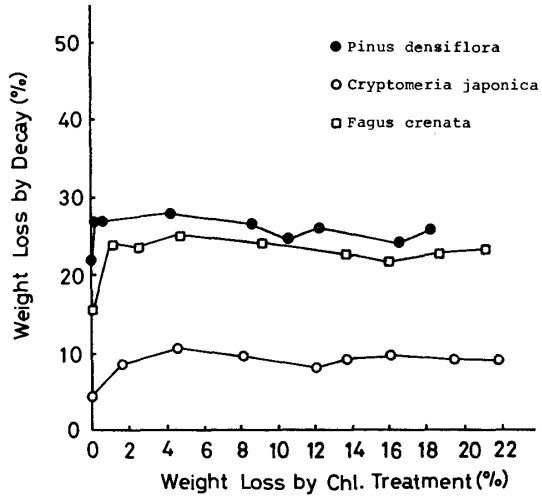

Fig. 23. Weight loss in wood exposed to *Coniophora puteana* after different degrees of delignification.

4% of weight loss, equivalent to 12% of delignification, the acceleration of decay was about 30% for *C. versicolor* but only about 10% for *C. globosum*. However, the curve for *C. versicolor* reached the maximum level during the middle stage of delignification, whereas the curve for *C. globosum* still considerably steep at over 40% of delignification. In cases of the two softwoods exposed to *P. coccineus*, the acceleration of decay was also found significantly at the first stage of delignification, although maximum levels in both woods for this fungus were lower than those for *C. globosum* and *C. versicolor*.

In cases of both woods exposed to the two brown rot species, *S. lacrymans* and *C. puteana*, the acceleration was undetected (*P. densiflora*) or only slight (*C. japonica*). The differences of weight losses between untreated and treated woods of *C. japonica* was much less for the two brown rot species.

In all cases of *F. crenata*, the acceleration was significant at some stage of delignification. However, the extent of acceleration for this wood was generally smaller than that for the two softwoods. This suggests that the delignification treatment is less effective for the fungal attack of the originally susceptible wood such as *F. crenata*.

DUNCAN[9] found that leaching of redwood (*Sequoia sempervirens*) with sodium hypochlorite solution (containing 2 ppm of available chlorine) facilitated attack by many isolates of soft rot fungi including *Chaetomium funicolum* and by a white rot fungus, *Poria nigrescens*, but it had no apparent effect on the wood-attacking ability of a brown rot fungus, *Poria oleraceae*. BAILEY et al.[76] reported that partial delignification of softwoods (*Picea abies* and *Pinus sylvestris*) with acidified sodium chlorite accelerated attack by soft rot fungi, *C. globosum*, *Ceratocystis* sp. and *Paecilomyces* sp.,

but it was not effective for attack by brown rot fungi, *Coniophora cerebella*, *Poria vaporaria* and *Polyporus scweinizii*. NOUVERTNÉ[102] obtained similar results on the extent of degradation of delignified softwoods by three species of soft rot fungi. In their investigations, loss of lignin by the delignification treatment was not determined experimentally and the observations were done only at one or few stages of delignification. In this experiment, effect of the chlorite delignification was examined at various stages of delignification. Similar results were obtained here and then it can be concluded that the delignification treatment of softwoods is effective for acceleration of wood-attacking capacity of soft rot- and white rot fungi but not for that of brown rot fungi.

Although white rot fungi are regarded as lignin-degrading fungi, they have a preference for hardwoods containing smaller amount of lignin than softwoods. It can be assumed that white rot fungi have two types of enzyme system. The first is involved in breakdown of polysaccharides and the second is in breakdown of lignin. Brown rot- and soft rot fungi have certainly the first system only, or have the first and the incomplete system of the second. White rot fungi are regarded as an advanced group, evolved out of a primitive group which lacks extracellular oxidase and prefers softwoods to hardwoods[103]. If the second system which is associated with lignin degradation is developed gradually in the course of evolution, this system may be still minor for the survival of white rot fungi in the natural habitat compared with the first system. FUKUDA and HARAGUCHI[74] and KIRK et al.[75] presented that the lignin-degrading ability of white rot fungi is an effective means to gain access to the cellulose in lignified cell wall but does not serve effectively to metabolize lignin itself as energy-and nutrient sources. According to this theory, removal of lignin from cell wall may facilitate the action of cellulolytic enzyme system in the attack of softwoods by white rot fungi. Such an accelerative effect was found here for the two softwoods exposed to the white rot fungi used.

Brown rot fungi have a limited ability to degrade lignin, although they are associated most frequently with the decay of softwoods containing higher amount of lignin than hardwoods. However, delignification treatment of softwoods had no effect on the wood-attacking ability of the brown rot fungi used. KOENIGS[104] found that low concentrations of H_2O_2 and Fe^{++} caused rapid weight loss of sweetgum (*Liquidamber styraciflua*) and loblolly pine (*Pinus taeda*) and that the degree of polymerization of cellulose in treated woods decreased rapidly at low weight loss and then diminished gradually. This effect was similar to that in wood created by brown rot fungi[30]. Out of this fact and the evidence with production of H_2O_2 from native substrates in wood by brown rot fungi[105], he proposed that these fungi might attack cellulose and partly decay wood via an H_2O_2-Fe^{++} system. This suggests

that brown rot is differentiated from white rot and soft rot by the rather oxidative system for the decomposition of cellulose in wood.

Acceleration of attacking capacity on softwoods by delignification was evidenced in both soft rot- and white rot fungi, but acceleration pattern varied apparently with wood and fungal species. It can be considered, therefore, that in the rapid and shorter acceleration (*P. densiflora* vs. *C. globosum* and *C. japonica* vs. *C. versicolor*) a modification of lignin may play an important role, and that a removal of lignin may act as a main agent in the slow and longer acceleration (*P. densiflora* vs. *C. versicolor* and *C. japonica* vs. *C. globosum*).

5. Role of lignin in decay resistance of wood against *Chaetomium globosum*[106]

In this chapter, the pattern of lignin removal from partially delignified woods by a soft rot fungus, *Chaetomium globosum* (IAM 8059), and a white rot fungus, *Coriolus*

Table 17. The loss of weight in the samples for lignin analysis caused by chlorite treatment and exposure to fungal attack.

Fungus \ Wood	*Pinus densiflora*		*Cryptomeria japonica*		*Fagus crenata*	
	Weight loss by chlorite treatment (%)	Weight loss by decay (%)	Weight loss by chlorite treatment (%)	Weight loss by decay (%)	Weight loss by chlorite treatment (%)	Weight loss by decay (%)
Chaetomium globosum	0*	3.50	0*	0.00	0*	36.50
	0.46	24.11	2.53	3.83	2.52	40.45
	3.56	34.74	4.50	13.67	3.75	46.70
	6.48	36.35	5.28	16.31	10.64	40.62
	8.70	39.24	7.21	22.93	14.02	41.44
	13.61	43.66	7.96	32.21	17.81	54.81
	15.41	43.78	11.37	34.35		
	18.37	43.64	13.88	45.91		
	20.29	40.89	16.05	50.53		
Coriolus versicolor	0*	14.00	0*	11.50	0*	45.50
	2.59	18.61	3.48	41.67	4.17	42.70
	3.56	19.91	4.21	41.32	5.40	45.78
	7.58	24.68	7.35	37.20	6.56	48.71
	8.49	28.82	10.26	41.23	10.77	56.75
	9.45	29.65	10.79	39.67	13.48	52.58
	12.35	40.91	11.31	42.17	15.26	63.97
	15.09	36.39	11.85	40.37		
	17.00	39.35	12.92	42.92		
	18.06	32.28	15.06	37.45		

All values are expressed on the basis of the weight of extractive-free wood before chlorite treatment.
0*: Extracted with ethanol-benzene, not treated with sodium chlorite and acetic acid.

versicolor (FES 1030), has been studied on two softwoods, *Pinus densiflora* and *Cryptomeria*, and one hardwood, *Fagus crenata*, with reference to the different acceleration pattern of wood decay descibed in Chapter 4. On the basis of the results obtained, role of lignin in different decay resistance of wood against *C. globodum* has been discussed, comparing with that against *C. versicolor*.

Table 17 shows the loss of weight by chlorite treatment and fungal attack of each group for lignin analysis. Figs. 24~26 show the results with the loss of lignin from each group of the three wood species subjected to chlorite treatment and fungal

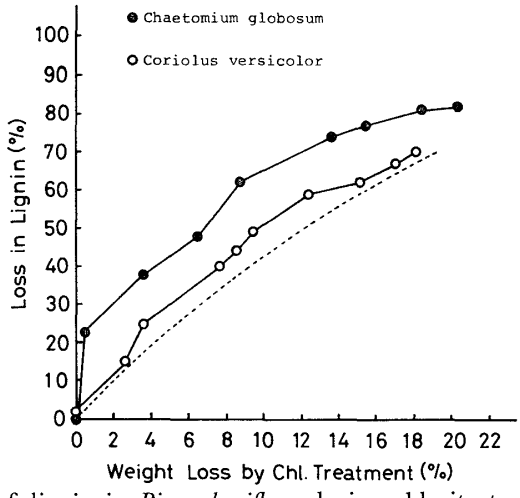

Fig. 24. Decrease of lignin in *Pinus densiflora* during chlorite treatment and exposure to fungal attack. Dotted line represents the pattern of lignin removal from wood shavings of *P. densiflora* during chlorite treatment.

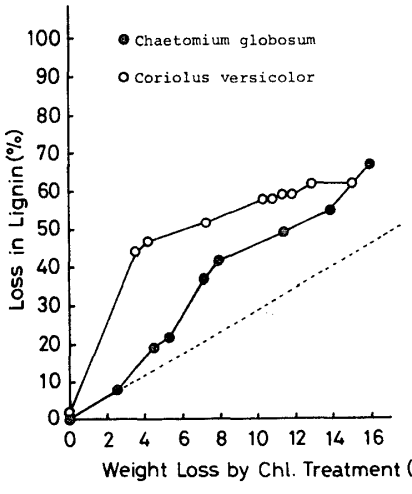

Fig. 25. Decrease of lignin in *Cryptomeria japonica* during chlorite treatment and exposure to fungal attack. Dotted line represents the pattern of lignin removal from wood shavings of *C. japonica* during chlorite treatment.

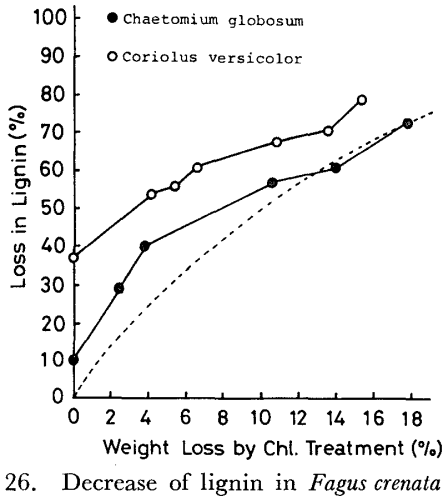

Fig. 26. Decrease of lignin in *Fagus crenata* during chlorite treatment and exposure to fungal attack. Dotted line represents the pattern of lignin removal from wood shavings of *F. crenata* during chlorite treatment.

Table 18. Klason and acid-soluble lignin contents of sound woods*.

Wood	Lignin content (%)		
	Klason	Acid-soluble	Total
Pinus densiflora	28.33	0.26	28.59
Cryptomeria japonica	33.67	0.37	34.04
Fagus crenata	26.47	2.14	28.61

* An average of the values from three separate Klason hydrolyses of 0.3 mm thick wood shavings.

attack. Percent of lignin loss is expressed on the basis of the original amount of lignin in sound wood shown in Table 18. Difference between solid and dotted lines at a certain point on the ordinate shows a rough estimate of the lignin loss caused fungal attack. Figs. 27~29 show the ratio of lignin loss to weight loss (LL: WL ratio) in each group of the three wood species exposed to fungal attack after chlorite treatment. The ratio was calculated by dividing difference between solid and dotted lines in Figs. 24~26 by corresponding percent of weight loss in Table 17. The

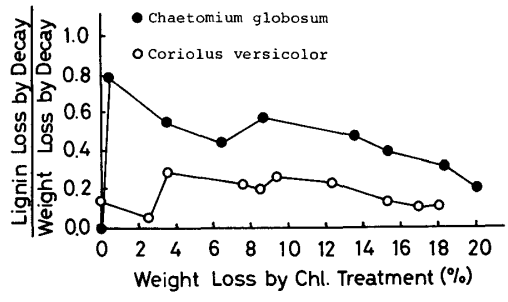

Fig. 27. The ratio of lignin loss to weight loss caused by fungal attack on chlorite treated wood of *Pinus densiflora*.

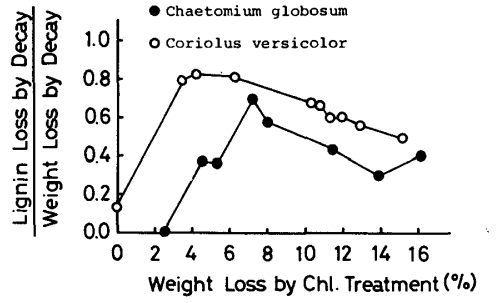

Fig. 28. The ratio of lignin loss to weight loss caused by fungal attack on chlorite treated wood of *Cryptomeria japonica*.

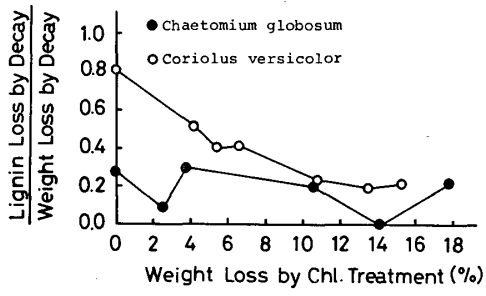

Fig. 29. The ratio of lignin loss to weight loss caused by fungal attack on chlorite treated wood of *Fagus crenata*.

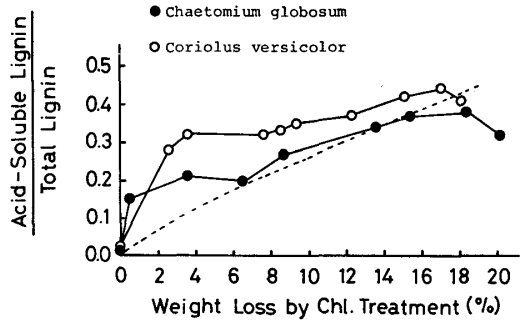

Fig. 30. The ratio of acid-soluble lignin to total lignin in *Pinus densiflora* after chlorite treatment and exposure to fungal attack. Dotted line represents the pattern of accumulation of acid-soluble lignin in wood of *P. densiflora* during chlorite treatment.

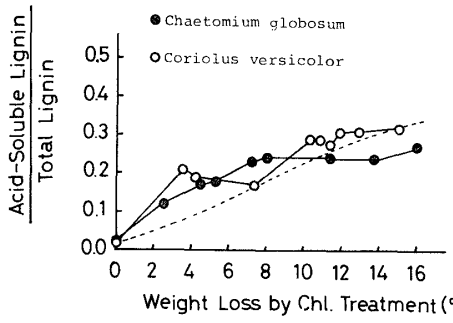

Fig. 31. The ratio of acid-soluble lignin to total lignin in *Cryptomeria japonica* after chlorite treatment and exposure to fungal attack. Dotted line represents the pattern of accumulation of acid-soluble lignin in wood shavings of *C. japonica* during chlorite treatment.

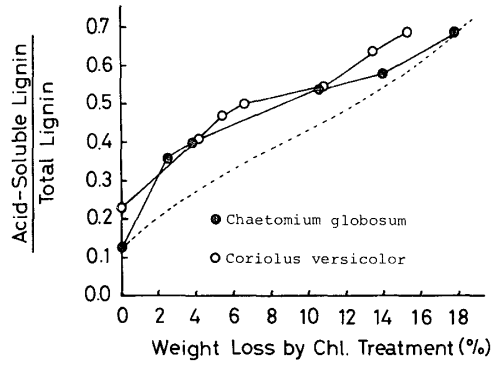

Fig. 32. The ratio of acid-soluble lignin to total lignin in *Fagus crenata* after chlorite treatment and exposure to fungal attack. Dotted line represents the pattern of accumulation of acid-soluble lignin in wood shavings of *F. crenata* during chlorite treatment.

ratio reaches 1.0 when the lignin and non-lignin components (carbohydrates) are removed at the same relative rates from wood by fungus. Figs. 30~32 show the data on the ratio of acid-soluble lignin to total lignin (SL: TL ratio) in each group of the three wood species. The ratio was calculated by dividing percent of acid-soluble lignin by percent of total lignin. Percent of the two kinds of lignin was based on the weight of wood after decay.

A considerable amount of lignin was removed from *P. densiflora* by *C. globosum* during the first 0.46% of weight loss by chlorite treatment and the LL: WL ratio was highest at this stage. Removal of lignin from *P. densiflora* by *C. versicolor* was slower than by *C. globosum* throughout all the stages. That partly reflected the lower acceleration of wood decay by the former. However, the LL:WL ratio for *C. versicolor* was also smaller than for *C. globosum* at every stage of delignification with an exception at 0% of weight loss (non-chlorite treatment). On the other hand, the SL:TL ratio for *C. versicolor* was always larger than for *C. globosum*, which suggests that solubilized lignin derived from insoluble Klason lignin was concentrated because of the lesser action of *C. versicolor* on this substance.

In the case of *C. japonica*, the pattern of lignin removal was nearly reverse for the two fungi. During the first 3.48% of weight loss by delignification, large amount of lignin was removed by *C. versicolor*. The slower rate of lignin removal by *C. globosum* was coincident with the slower acceleration of wood decay by the fungus. The LL:WL ratio for *C. versicolor* reached the maximum level at about 4% of weight loss by delignification. The ratio for *C. globosum* reached maximum level at about 7% of weight loss, and was always smaller than for *C. versicolor*. The SL: TL ratio for *C. globosum* was smaller than that for *C. versicolor* in a range of over 10% of weight

loss, and that for non-decayed wood shavings at greater extent of weight loss. This suggests that the acid-soluble lignin was rapidly depleted by *C. globosum* at these stages.

Although a considerable amount of substances was removed from non-delignified softwoods by *C. versicolor*, no removal of lignin was detected. The SL: TL ratio for *C. versicolor* was nearly equal to the sound wood. These results assume that the lignin remaining in non-delignified softwoods is mostly unaltered. This suggestion extends to the cases of the two non-delignified softwoods exposed to *C. globosum*. It is well known that white rot fungi metabolize all the major constituents of lignified cell walls. However, various species of white rot fungi differ in relative rates at which they remove the major components[107]. Sometimes a distinction is made between white rot fungi and simultaneous rot fungi. The former decompose wood successively, beginning with lignin and hemicelluloses and deteriorating the cellulose only at a later stage, while the latter decompose all substances of the lignified cell wall simultaneously[35]. Differences between the two types have been found by fluorescense microscopy[108] and chemical investigations of wall degradation[109~112]. However, it seems that these differences occasionally fluctuate with wood species[108] and that these types are not phylogenetically fixed characters. SEIFERT[78], in the analysis of chemical changes during decay processes, considered that white rot and simultaneous rot are synonymous. A white rot fungus, *C. versicolor*, used in this investigation, is often recognized as an example of a group which removes the three major components approximately simultaneously[30,113]. Loss of lignin and the LL: WL ratio in non-delignified wood of *F. crenata* attacked by *C. versicolor* apparently demonstrate that wood constituents are removed at approximately the same relative rates. It seems therefore that such a simultaneous removal of the major constituents occurs only in hardwoods but a preferential degradation of non-lignin components sometimes occurs in non-lignified or original softwoods.

The slower rate of lignin removal and the lower LL: WL ratio in non-delignified wood of *F. crenata* attacked by *C. globosum* agreed with the results obtained by SAVORY and PINION[7], and LEVI and PRESTON[29]. Soft rot fungi do not attack softwoods so rapidly or extensively as they do hardwoods. A large number of white rot fungi, including *C. versicolor* and *Pycnoporus coccineus*, prefer hardwoods to softwoods. Hence, it is possible to assume that the lignin in softwoods is more or less hindrance in both types of wood-decaying fungi. Of the two types of acceleration pattern of decay observed in the two softwoods, the rapid and shorter acceleration was accompanied with the rapid rate of lignin removal and the higher LL: WL ratio during first stage of delignification. In such a case, hindrance by lignin may be rather qualitative than quantitative, so that a certain modification of lignin which was

caused by the chlorite treatment for a short time seems to act as a trigger for succeeding degradation of the modified lignin. On the other hand, the slow and longer acceleration of decay was accompanied with slow or poor overall removal of lignin. In the case of *P. densiflora* attacked by *C. versicolor*, the lignin was removed at apparently slower rate than non-lignin components, and removal or modification of lignin acts largely for facilitating to gain access to the carbohydrates. In such a case, hindrance by lignin may act rather quantitatively. In the case of *C. japonica* attacked by *C. globosum*, however, hindrance by lignin may be rather qualitative at least during the first 7% of weight loss by delignification, since the LL: WL ratio increased in proportion to the extent of delignification and reached the maximum level at about 7% of weight loss. This suggests that the lignin is a greater qualitative hindrance in *C. globosum* for *C. japonica* than for *P. densiflora*.

Although *F. crenata* is highly susceptible to both fungi, the rate of lignin removal and the LL: WL ratio were always slower and smaller in *C. globosum* than in *C. versicolor*. If it is assumed that the lignin is also hindrance in both fungi even in this easily attackable hardwood, removal of lignin by chlorite treatment may help both fungi for reducing the hindrance. This hindrance-reducing system may be less operative as the amount of lignin decreases. This was confirmed in *C. versicolor* by the constant decrease in the LL:WL ratio but not in *C. globosum*. In *F. crenata* attacked by *C. globosum*, the poor removal of lignin and the lower LL:WL ratio were demonstrated throughout the stages. From the results, it can be considered that the lignin is not hindrance in *C. globosum* in *F. crenata* and removal of lignin by chlorite treatment is less helpful for attack on the wood by the fungus.

The greater resistance of softwoods to soft rot attack has been attributed to a physical blocking of the enzymes by the higher lignin content[4]. When the extent of acceleration of decay by *C. globosum* for 44 softwood species subjected to a six-hour delignification was compared, it was noticeably proportional to the extent of delignification. This seems to support the proposal of physical blocking action by lignin. However, the acceleration was often detected at very early stage of delignification. As has been mentioned, the pattern of acceleration varied with wood and fungal species. From these and the results with the pattern of lignin removal by soft rot- and white rot fungi, it can be concluded that the lignin in softwoods is more or less hindrance in both types of wood rot fungi. With respect to a soft rot fungus, *C. globosum*, hindrance by lignin may be rather qualitative than quantitative, because of the rapid increase of the LL: WL ratio in both softwoods. For a white rot fungus, *C. versicolor*, however, hindrance by lignin may act qualitatively on *C. japonica* but quantitatively on *P. densiflora*. Quantitative hindrance is considered simply in terms of a physical blocking of the cellulolytic enzymes by lignin. Qualitative hin-

drance seems to be related to the chemical and topochemical natures of lignin and/or the nature of the lignin-carbohydrate association.

Hardwood lignin contains both guaiacyl and syringyl groups but softwoods lignin contains quaiacyl group only[93]. MUSHA and GORING[114] demonstrated by ultraviolet microscopy that the walls of fibres and ray cells contain mostly syringyl group, and that the vessel walls and cell corner regions contain mostly guaiacyl group. Recently, KIRK et al.[115] reported that *C. versicolor* firstly degraded syringyl-rich lignin and then guaiacyl-rich lignin in the attack of birch wood (*Betula alleghaniensis*), through the progressive action of enzymes from the lumen surfaces toward the middle lamella. However, such successive degradation only reflects the distribution of lignin residues in birch wood, and does not show a greater susceptibility of the syringyl group and hardwoods to this fungus. Although softwood lignin contains guaiacyl group only, microscopic distribution of the lignin in wood tissue and the association with carbohydrates probably vary with species at some extent. The mechanism of lignin degradation has been known to be largely oxidative but it has not been completely understood[41,116]. Knowledge about the depletion of lignin by soft rot fungi is especially meager. To define more accurately the significance of lignin in different decay resistance of wood, a further knowledge of factors varying with wood and fungal species is needed: such as chemical and topochemical natures of lignin, the nature of the lignin-carbohydrate association, topochemical effect of delignification, and fungal enzyme systems involved in breakdown of wood components.

Conclusion

Characteristics of wood decay by a soft rot fungus, *Chaetomium globosum* KUNZE have been studied, with reference to the changes in physical- and chemical properties of wood, carbohydrate utilization by this fungus, decay resistance of various softwoods and hardwoods, and some factors concerning different decay resistance among these woods.

Prior to these studies, investigations were made on the effects of variations in amount of carbon- and nitrogen sources, kind of carbon sources and size of test blocks, for seeking the cultural conditions which allow the higher wood-decaying capacity of this fungus. Results obtained show that wood-decaying capacity of this fungus is not exhibited in a nutritional condition which is favorable to brown rot- and white rot fungi.

It can be seen from the infrared spectral analysis of decayed wood that *C. globosum* is more active against hardwood lignin than is a brown rot fungus used, although preferential depletion of carbohydrates with little lignin attack has been emphasized

as the similarity of soft rot to brown rot. Rapid decrease of the absorption at 1730 cm^{-1} in *Fagus crenata*, assigned to the C=O stretching vibration of carboxyl and acetyl groups in glucuronoxylan, and the rapid utilization of xylose and hardwood xylan suggest a possible relation to the greater susceptibility of hardwoods to soft rot fungi. The ability of *C. globosum* to reduce the strengths of wood was rated in the middle of brown rot and white rot. This seems to reflect these degradation patterns of wood constituents and the mode of attack on lignified cell wall which is generally characterized by cavity formation in secondary wall.

Decay resistance of various wood species (138 spp. of temperate hardwoods, 64 spp. of tropical hardwoods and 45 spp. of softwoods) against *C. globosum* was estimated with reference to methanol extractives in wood. With respect to temperate hardwoods, variances of decay resistance among species and families were not so much different from those for Basdiomycotina reported in the literature. In contrast with the case of temperate hardwoods, it was generally found that dense and/or extractive-rich species became more susceptible to decay after treatment with hot methanol. This was shown more notably in the case of white rot fungus, *Coriolus versicolor* QUÉL. Very low ability of *C. globosum* to degrade softwoods was evidenced for all wood species used. The greater part of species retained high resistance even after treatment with hot methanol. Also *C. versicolor* could not cause severe weight loss of softwoods. However, extractive-rich species were more resistant against this white rot fungus, and the greater part of these species became less resistant after extraction with hot methanol. It can be concluded from these results that the role of extractives in softwoods with higher resistance against soft rot fungi is generally insignificant.

Effects of some biological- and chemical treatments of softwoods on the wood-decaying capacity of *C. globosum* were investigated. Pre-exposure of softwoods to 9 Basidiomycotina and 1 Deuteromycotina did not facilitate attack by *C. globosum*. Attacking capacity of this fungus on softwoods was not enhanced by treatments with both mild alkali and acid. However, treatment of softwoods with acidified sodium chlorite was very effective for acceleration of attacking capacity of this fungus. Such acceleration was found even in the case of lignolytic white rot fungi but not in the case of brown rot fungi. From the patterns of decay acceleration and lignin removal by soft rot- and white rot fungi, it can be concluded that lignin in softwoods is more or less hindrance in both types of wood rot fungi. With respect to a soft rot fungus, *C. globosum*, the hindrance by lignin may be rather qualitative than quantitative. However, for a white rot fungus, *C. versicolor*, the hindrance may act either qualitatively or quantitatively varying with wood species exposed. Quantitative hindrance is considered simply in terms of a physical blocking of the cellulolytic

enzymes by lignin. Qualitative one seems to be related to the chemical and topochemical natures of lignin and/or the nature of the lignin-carbohydrate association, although knowledge of these natures is not fully available at present. To define more accurately the significance of lignin in different decay resistance of wood, a further knowledge of factors, including these natures, topochemical effect of delignification and fungal enzyme systems, is needed.

Besides in the way they attack wood, soft rot fungi have a number of distinctive physiological and ecological characteristics. As exemplified by *C. globosum*, they differ from other wood-decaying fungi in the way they modify the wood chemically and physically, resembling alternatively white rot fungi and brown rot ones yet behaving peculiarly. As has been mentioned, soft rot species such as *C. globosum* can attack hardwoods more easily than softwoods. However, partial delignification and splitting of the lignin-carbohydrate association, which is, by way of example, observed in wood cooling towers wetted by chlorine-containing water, will decrease the resistance of softwoods to soft rot.

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