燻煙熱処理針葉樹材を用いたきのこの菌床栽培

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論　文

燻煙熱処理針葉樹材を用いたきのこの菌床栽培 *1

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Mushroom Cultivation Using Smoke-Heated Softwood Sawdust

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Summary

The uses of smoke-heated sugi (*Cryptomeria japonica* D. Don.) and karamatsu (*Larix leptolepis* Gord.) sawdust for the sawdust-based cultivations of shiitake ((*Lentinus edodes* (Berk.) Sing.)) and hiratake ((*Pleurotus ostreatus* (Fr.) Quel.)) mushrooms were investigated in order to offset the shortage of hardwood sawdust as a cultural substrate.

In chemical analyses of smoke-heated sawdust of sugi and karamatsu, the hot-water, 1% sodium hydroxide, and ethanol-benzene extracts decreased by smoke heatings. This probably leads to active mycelial growth of shiitake, suggesting that smoke-heated softwood sawdust can be used for shiitake mushrooms.

In shiitake mushroom-cultivation using both sugi and karamatsu smoke-heated sawdust, yields of fruiting bodies decreased with increases in mixing ratios of softwood sawdust. A comparison of the effects on the yields of fruiting bodies between sugi and karamatsu sawdust by smoke heating indicated that sugi sawdust is more effective than that of karamatsu. The use of sugi and karamatsu sawdust treated by smoke heating could be possible in the sawdust-based cultivation of shiitake mushrooms, if the mixing weight ratio of 3:1 (beech:softwood) or 1:1 is used.

In the sawdust-based cultivation of hiratake using smoke-heated sugi sawdust, yields of fruiting bodies were almost the same or slightly more than those of control media. In karamatsu sawdust beds, relatively large yields of fruiting bodies were obtained with almost all of the media, in which great differences were not found between the smoke-heated and the non-treated wood meals compared to those of sugi. These results suggest that smoke-heated sawdust of both sugi and karamatsu are useful for the cultivation of shiitake and hiratake mushrooms. In addition, it is expected that the use of smoke-heated softwood sawdust in the sawdust-based cultivation of mushrooms largely reduce the cost of mushroom cultivation.

Keywords : Smoke-heating, Mushroom cultivation, Softwood sawdust, *Lentinus edodes* (Berg) Sing, *Pleurotus ostreatus* (Fr.) Quel.

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要　旨

燻煙熱処理したスギとカラマツを用いて，シイタケとヒラタケの菌床栽培を行い，培地としての有効性を考察した。燻煙熱処理によって菌糸の伸長を阻害する成分が含まれると思われる抽出物の量が減少したが，主要化学成分には大きな変化は見られなかった。スギおよびカラマツともに，燻煙熱処理によってシイタケ菌糸の成長が促進されるのが認められた。シイタケ栽培での子実体収量は，ブナオガ粉に対する針葉樹オガ粉の混合割合が増加するにつれて減少したが，混合割合が3：1あるいは1：1のとき十分な収量が得られた。燻煙熱処理による効果は，カラマツよりもスギを用いた培地で見られた。シイタケでは，スギオガ粉のみの培地を除いて，ブナオガ粉培地と同等の収量が得られた。ヒラタケでは，スギオガ粉のみの培地を除き，スギ，カラマツ共燻煙熱処理オガ粉の混合割合の違いにかかわらず有効であった。これらの結果より，これまで利用されなかった針葉樹を燻煙熱処理することによってきのこの栽培に十分使用でき，さらに栽培コストの削減と栽培期間の短縮を可能にすることが示唆された。

キーワード：燻煙熱処理，きのこの栽培，針葉樹木粉，シイタケ，ヒラタケ

1. Introduction

Mushrooms are becoming very popular as a health food, and the production of various kinds of mushrooms gradually is increasing in Japan using sawdust-based cultivation. However, the shortage of hardwood sawdust is now a serious problem for mushroom cultivators.

Large amounts of thinned trees, tree tops, and branch woods of softwoods are left in the forest. Effective utilization of this slash woods should be developed. It is well known that softwood slash is not suited to mushroom cultivation compared to that of hardwoods because most softwoods contain some inhibitors to mycelial growth. Therefore, selection of mushroom species and softwoods suitable as substrates of mushroom cultivation has not been made so far. The use of softwood sawdust for mushroom cultivation is limited to a few mushrooms such as hiratake ((*Pleurotus ostreatus* (Fr.) Quel.)) (Kinuta 1992) and enokitake ((*Flammulina veltipes* (Fr.) Sing.)) (Nakamura 1992). If sugi (*Cryptomeria japonica* D. Don.) sawdust is aged with water, cultivation of these mushrooms is possible (Kinjo and Kondo 1978; Sawa 1991). In nameko (*Pholiota nameko* S. Ito and Imai) mushrooms, mycelial growth on softwood medium was less than that on beech (Shoji and Ohtake 1968; Arita and others 1969; Sawa 1984). In the sawdust-bed cultivation of maitake ((*Grifola frondosa* (Fr.) S. F. Gray)), sugi sawdust gave smaller yields of fruiting bodies than did beech sawdust (Tonomura 1980). Kunitomo (1986) reported that in maitake cultivation, the use of beech sawdust mixed with 40% of sugi sawdust, gave almost the same yield of fruiting bodies as did those of beech sawdust alone. In shiitake ((*Lentinus edodes* (Berk.) Sing.)), mycelial growth was considerably less in softwood substrates than in beech substrate, except for sapwood sawdust of tsuga (*Tsuga sieboldii* Carr.) and karamatsu (*Larix leptolepis* Gord.) (Nishikado and others 1942; Nakamura and Iioka 1951; Ishikawa 1967). In shiitake mushroom cultivation, some investigators reported that karamatsu sawdust could give relatively large yields of fruiting bodies among softwood species (Sawa 1980; Tajima and Shinoda 1981; Nakazawa and others 1984; Sakamukai and Nonaka 1985).

Kinjo and Kondo (1979) reported that mycelial growth was inhibited by phenolic compounds because medium of softwood sawdust extracted with ethanol-benzene could promote the mycelial growth of shiitake. The extractives, such as phenolics, terpenes, flavonoids, and so on, have been shown to be inhibitors of the mycelial growths of many mushrooms. Nakajima and others (1980) and Yoshimoto (1983) showed that sugi and karamatsu woods contain ferruginol and flavonol respectively, as inhibitors. Mycelial growth of shiitake was inhibited more by ferruginol than were those of hiratake and enokitake (Kawachi and others 1991). Sawa (1991) succeeded in obtaining the same yield of fruiting bodies as that of hardwood medium in the sawdust-based cultivation of shiitake using softwood, which were treated by water-sprinkling, water-extracting, and seasoning for 3 to 6 months to reduce the amounts of mycelial-growth inhibitors. Ishii and others (1996) reported that increasing the moisture content of softwood medium up to 75% promoted the mycelial growth of shiitake, the mycelial growth almost being the same as that of hardwood.

On the other hand, selection and production of mushroom strains suitable for the cultivation of mushrooms using softwood meals have been attempted by using biotechnological techniques (Takahashi 1984; Kadoya and Sawa 1991; Katoh and Sawa 1992; Kadoya 1992). The results which have been obtained so far suggest the possibility of mushroom cultivation using softwood sawdust, although the yields of fruiting bodies obtained were relatively small. Further research is needed to obtain the large yields of mushrooms by softwood-sawdust cultivation.

In this paper, effective utilization of the unused softwoods from thinning was examined for mushroom cultivation. Sugi and karamatsu woods were smoke-heated and used for the sawdust-based cultivation of some mushrooms. Resulting effects obtained by using smoke-heated softwoods on the cultivation of mushrooms were discussed.

2. Materials and Methods

2.1. Smoke-heating of logs

Sugi logs, 3 m in length, were obtained from the Nasu Forest Owners association, Tochigi Prefecture. Karamatsu logs, 3 m in length, were harvested from the Nikko stand of the Utsunomiya University Forest. After being smoke-heated for 35 hrs in a kiln with increased far-infrared radiation (Ando 1996), the logs of both species were ground for collecting sawdust. The sawdust obtained were dried in the field before use, and they were used for the cultivation of mushrooms.

2.2. Chemical anlyses

To examine the influence of smoke-heating on the chemical changes of wood components, hot water, 1% sodium hydroxide, and ethanol-benzene extracts and main wood components (holocellulose, alpha-cellulose, and lignin) were determined according to ordinary methods (Nakano 1956).

2.3. Mycelial growth

To select strains suitable for softwood sawdust-based cultivation, mycelial growths of five strains (hiratake, *Pleurotus ostreatus* Quel.; shiitake, *Lentinus edodes* Sing.; enokitake, *Flammulina velutipes* Karst.; maitake, *Grifola frondosa* S.F. Gray; and bunashimeji, *Hypsizigus mamoresus* Bigelow) were compared on the 100% softwood substrate prepared from logs treated or non-treated by smoke-heating.

Moisture contents of the substrates were adjusted to 65%. After being autoclaved at 120 ℃ for 30 min, spawn of about 5 g was inoculated onto the medium, and then incubated at 28 ℃ in the dark.

2.4. Sawdust-based cultivation of mushrooms

Sawdust beds were prepared by mixing fresh beech sawdust with smoke-heated softwood sawdust in the various weight ratios listed in

Table 1. Moisture contents of substrates were adjusted to 65%. Each substrate of 1 kg containing 70 g of wheat bran was packed in a polypropylene bag equipped with a porous sterile filter. Five sawdust beds were used for each experimental condition.

Mushroom cultivation using the sawdust beds was performed at the Forest Research Center of Tochigi Prefecture. Strains used in the experiment were shiitake (Hokken No. 600) and hiratake (Mori No. H7). These strains were suitable for sawdust-based cultivation. Spawn was inoculated aseptically onto the sawdust beds in a laminar flow hood. The beds were incubated for vegetative growth for certain periods; 97 days for shiitake and 37 days for hiratake. Temperature was maintained at 22 ℃, and relative humidity at 70% by overhead misting.

For the fruiting of shiitake, after 97 days of incubation, each polypropylene bag was removed. Sawdust beds sufficiently colonized with fungi were transferred to a fruiting room in which the temperature was maintained at 15 ℃, and the relative humidity at 95%. After culture for 11 days, developed fruiting bodies were harvested from the beds, and their fresh weights were measured.

For the fruiting of hiratake, after the sawdust beds were cultured for 37 days, they were transferred to a fruiting room in which temperature and relative humidity were maintained at 15 ℃ and 95%, respectively. The mycelial mat on each bed was scratched for fruiting, and some water was poured on the beds. Then the sawdust beds were covered with porous polyfilm to maintain the humidity during fruiting.

3. Results and Discussion

3. 1. Extracts

Mycelial growth is inhibited frequently by phenols or monoterpenes present in softwood (Kinjo and others 1979; Tajima 1982; Yoshimoto 1983). Although ferruginol in sugi wood also inhibits the mycelial growth of shiitake, it does not inhibit that of hiratake (Nakajima and others 1980; Kawachi and others 1991). Flavonoids in karamatsu wood also inhibit the mycelial growths of many mushrooms. Thus, mycelial growth is less active in softwood meals than in hardwood meals. This makes mushroom production using softwood sawdust very difficult.

In the present investigation, to examine the availability of smoke-heated softwood sawdust for mushroom cultivation, extracts and main chemical components were compared between smoke-heated and control sawdust. The results are shown in Table 2.

The decrease in hot-water extracts by smoke-heating was 12.0% in sugi and 7.5% in karamatsu. It has been found to date that water-soluble carbohydrates, tannins, and gly-

Table 1. Weight ratios of beech to softwood sawdust in the sawdust-bed cultivation of mushrooms.

Sawdust beds	Beech sawdust (g)	Softwood sawdust (g)	Mixing ratios
A	280	0	-
B	210	70	3 : 1
C	140	140	1 : 1
D	70	210	1 : 3
E	0	280	-

Each sawdust-bed contained 70 g of wheat bran.
Moisture content was adjusted to 65 %.

Table 2. Chemical analyses of sawdust.

	Sugi (%)			Karamatsu (%)		
	Non-treated	Smoke-heated	Increase ratio	Non-treated	Smoke-heated	Increase ratio
Hot-water ext.	5.0	4.4	-12.0	8.0	7.4	-7.5
1 % sodium hydroxide ext.	15.8	12.1	-23.4	17.4	15.6	-10.3
Alcohol-benzene ext.	1.8	1.7	-5.6	2.1	1.9	-9.5*
Holocellulose	71.8	73.0	1.7*	72.9	71.3	-2.2*
α-cellulose	47.7	47.7	0.0	48.8	45.4	-7.0
Lignin	33.2	32.8	-1.2*	29.4	29.3	-0.3*

* No significance (5 % level)

cosides were included in the hot-water extracts (Yasuoka 1985). In both the non-treated and smoke-heated sawdust, the amounts extracted with 1% sodium hydroxide were greater than those of hot-water extracts. Sodium hydroxide extracts largely decreased in the smoke-heated sawdust, the decrease ratios being 23.4% in sugi and 10.3% in karamatsu. The decreases in ethanol-benzene extracts by smoke-heating were 5.6% in sugi and 9.5% in karamatsu. In ethanol-benzene extracts of softwood, fats, oil, pigments, resin, and essential oil have been detected to date (Yasuoka 1985). Okuyama and others (1988) reported that the hot-water and ethanol-benzene extracts increased in sugi logs that were smoke-heated. It is considered that much of the extracts flowed out with the hot water from the end surfaces of logs. Kinjo and Kondo (1979) also reported that softwood sawdust treated with ethanol-benzene mixture promoted active mycelial growth. These findings and the results obtained here suggest that smoke-heated softwood could be used for the cultivation of various mushrooms.

3. 2. Chemical components

Great differences in the main chemical components were not found between smoke-heated and non-treated sawdust (Table 2). Okuyama and others (1988) indicated that heat deterioration of chemical wood components occurs during prolonged smoke-heating, particularly in hemicellulose, resulting in increases of hot-water extracts. In this experiment, almost no decrease of hemicellulose contents was found in both sugi and karamatsu that was smoke-heated. Hemicellulose is very important in the mycelial growth, especially in the early stage of decay, because white-rot fungi degrade hemicellulose preferentially as a nutrient prior to the degradation of cellulose (Kirk and Moore 1972; Blanchette 1984; Otjen and Blanchette 1987; Yoshizawa and others 1990, 1992). It is considered that smoke-heated sawdust could be used as a substrate for the sawdust-based cultivation of various mushrooms. This consideration is supported also by the decreases in the amounts of extracts which seem to inhibit mycelial growth as described above.

3. 3. Mycelial growth

To select fungi which grow well on softwood substrate, mycelial growths of five fungi were compared using the sawdust of sugi and karamatsu with／without smoke-heating treatment. The results are shown in Figs. 1-4.

In non-treated sugi sawdust, only hiratake showed vigorous mycelial growth compared to

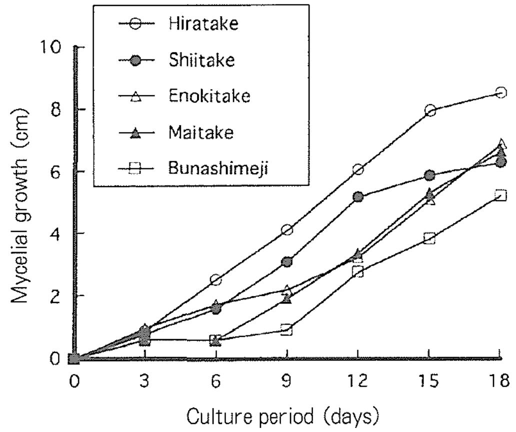

Fig. 1. Mycelial growths of five fungi cultured in non-treated sugi sawdust(100%).

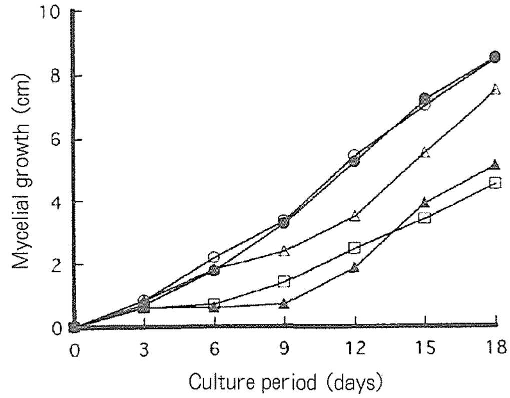

Fig. 2. Mycelial growths of five fungi in smoke-heated sugi sawdust (100%). Symbols: Refer to Fig. 1.

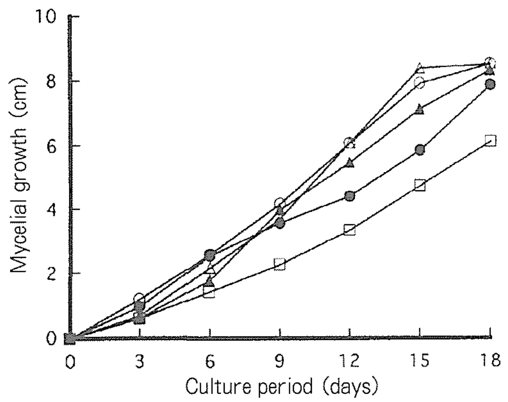

Fig. 3. Mycelial growths of five fungi cultured in non-treated karamatsu sawdust (100%). Symbols: Refer to Fig. 1.

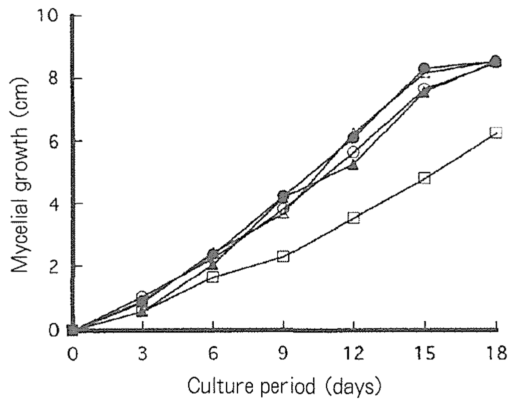

Fig. 4. Mycelial growths of five fungi cultured in smoke-heated karamatsu sawdust (100%). Symbols: Refer to Fig. 1.

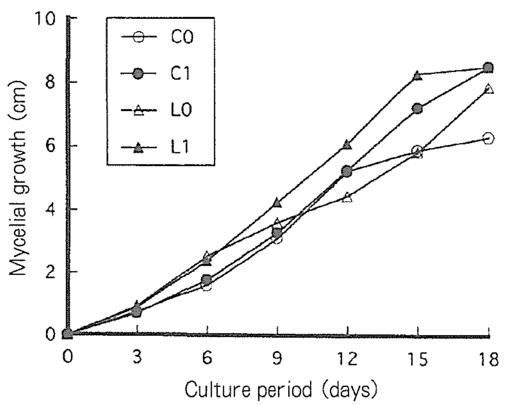

Fig. 5. Mycelial growths of shiitake cultured in softwood sawdust (100%). Note: C0; non-treated sugi, C1; smoke-heated sugi, L0; non-treated karamatsu, L1; smoke-heated karamatsu.

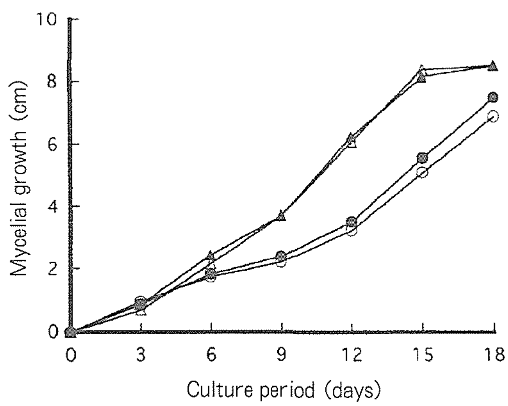

Fig. 6. Mycelial growths of enokitake cultured in softwood sawdust (100%). Symbols: Refer to Fig. 5.

the other four fungi. In the smoke-heated sawdust, mycelia of shiitake grew vigorously, but almost no difference was found between hiratake and shiitake. The smoke-heated sawdust did not give significant promotion of mycelial growth of enokitake, maitake, and bunashimeji.

In the non-treated karamatsu sawdust, mycelia of hiratake, enokitake, and maitake showed relatively active growth compared to those of shiitake and bunashimeji, whereas mycelia of shiitake grew actively on the smoke-heated sawdust. Bunashimeji showed almost no promotion of mycelial growth on the smoke-heated sawdust, this fact being similar to the results using sugi sawdust. This indicates that softwood sawdust is unsuitable for the mycelial growth of bunashimeji. In contrast, it is of interest to note that the mycelial growth of shiitake was promoted by using the smoke-heated sawdust in both sugi and karamatsu (Fig. 5). In enokitake, karamatsu sawdust gave better mycelial growth than did that of sugi (Fig. 6). There was almost no difference in mycelial growth between the non-treated and smoke-heated sawdust in both sugi and karamatsu.

Mycelial growth is less active in softwood medium than in hardwood medium (Nishikado and others 1942; Nakamura and Iioka 1951; Ishikawa 1967; Kinjo and Kondo 1979). It has been found that the use of softwood in the cultivation of mushrooms is very difficult, especially for shiitake and maitake. However, a supplemental experiment showed interesting results in that mycelia of shiitake grew vigorously on the smoke-heated sugi sawdust, the growth of which was almost similar to that of beech sawdust (Fig. 7). This fact indicates that smoke-heated softwood sawdust has the possibility of cultivating certain mushrooms as a substitute for hardwood sawdust. This would be because inhibitors of mycelial growth largely decrease or thermal structural changes of them occurs by smoke-heating.

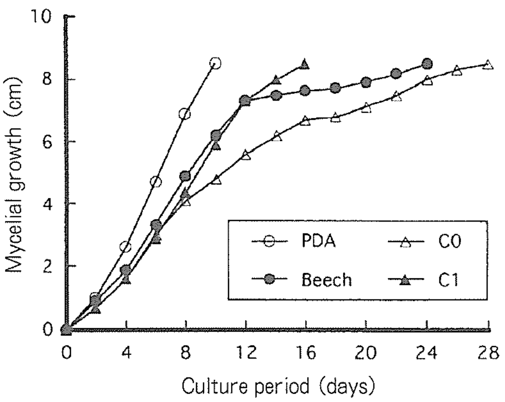

Fig. 7. Mycelial growths of shiitake cultured in the smoke-heated sugi sawdust. C0: non-treated sugi, C1: smoke-heated sugi.

Of the five fungi tested here, hiratake and shiitake which showed relatively good mycelial growth on the sawdust of sugi and karamatsu were selected for sawdust-based cultivation.

3. 4. Yields of fruiting bodies

Yields of fruiting bodies (fresh weight) in the sawdust-based cultivation of shiitake mushrooms using softwood sawdust are shown in Figs. 8 and 9. In both sugi and karamatsu sawdust, yields of fruiting bodies decreased with increases in the mixing ratio of softwood sawdust, irrespective of the use of sawdust treated with or without smoke-heating.

In shiitake mushroom-cultivation using sugi sawdust, the medium B-C1 with a mixing ratio of 3:1 (beech to smoke-heated sawdust) gave almost the same yield of fruiting bodies as those of beech sawdust (Fig. 8). The culture media B and C containing smoke-heated sugi sawdust, the mixing ratios being 3:1 for medium B and 1:1 for medium C, gave larger yields of fruiting bodies than did the culture media containing non-treated sawdust with the same mixing ratios. In the culture medium C, great variation in the yield of fruiting bodies was found among the culture media. This indicates that decay progress among the media apparently was different, resulting in some differences in the yields of fruiting bodies. If the culture conditions were controlled well, larger yields of fruiting bodies could be expected.

On the other hand, in shiitake mushroom cultivation using karamatsu, all of the culture media gave smaller yields of fruiting bodies than did beech sawdust medium (Fig. 9). Among the media used, medium B containing smoke-heated karamatsu sawdust with a mixing ratio of 3:1 gave larger yields of fruiting bodies than did the culture medium containing non-treated sawdust.

A comparison of the effects on the yields of fruiting bodies between sugi and karamatsu smoke-heated sawdust indicates that sugi sawdust is more effective than that of karamatsu. However, the results suggest that sugi and karamatsu sawdust treated with smoke-heating could be sufficient in the sawdust-based cultivation of shiitake mushroom, if the mixing ratio of at least 3:1 is used. In addition, it is considered that in smoke-heated sugi sawdust a mixing ratio of 1:1 also is useful.

In the sawdust-based cultivation of hiratake mushrooms using softwoods are shown in Figs. 10 and 11. In the sawdust-based cultivation using sugi sawdust, relatively large yields of fruiting bodies were obtained with the media C-

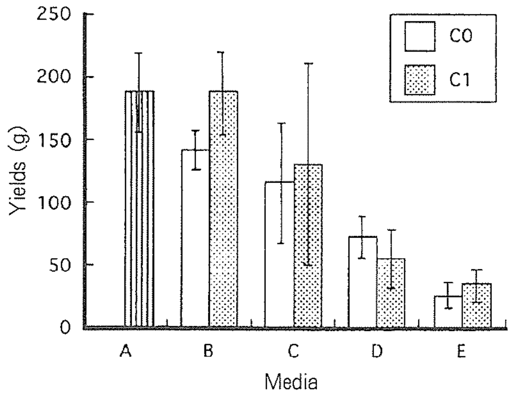

Fig. 8. Yields of fruiting bodies of shiitake using sugi sawdust. Note: Refer to Fig. 5 and Table 1 for details.

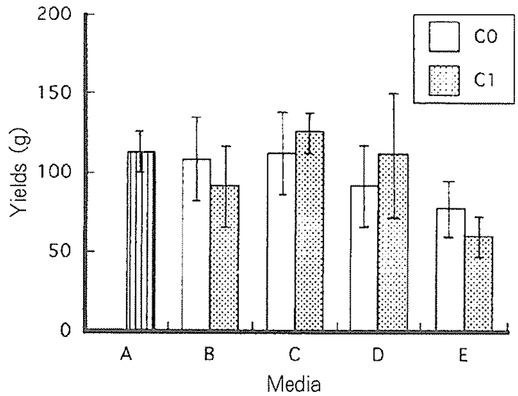

Fig. 10. Yields of fruiting bodies of hiratake using sugi sawdust. Note: Refer to Fig. 5 and Table 1 for details.

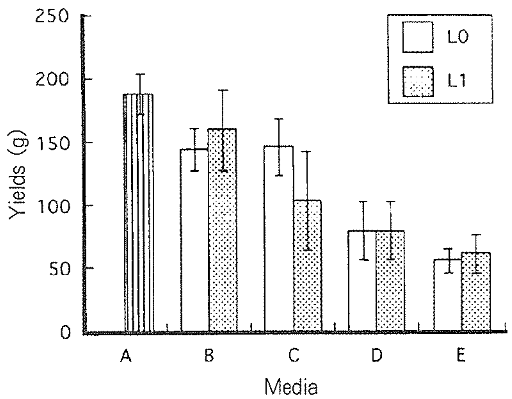

Fig. 9. Yields of fruiting bodies of shiitake using karamatsu sawdust. Note: Refer to Fig. 5 and Table 1 for details.

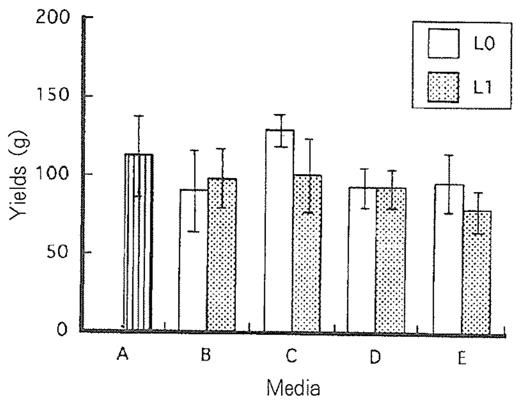

Fig. 11. Yields of fruiting bodies of hiratake using karamatsu sawdust. Note: Refer to Fig. 5 and Table 1 for details.

C1 and D-C1 containing smoke-heated sawdust with mixing ratios of 1:1 and 1:3, respectively. The yields of fruiting bodies were almost the same or slightly more than those of the control media.

In karamatsu sawdust beds, relatively large yields of fruiting bodies were obtained with almost all of the media in which great differences were not found between the non- and smoke-heated wood meals compared to those of sugi, except for the medium C-L0 which gave larger yields than did the medium C-L1. Differences in the mixing ratios of karamatsu sawdust also gave almost no great differences in the yields of fruiting bodies as well as those of shiitake mushrooms, except for media E-C0 and -C1 containing sugi sawdust alone.

Kinuta (1992) reported that sugi sawdust could be used in the case of hiratake mushrooms. The results obtained here are in good agreement with the previous results. These facts suggest that in hiratake mushroom cultivation, both smoke-heated sugi and karamatsu are useful, irrespective of the difference in the mixing ratios, except in the case using sugi sawdust alone.

Weight losses of sawdust beds decreased with increases in mixing ratios of softwood sawdust in the cultivation of the shiitake and hiratake mushrooms (Table 3), suggesting that the ability of both fungi to degrade softwood is relatively small compared to their abilities of degrading hardwood. Especially, the shiitake fungus could not degrade sugi wood meals sufficiently without smoke-heating treatment compared to the hiratake fungus. These results are in agreement with those found in the yields of fruiting bodies (Figs. 8 and 10). The mycelial growth of shiitake is slower in sugi sawdust medium than in that of karamatsu (Nakazawa and others 1984). In this experiment, however, slightly larger effects on the cultivation of shiitake mushrooms by smoke-heating were obtained with sugi compared to those with karamatsu. This effect may have been due to the differences in the amounts of hot-water and 1% sodium hydroxide extracts.

In hiratake cultivation, on the other hand, almost no effects on the yields of fruiting bodies by smoke heating were found, because the mycelium of hiratake can grow better in softwood sawdust medium than in that of shiitake. Extracts present in softwood may not inhibit the mycelial growth of the hiratake fungus compared to that of shiitake.

Ishii and others (1996) reported that the

Table 3. Weight losses on shiitake and hiratake mushroom cultivation per 1 kg sawdust bed.

		Sugi (%)		Karamatsu (%)	
	Sawdust beds	Shiitake	Hiratake	Shiitake	Hiratake
A	Beech	41.3	31.1	41.3	31.1
B	Non-treated	43.4	27.7	34.5	30.8
	Smoke-heated	33.3	25.4	25.8	27.4
C	Non-treated	28.0	25.4	31.9	25.4
	Smoke-heated	30.7	27.1	28.3	27.1
D	Non-treated	26.1	24.3	25.4	24.3
	Smoke-heated	20.5	30.7	24.8	30.7
E	Non-treated	17.9	22.3	15.1	22.3
	Smoke-heated	21.2	22.3	27.9	22.3

A-E : Refer to Table 1 for details.

mycelial growth of the shiitake fungus could be promoted by increasing the moisture content of the medium up to 75%. In the case of the sawdust-bed cultivation of shiitake mushrooms, aging of the softwood meals also was effective in increasing the yields of fruiting bodies (Sawa 1991). It is necessary to improve the physical conditions of the substrates for maintaining sufficient aeration and the water contents in the sawdust beds, as well as the conditions of the smoke-heated softwood for further reducing the amounts of inhibitors to mycelial growth. If mycelial growth could be promoted more in the sawdust beds containing smoke-heated softwood sawdust, it is possible to greatly reduce the substrate costs for the cultivation of shiitake mushrooms using smoke-heated softwood.

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