

Conversion of conifer wastes into edible and medicinal mushrooms

Suki C. Croan

Abstract

Mushroom-producing white-rot fungi can be used to convert wood waste into gourmet and medicinal mushrooms. White-rot fungi do not always readily colonize on conifer wood because of its extractives content. This study evaluated the resinous extractive content of loblolly pine (*Pinus taeda*), ponderosa pine (*Pinus ponderosa*), and an unknown species of southern yellow pine before and after treatment with the extractive-degrading fungi *Aureobasidium* spp., *Ceratocystis* spp., and *Ophiostoma* spp. Fungal treatment removed 70 to 99.9 percent of extractives. Scanning electron microscopy showed heavy mycelial growth of the colorless isolate *Ophiostoma piliferum*, with good sporulation, on the surface of loblolly and southern yellow pine chips, in the resin canals, and in the parenchyma cells within 4 to 5 days. The treated wood chips were used to cultivate lignolytic mushroom-producing, white-rot basidiomycetes of various *Pleurotus* species and two other fungi, *Grifola frondosa* and *Hericium erinaceus*. The results show that lignolytic white-rot basidiomycetes can easily colonize and produce mushrooms on treated conifer wood chips.

Lentinus edodes, compared to 13 and 25 percent of wheat and milk, respectively (Ogundana and Okogbo 1981). *Pleurotus* mushrooms are considered to be one of the most efficient producers of food protein (Ogundana and Okogbo 1981, Manzi 1999, Dabbour and Takburi 2002) and an excellent source of dietary fiber (Cheung and Lee 2000, Mattila and Pizzoferrato 2000). *Pleurotus* mushrooms also produce vitamins (B1, B2, B12, C, D, folate, and niacin) (Mattila et al. 2001) and mineral elements (Manzi 1999, Mattila et al. 2001). These nutritious and medicinal mushrooms (Cochran 1978, Chang and Buswell 1996, Ishisuka et al. 1997, She et al. 1998, Wasser and Weis 1999) are in great demand by the gourmet mushroom industry (Chang 1999).

Wood waste, including thinned material from stagnated and overstocked small-diameter wood, poses a serious threat to forest health by increasing fuel load, which can result in forest fires that severely damage the ecosystem. In the year 2000, there were 122,827 fires, 8,422,237 acres burned, and an estimated \$1.3 billion in fire control costs (Bosworth 2001). At the same time, the global demand for energy and nutritious food has been increasing, and there is a growing shortage of natural resources. Thinned material from stagnated and overstocked small-diameter wood stands can serve as a valuable resource for the production of nutritious gourmet and medicinal mushrooms.

White-rot fungi that produce edible and medicinal specialty mushrooms have been cultivated from hardwood in Asia for centuries. The production of

Pleurotus or oyster mushrooms has been increasing at a rapid rate worldwide. *Pleurotus* mushrooms represent 14 percent of the total world production of edible mushrooms, amounting to 368,000 tons in 1997 (Chang 1999). The production of *Pleurotus* mushrooms is a relatively new enterprise in the United States; 881 tons were produced in 1995, an increase of 94 percent from the previous year (Royse 1997).

Pleurotus mushrooms are very efficient producers of protein. Protein constitutes 30 percent of the dry weight of *Pleurotus* mushrooms and 18 percent of

The medical uses of *Pleurotus* mushrooms include their potential to act as antitumor agents (Cochran 1978; Gunde-Cimerman 1999; Kawamura et al. 2000; Zhang et al. 1994, 2001) and antifungal and antiviral agents (Gunde-Cimerman 1999) as well as their ability to lower cholesterol (Chovot 1997, Cheung 1998, Bobek et al. 1998a, Wasser and Weis 1999) and reduce colon carcinoma (Bobek et al. 1998b, Gunde-Cimerman 1999). *Pleurotus* mushrooms are fast colonizers that degrade a wide variety of lignin in lignocellulosic wood waste. They produce extracellular

The author is a Mycologist, USDA Forest Serv., Forest Prod. Lab., One Gifford Pinchot Drive, Madison, WI 53726-2398. The author wishes to thank the following staff at the Forest Prod. Lab. for their assistance: Roger C. Pettersen for analysis of wood extractives, Thomas A. Kuster for scanning electron microscopic examinations, and John Haight for technical assistance. This paper was received for publication in August 2002. Article No. 9532.
©Forest Products Society 2004.
Forest Prod. J. 54(2):68-76.

enzymes, including lignin peroxidase, manganese peroxidase, and laccase. These enzymes are nonspecific with regard to substrate and are referred to as lignin-modifying and/or lignin-degrading enzymes. As a result of fungal lignolytic action on wood, the spent substrates together with the remaining fungi can become a source of hemicelluloses and cellulose that can be used as carbohydrate for animal feed (Bisaria et al. 1997) or fertilizers (Stewart et al. 1998).

Southern yellow pine (SYP) consists of 11 species: longleaf pine (*Pinus palustris*), short leaf pine (*P. echinata*), slash pine (*P. elliotii*), southern Florida slash pine (*P. elliotii* var. *densa*), loblolly pine (*P. taeda*), pitch pine (*P. rigida*), spruce pine (*P. grabra*), Virginia pine (*P. virginiana*), sand pine (*P. clausa*), pond pine (*P. serotina*), and table mountain pine (*P. pungens*). Four of these species (loblolly pine, short leaf pine, long leaf pine, and slash pine) account for about 93 percent of all standing timber in the southeastern and south-central states (Hoadley 1990). SYP usually contains a high concentration of wood extractives or pitch deposits, ranging from 3 to 9 percent of total dry weight of the wood (Koch 1972). In exceptional cases, the extractives may constitute up to 40 percent, depending on the season when they were harvested (Zabel and Morrell 1992). Some extractives (i.e., monoterpenes) are toxic to certain fungi and insects (Raffa and Smalley 1995, Payne et al. 2001); other extractives impart color and fragrance to wood.

Wood-extractive-degrading fungi are initial wood colonizers that cause black discoloration in sapwood as well as in wood chips, logs, sawn timber, veneer, sawdust, and wood products. The fungi penetrate rapidly and deeply into the sapwood of conifers, assimilating available nutrients (primarily nonstructural wood components), but they have little or no effect on the cellulose, hemicellulose, or lignin content of the wood (Sjostrom 1981; Blanchette et al. 1992; Brush et al. 1994; Rocheleau et al. 1998, 1999). Although these fungi do not degrade the major components of wood, their metabolic action substantially reduces wood extractives (Blanchette et al. 1992, 1996). These nonstructural components of wood extractives consist of simple carbohydrates (sugar and starch), phenolic compounds (such as stilbenes, tannins, phlobaphenes, flavanoids, and lignans), lipids (triglycerides, fatty ac-

ids, resin acids, sterols, and steryl esters), and waxes, alkaloids, and terpenes, which are found in parenchyma cells and in the lumen of other cells (Sjostrom 1981, Zabel and Morrell 1992, Gao and Breuil 1995).

Pleurotus mushrooms have been cultivated on various agricultural lignocellulosic wastes or hardwood but never on conifer wood. The primary objective of our research was to evaluate the mycelial growth of mushroom-producing *Pleurotus* species on conifer wood waste after treating the wood with extractive-degrading fungi. The secondary objective was to convert conifer wood residues, wood wastes, and underutilized forest resources into a value-added resource for the production of gourmet and medicinal mushrooms.

Methods and materials

Fungi

Dikaryotic isolates of the following mushroom-producing white-rot fungi were obtained from the culture collection of the Center for Forest Mycology Research at the Forest Products Laboratory (USDA Forest Serv., Madison, Wisconsin): *Grifola frondosa* (Dicks:Fr.) S.F. Gray (FP-101988, SC 10), *Hericium erinaceus* (Bulliard: Fries) Persoon (FP-140075, SC 13), *Pleurotus citrinopileatus* (Singer) (FP-102361, SC 17), *P. cystidiosus* O.K. Miller (FP-140088, SC 18 and FP-140088, SC 19), *P. euosmus* (Berkley apud Hussey) Saccardo (FPRL-66, SC 20), *P. ostreatus* (Jacquin: Fries) Kummer (FP-101509, SC 21 and FP-140084, SC 23), *P. populinus* Hilber and Miller, (FP-102755, SC 24), *P. pulmonarius* (Fries) Quelet (RLG-10645, SC 25 and FP-140087, SC 36), *P. sapidus* Kalchbr., (FP-4-103, SC 27, FP-140080, SC 28, and FP-140094, SC 55), and *P. sajor-caju* (Fr.) Sing. (FP-140078, SC 29).

Pleurotus cornucopiae (Paulet ex Fries, WC-608, SC 52) and *P. eryngii* (De Candolle ex Fries) Quelet *sensu lato* (WC-827, SC 52) were obtained from Dr. D. J. Royse, Penn State University (University Park, Pennsylvania). The wood extractive-degrading fungi *Aureobasidium pullulans* (deBary) Arnand [MDX 18], *Ceratocystis coerulea* (Munch) Bakeshe [C-262], and *C. pilifera* (Fries) Moreau [RWD 9472B] were obtained from the Center for Forest Mycology Research at

the Forest Products Laboratory. A colorless isolate of *Ophiostoma piliferum* (Cartapip 97) was obtained from AgraSol, Inc. (Raleigh, North Carolina).

The fungi were maintained on 1.5 percent (w/v) malt extract (Bacto, Difco, Detroit, Michigan) and 2 percent (w/v) agar (Bacto, Difco; MEA). Malt extract agar 90-mm-diameter plates were inoculated with a mycelium/agar plug (6 mm diameter) of a young, actively growing margin of the colony at the center of the plate and incubated at 24°C in the dark for 1 to 2 weeks or until mycelial growth had covered the entire surface of the plate.

Treatment of chips

Loblolly pine (*Pinus taeda*) chips were obtained from Bowater, Inc. (Catawba, SC). Chips of an unknown species of SYP (*Pinus* spp.) were obtained from the Bienville National Forest in Mississippi. Ponderosa pine (*P. ponderosa*) chips were obtained from the Coconino National Forest near Flagstaff, Arizona. All chips were frozen until used.

Small-scale treatment. – One hundred grams (dry weight 45% to 50%) of frozen SYP chips of various sizes (0.5 to 2 cm by 0.2 to 0.25 cm) and distilled water were added to Pyrex storage dishes (Corning No. 3250) to produce a final moisture content of 60 percent. Each dish was autoclaved and inoculated with actively growing mycelia from half an MEA plate inoculated with the extractive-degrading fungi *Aureobasidium* spp. or *Ceratocystis* spp. A colorless isolate of *Ophiostoma piliferum* (Cartapip 97) was inoculated 2×10^8 spores ($1 \times$) and 4×10^8 spores ($2 \times$) per storage dish. After thorough mixing, the inoculated wood chips were incubated at 24°C in the dark for 30 days and then autoclaved to kill the extractive-degrading fungi. The experiment was performed in triplicate.

To evaluate the growth of mushroom-producing fungi, treated SYP chips were inoculated with actively growing mycelia of *Grifola frondosa*, *Hericium erinaceus*, or *Pleurotus ostreatus* scraped from half the surface of an MEA plate. After thorough mixing, the chips were incubated at 24°C in the dark. Mycelial growth was measured after an incubation period of 50 days.

Large-scale treatment. – Four kilograms (dry weight 45% to 50%) of frozen conifer wood chips of various

sizes and distilled water were added to air-permeable polyethylene bags (610 by 914 mm) to produce a final moisture content of 60 percent. The bags were inoculated with 2×10^9 spores of the colorless isolate (Cartapip 97) per kilogram wet wood chips. The chips were mixed manually and incubated at 24°C in the dark for 4 to 5 days. At the end of the incubation period, the chips were autoclaved at 121°C for 45 minutes.

Determination of resinous extractives

After treatment, pine chips were oven-dried at 50°C and ground into 30-mesh sawdust with a Wiley mill (Authur H. Thomas Co., Scientific Apparatus, Philadelphia, Pennsylvania). The oven-dried sawdust (dry weight) was extracted overnight in a Soxhlet extractor with diethyl ether (Brush et al. 1994) or dichloromethane (T 204 cm-97) (TAPPI 1997).

Scanning electron microscopy

Treated loblolly pine chips were cut radially using razor blades, mounted on aluminum stubs using silver paste, and gold coated using a Denton Desk-1 (Denton Vacuum, Inc., Cherry Hill, New Jersey) sputter coater. Samples were examined and photographed using Polaroid film in a JEOL JSM-840 (JEOL Ltd., Tokyo, Japan) scanning electron microscope at 15 kV.

Grain spawn and fruiting body production

Grain spawn and mushroom production were prepared as outlined in a previous publication (Croan 2000). A mixture of 500 g barley, 5 g gypsum (calcium carbonate or calcium sulfate), and 500 to 600 mL water in a polypropylene autoclavable bag (20.2 by 42 cm) with a microporous filter patch was used

for spawn production. After the bags were inoculated with various *Pleurotus* species, they were sealed with a thermal impulse sealer and incubated in the dark at 24°C for 1 to 3 weeks or until mycelial growth had covered the surface of the grain.

Pretreated pine chips of various sizes with or without 20 percent wheat bran, 1 percent gypsum, and 3 percent glucose were placed in autoclavable bags with a microporous filter patch. Distilled water was added to increase moisture content of the mixture to 60 percent, resulting in a final weight of 1,000 g. The bags were then inoculated with grain spawn of various *Pleurotus* species at approximately 20 percent wet weight. The bags were manually mixed, sealed with thermal impulse sealer, and incubated at 24°C in the dark for 1 to 2 weeks or until the mycelium had completely colonized the substrate. The experiment was replicated three to five times.

The bags were placed in a refrigerator for 1 to 2 days, and then were cut open, exposing the colonized substrate to the air, and placed in an incubator. The temperature was maintained at 20° to 22°C under a standardized light cycle (approximately 10 hr. light, 14 hr. dark) using a fluorescent ceiling light (two 20-W, standard, cool white bulbs). Humidity and moisture were maintained at 70 to 95 percent with a constant vapor-like spray of distilled water for an hour every 6 hours, using a Herri-michifier (part L122-74, trio model 707). Fruiting bodies were harvested when the caps reached 5 to 10 cm in diameter. Fruiting bodies were harvested for up to three flushes. The dry weight of the substrates was used to calculate the percentage of biological efficiency. Biological efficiency is the fresh weight of the harvested mushrooms divided by the dry

weight of the substrate and then multiplied by 100.

Determination of lignin

Total lignin content in the spent substrates was extracted with 72 percent H_2SO_4 . Acid-insoluble lignin (Klason lignin) was measured gravimetrically (Effland 1977), and acid-soluble lignin was determined by spectrophotometry based on absorption of ultraviolet radiation (TAPPI standard method T 222 om-88. (TAPPI 1988).

Results and discussion

Three mushroom-producing basidiomycetes, *Griofola frondosa*, *Hericium erinaceus*, and *Pleurotus ostreatus*, were selected for small-scale treatment of conifer chips in Pyrex storage dishes. The fungi did not grow or grew poorly on surfaces of untreated chips, even though these versatile fungi are fast colonizers that can degrade a wide variety of lignin in various lignocellulosic substrates. Martinez-Inigo et al. (1999) found that Scots pine extractives were toxic to various wood-inhabiting fungi. Resin acids accounted for 88 percent of total extractives. Fungal growth was enhanced by removing the extractives. Similarly, certain resin acids in pine cone extractives were found to be toxic to wood-inhabiting fungi (mold, sapstain, and wood-rotting fungi) (Micales et al. 1994).

When conifer chips were treated with the extractive-degrading fungi *Aureobasidium pullulans*, *Ceratocystis coerulea*, and *Ophiostoma piliferum*, and a colorless isolate of *O. piliferum* (Cartapip 97), rapid and heavy filamentous mycelial growth occurred on the entire surfaces of SYP chips (**Fig. 1**). The fungi removed 70 to 99.9 percent of the extractives (**Table 1**). In addition to heavy mycelial growth, *Griofola frondosa*, *Hericium erinaceus*, and *Pleurotus ostreatus* produced abnormal fruiting bodies, aerial spines, or aerial mycelia (**Fig. 2**). The aerial spines of *Hericium erinaceus* were 24 to 35 mm high and extended 28 to 33 mm beyond the surface of the chips in deep dishes. By contrast, the aerial spines of *G. frondosa* remained relatively flat although they were elongated (20 to 28 mm). *Pleurotus ostreatus* produced flat and shorter aerial spines (8 to 15 mm) and abnormal fruiting bodies (20 to 50 mm by 5 to 25 mm) with many small hyphal proliferations.



Figure 1. – Mycelial growth of *Griofola frondosa* on SYP chips: (a) chips treated with extractive-degrading fungi; (b) untreated chips.

Table 1. – Removal of wood extractives from southern yellow pine chips by extractive-degrading fungi.

Treatment	Remaining extractives	Removed extractives
	----- (%) -----	
No treatment (control)	9.02 ± 0.04	0
<i>Aureobasidium pullulans</i> MDX-18	2.73	69.7
<i>Ceratocystis coerulea</i> C-256	2.69	70.2
<i>C. piliferum</i> RWD9427B	0.05	94.5
<i>Ophiostoma piliferum</i> (colorless isolate)		
1 × inoculation (2 × 10 ⁸ per dish)	0.009	99.9
2 × inoculation (4 × 10 ⁸ per dish)	0.009	99.9



Figure 2. – Abnormal fruiting bodies and aerial spines: (a) *Grifola fondosa*, (b) *Hericium erinaceus*, and (c) *Pleurotus ostreatus*.

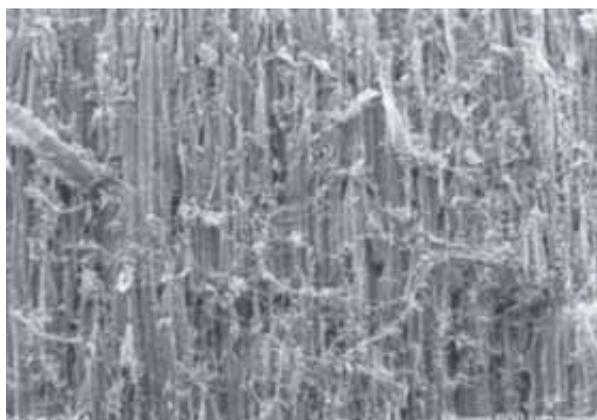


Figure 3. —Filamentous 25-day-old mycelial growth of *Ophiostoma piliferum* on SYP chips.

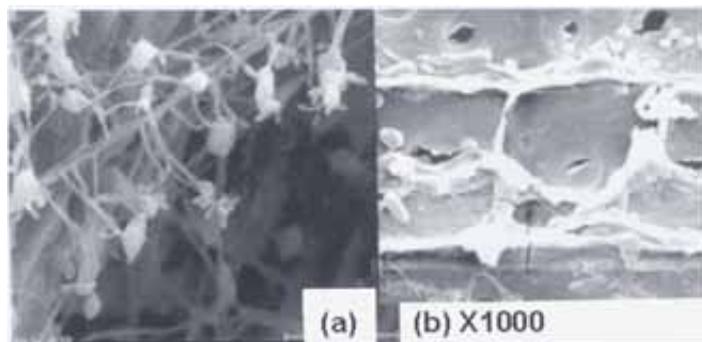


Figure 4. – Sporulation of *O. piliferum*: (a) 6-day-old spores on SYP chips; (b) 10-day-old spores on ponderosa pine chips.

Aerial spines and abnormal fruiting bodies were produced on treated SYP chips in storage dishes without special treatment, e.g., light/dark cycles, added humidity, or decreased temperature. They may have been caused by the accumulation of carbon dioxide, which can occur in deep dishes. Aerial spines and abnormal fruiting bodies were initially very pale yellow to white; as the cultures aged, spines and fruiting bodies became yellowish brown.

The lyophilized colorless isolate of *Ophiostoma piliferum* (Cartapip 97) was selected for large-scale treatment of conifer chips because it can be easily obtained in large quantities and is simple to use. Wood extractives are primarily low molecular weight compounds that are easily extracted by solvents such as acetone, alcohol, diethylether, dichloromethane, benzene, or water. The percentage of extractives varies, depending upon the solvents used for extraction, the batch of wood chips received, the season, and the amount of rainfall when they were harvested (Terry Conners, Mississippi Forest Products Laboratory, personal communication).

In our study, extractives content was 3.05 to 9.02 percent, depending on the particular batch of chips. Treatment with Cartapip 97 (8 × 10⁹ spores per 4 kg chips) removed 30 to 90 percent of extractives within 4 to 5 days, which induced excellent mycelial growth and mushroom production. SEM examination revealed that *O. piliferum* had colonized the entire surfaces of chips within 2 days, producing heavy filamentous mycelial growth (Fig. 3) with sporulation (Fig. 4). The treatment removed 25.9 percent of extractives within 2 days. The mycelium of *O. piliferum* passed from one cell to the next through bordered pits and tracheids (Fig. 5). Heavy mycelial growth was observed in radial tracheid cells (Fig. 6) and ray paracyma cells (Fig. 7) within 5 to 10 days.

All *Pleurotus* species showed dense and heavy filamentous mycelial growth on the entire surfaces of treated conifer chips. After 6 days, the fungi grew faster on treated conifer chips than on hardwood sawdust (red oak, *Quercus* spp.) (Fig. 8). In addition, various *Lentinula edodes* species showed the same filamentous mycelial growth on entire surfaces of treated conifer chips and grew

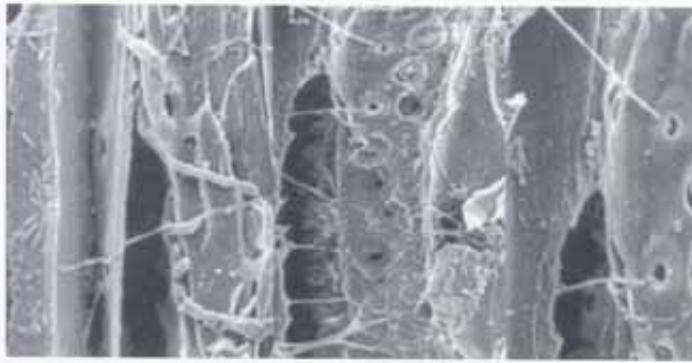


Figure 5. – Mycelial growth of *O. piliferum* in bordered pits and tracheid cells of SYP chips.

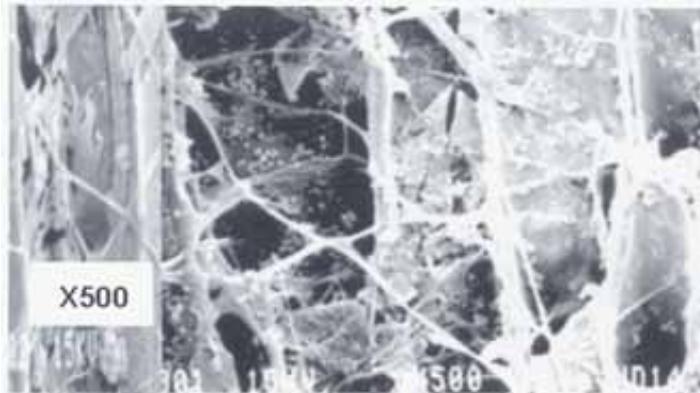


Figure 6. – Mycelial growth of *O. piliferum* in five tracheid cells of ponderosa pine chips, radial section.

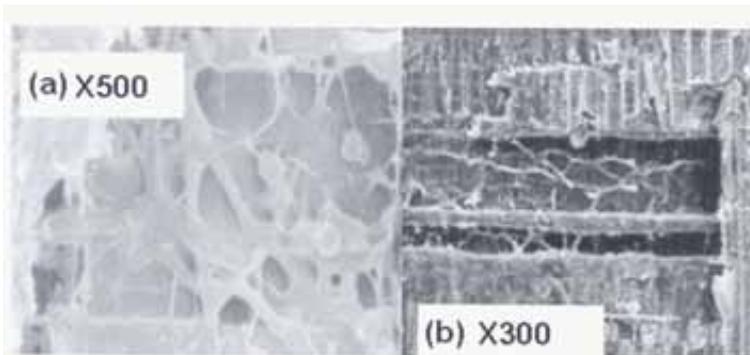


Figure 7. – Mycelial growth of *O. piliferum* in ray parenchyma cells of (a) ponderosa pine chips, and (b) SYP chips.

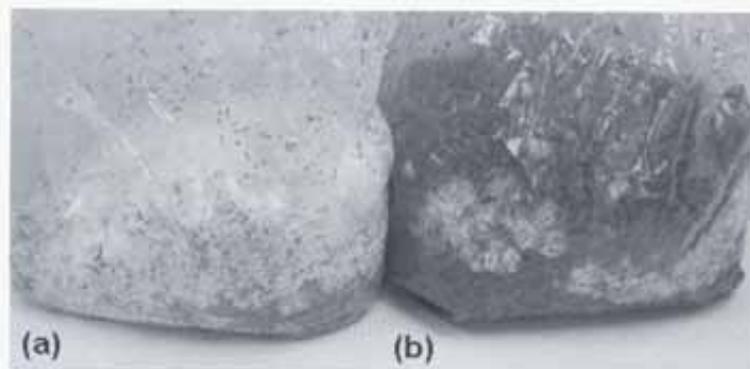


Figure 8. – Mycelial growth (6 days old) of *Pleurotus pulmonarius* on (a) treated loblolly pine chips, and (b) red oak sawdust.

faster on treated chips than on red or white oak sawdust (unpublished data).

Pleurotus mushrooms were produced on treated conifers. The species fell into three groups, according to the length of time required for fruiting (Table 2). The caps of the mushrooms were generally oyster- to fan-shaped, and their color ranged from creamy white, tan, or yellow to grayish-blue, brown, and almost black (Table 3). The white stems were usually positioned at the cap margin (100 to 50 mm).

In addition to mushrooms, *P. cystidiosus* (SC 19) produced numerous sterile cells on black caps. On treated pine chips supplemented with 20 percent bran, *P. cystidiosus* also produced conidiophores bearing conidia at the apex (called synnemata or coremia by Miller [1969]) (Table 3, Fig. 9). The SC 19 strain of *P. cystidiosus* did not produce synnemata on MEA plates. Another strain, SC 18, produced numerous synnemata around the colony and agar plug of the inoculated area on MEA plates. However, this fungus grew very poorly on treated wood chips and did not form synnemata or fruiting bodies. The cap clusters of *P. eryngii*, the king oyster mushroom (SC 54), were divided into two types: large and small fruiting bodies (Table 3, Fig. 11).

The biological efficiency of the mushrooms produced by *P. pulmonarius* (SC 36) was 155 and 159 percent for red oak sawdust and SYP chips, respectively, supplemented with 20 percent bran. Mushroom production by *P. pulmonarius* was about the same for the red oak sawdust and treated conifer chips (Table 4, Fig. 14). The basidiomata were harvested three times. The first harvest was typically the largest one, but the second harvest was sometimes larger than the first. The third harvest was always small (Fig. 14). In general, the mushroom caps were relatively flat with wavy margins. The stems of mushrooms on treated chips were relatively shorter compared to those on chips supplemented with 20 percent bran. Thus, the biological efficiency of the mushrooms on the treated chips supplemented with 20 percent bran was higher than that on unsupplemented treated chips (Table 4).

In 1977, about 6.16 million tons of mushrooms was produced worldwide (Chang 1999). Production increased more than 12 percent annually between

Table 2. – Time required for fruiting of mushrooms for various *Pleurotus* spp.

Group	Species	Time to fruiting
1	<i>P. citrinopileatus</i>	5 days to 1 week
	<i>P. cornucopiae</i>	
	<i>P. ostreatus</i> (SC 21, 23)	
	<i>P. populinus</i> (SC 24)	
	<i>P. pulmonarius</i> (SC 36)	
2	<i>P. eryngii</i>	1 to 2 weeks
	<i>P. sapidus</i> (SC 28, 55)	
	<i>P. sajor-caju</i> (SC 29)	
	<i>P. cystidiosus</i>	2 to 4 weeks
	<i>P. pulmonarius</i> (SC 25, 26)	
	<i>P. sapidus</i> (SC 27)	



Figure 9. – Fruiting bodies (a) and minute black synnemata (b) of *P. cystidiosus* (SC 19) on SYP chips supplemented with 20 percent wheat bran.

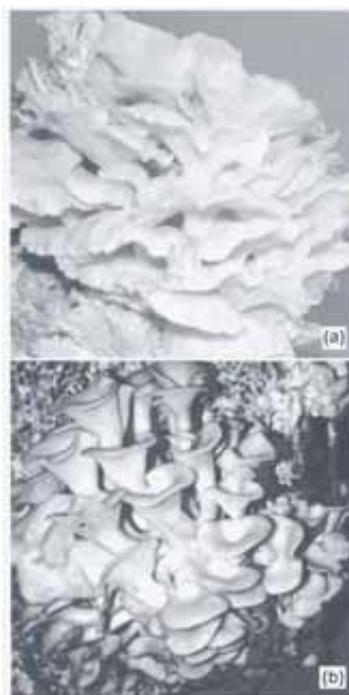


Figure 10. – Golden mushroom (*P. citrinopileatus*, SC 17) on SYP chips (a) and trumpet oyster mushroom (*P. cornucopiae*, SC 52) on loblolly pine chips, (b). Treatment was supplemented with 20 percent wheat bran.

1981 and 1997. Every ton of fresh mushrooms produces 1 to 2 tons of dry spent substrates (Rinker 2002). After mushrooms are harvested, millions of tons of spent substrates with their remaining fungi are available for other uses. Various uses for spent substrates are currently being investigated and evaluated in many countries. Our study constitutes one component of an ongoing, multifaceted international research effort.

In our study, treated conifer chip substrates were supplemented with additional glucose to protect holocelluloses in the treated chips. When additional glucose (35% glucose of dry pulp) was added to *Phanerochaete chrysosporium* stationary cultures on thermomechanical pulp, the utilization of cellulose and hemicellulose in lignocellulosic substrates was suppressed and only the lignin fraction was degraded (Yang et al. 1980). SYP chips were found to consist



Figure 11. – King oyster mushrooms (*P. eryngii*, SC 54) on loblolly pine chips supplemented with 20 percent wheat bran (a) and blue oyster mushrooms (*P. ostreatus*, SC 23) on SYP chips (b).

of 31.1 percent Klason lignin and 1.0 percent acid-soluble lignin.

After fruiting bodies of *P. ostreatus* (SC 23) were harvested for a third time, analysis showed that the spent substrates consisted of 12.1 to 16.1 percent Klason lignin and 0.54 to 0.72 percent acid-soluble lignin. Therefore, 51.9 to 61.1 percent Klason lignin was removed (Table 5). In addition, the chips in the spent substrates appeared to be brittle, suggesting that the remaining lignin might be partially degraded, selectively removed, or modified. Because ruminants may digest the spent substrates with fungi, the spent substrate may be used for animal feed (Moysen and Verachtert 1991, Zhang et al. 1995, Bisaria et al. 1997), animal bedding, soil conditioner (Stewart et al. 1998), or fertilizer (Stewart et al. 1998, Chiu et al. 2000). In addition, because the lignolytic enzyme systems of spent wood substrates with fungi can remove or modify lignin, the spent substrates may serve as a biopulping agent (Breen and Singleton 1999) and for bioremediation

Table 3. – Characteristics of *Pleurotus* mushrooms.

Species	SC number	Common name	Cap size (mm)	Description	Fig.
<i>P. cystidiosus</i>	19	Abalone or Miller's oyster mushroom	30 to 150	Caps convex to hollow with dark grayish brown center and yellowish brown smooth margins; long white stems	9
<i>P. citrinopileatus</i>	17	Golden oyster mushroom	20 to 70	Caps convex to plane; yellow and white stems centrally attached to caps	10
<i>P. cornucopiae</i>	52	Trumpet oyster mushroom	20 to 60	Caps trumpet-shaped, brilliant yellow, smooth margins; centrally attached stems	10
<i>P. eryngii</i>	54	King oyster mushroom	20 to 40	Caps small, tan to light brown, thick; thick, long, and stout stems	11
<i>P. ostreatus</i>	23,21	Blue oyster mushroom (SC 23), oyster mushroom (SC 21)	50 to 160	Caps grayish-dark blue in center, with light grayish-blue smooth margins (SC 23); lighter or pale (SC 21)	11
<i>P. euosmus</i>	20	Tarragon oyster mushroom	50 to 110	Caps broad and depressed in center, light bluish dark brown with tan smooth margins; very short stems	12
<i>P. pulmonarius</i>	25,26,36	Phoenix or Indian oyster mushroom	50 to 160	Caps grayish dark brown with curved inner surface and curved margins; long (70 to 150 mm) white stems	12
<i>P. sapidus</i>	55,28	Oyster mushroom	80 to 120	Caps light tan to creamy dark gray with smooth margins; short (20 to 50 mm) stems; lavender spore print.	13
<i>P. sajor-caju</i>	29	Oyster mushroom	80 to 180	Caps dark grayish–bluishblack, convex to hollow; short stems	13

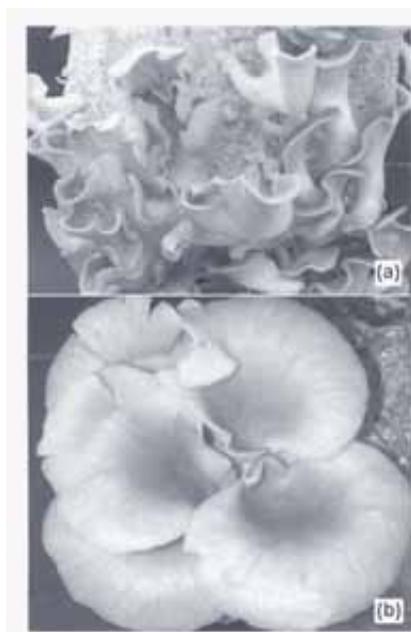


Figure 12. – Phoenix or Indian oyster mushrooms (*P. pulmonarius*, SC 36) (a) and tarragon oyster mushrooms (*P. euosmus*, SC 20) (b).

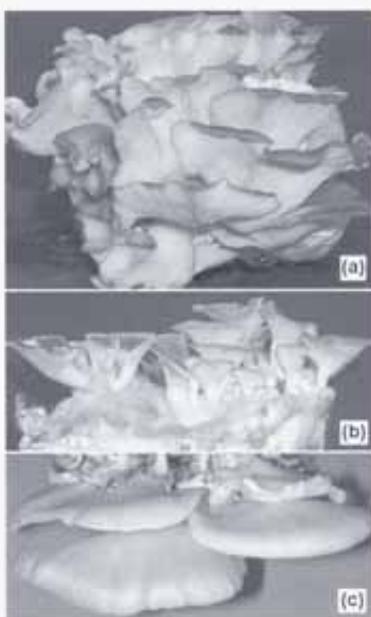


Figure 13. - Fruiting bodies of *I? sajor-caju* (SC 29) on SYP (a), *I? sapidus* (SC 28) on SYP supplemented with 20 percent bran (b), and *I? sapidus* (SC 55) on loblolly pine supplemented with 20 percent bran (c).

(Kirk et al. 1992, Lamar et al. 1994, Eggen and Majherczyk 1998, Semple et al. 1998, Eggen 1999, Eggen and Sveum 1999, Chiu et al. 2000, Marquez–Rocha et al. 2000).

Conclusions

Mushroom-producing basidiomycetes grow rapidly, degrading a variety

of lignin as well as hemicelluloses and cellulose in treated conifer lignocellulosic substrates. Mushroom-producing lignolytic white-rot fungi can convert conifers into a valuable resource, producing nutritious edible mushrooms, which are in great demand. Unused

woodwaste may pose a danger to the ecosystem. In conclusion, conifer wood substrates treated with extractive-degrading fungi, lyophilized *Ophiostoma piliferum*, can be recycled to produce valuable gourmet and medicinal mushrooms. The many potential uses of spent substrates can achieve total utilization of unused conifer residues, woodwaste, and underutilized forest resources.

Literature cited

- Bisaria, R., M. Madan, and P. Vasudevan. 1997. Utilisation of agro-residues as animal feed through bioconversion. *Bioresource Tech.* 59: 5-8.
- Blanchette, R.A., R.L. Farrell, and C.J. Behrendt. 1996. Biological control for wood products. Patent Application No. 5,532,164.
- _____, _____, T.A. Burnes, P.A. Wendler, W. Zimmerman, T.S. Brush, and R.A. Snyder. 1992. Biological control of pitch in pulp and paper production by *Ophiostoma piliferum*. *Tappi* 75(12):102-106.
- Bobek, P., S. Galbavy, and L. Ozdin. 1998b. Effect of oyster mushroom (*Pleurotus ostreatus*) on pathological changes in dimethylhydrazine-induced rat colon cancer. *Oncology Reports* 5:727-730.
- _____, L. Ozdin, and S. Galbavy. 1998a. Dose- and time-dependent hypocholesterolemic effect of oyster mushroom (*Pleurotus ostreatus*) in rat. *Nutrition* 14(3):282-286.
- Bosworth, D. 2001. Opportunities for forestry partnerships. nifc.gov/fireinfo/2000/index.html.
- Breen, A. and F.L. Singleton. 1999. Fungi in lignocellulose breakdown and biopulping. *Current Opinion in Biotechnology* 10:252-258.

Table 4. – Mushroom production on southern yellow pine chips, loblolly pine chips, and red oak sawdust.

Species	SC number	Substrate ^a	Total biological efficiency ^b (%)
<i>Pleurotus citrinopileatus</i>	17	P	94 (12)
		PB	113 (26)
<i>Pleurotus cornucopiae</i>	52	LB	188 (51)
<i>Pleurotus cystidiosus</i>	18	P	54 (7)
		PB	100 (6)
<i>Pleurotus euosmus</i>	20	P	109 (18)
		PB	137 (14)
<i>Pleurotus eryngii</i>	54	LB	131 (18)
<i>Pleurotus ostreatus</i>	21	P	90 (36)
		PB	138 (30)
<i>Pleurotus ostreatus</i>	23	P	132 (18)
		PB	177 (18)
<i>Pleurotus populinus</i>	24	P	82 (10)
		PB	83 (17)
<i>Pleurotus pulmonarius</i>	25	P	104 (15)
		PH	120 (13)
<i>Pleurotus pulmonarius</i>	36	P	116 (14)
		PB	159 (16)
		ROB	155 (26)
<i>Pleurotus sapidus</i>	28	P	120 (19)
		PB	142 (33)
<i>Pleurotus sapidus</i>	29	LB	154 (35)
<i>Pleurotus sajor-caju</i>		P	118 (14)
		PB	139 (22)

^a L = treated loblolly pine chips; P = treated southern yellow pine chips; RO = red oak sawdust; and B = supplemented with 20 percent wheat grain.

^b Percentage of biological efficiency = (fresh weight of harvested mushrooms/dry weight of substrate) × 100. Values in parentheses are standard deviations in percent

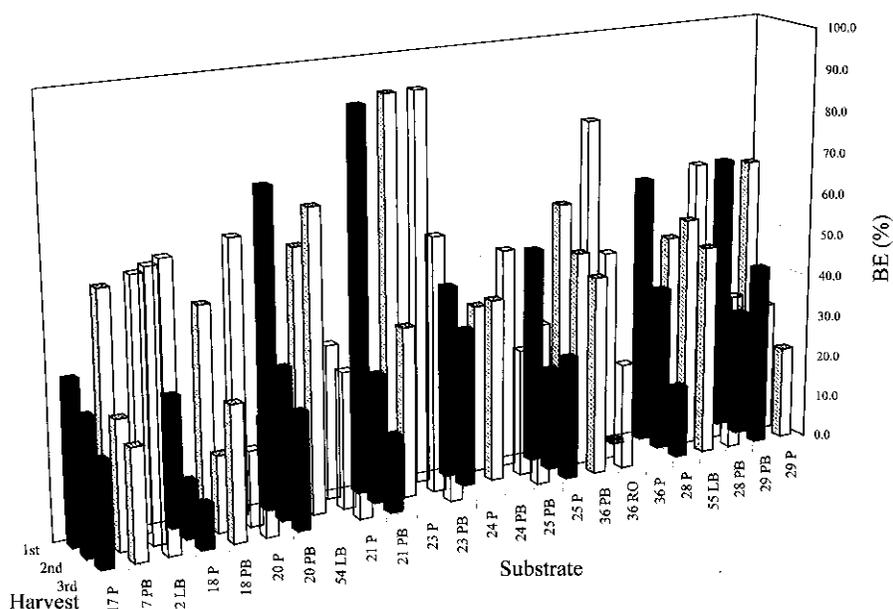


Figure 14. – Biological efficiency of various mushrooms on treated loblolly pine chips (L), treated southern yellow pine chips (P), and red oak sawdust (RO). B indicates supplemented with 20 percent wheat grain. See footnote to Table 4 for species identification.

Brush, T.S., R.L. Farrell, and C. Ho. 1994. Biodegradation of wood extractives from southern yellow pine by *Ophiostoma pili-ferum*. *Tappi* 77(1):155-159.

Chang, S.T. 1999. World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing. in China. *Inter. J. of Medical Mushrooms* 1:291-300.

_____ and J.A. Buswell. 1996. Mushroom nutraceuticals. *World J. of Microbiology & Biotechnology* 12:473-476.

Cheung, P.C.K. 1998. Plasma and hepatic cholesterol levels and fecal neutral sterol excretion are altered in hamsters fed straw mushroom diets. *J. of Nutrition* 128(9):1512-1516.

_____ and M.Y. Lee. 2000. Fractionation and characterization of mushroom dietary fiber (nonstarch polysaccharides) as potential nutraceuticals from sclerotia of *Pleurotus tuber-regium* (Fries) Singer. *J. of Agricultural and Food Chemistry* 48:3148-3151

Chiu, S.W., S.C. Law, M.L. Ching, K.W. Cheung, and M.J. Chen. 2000. Themes for mushroom exploitation in the 21st century: Sustainability, waste management, and conservation. *J. of Gen. Applied Microbiology* 46:269-282.

Chovol, V., L.O. Opletal, L. Jahodar, A.V. Patel, C.G. Dacke, and G. Blunden. 1997. Ergota-4,6,8,22-tetraen-3-one from the edible fungus *Pleurotus ostreatus* (oyster fungus). *Phytochemistry* 45:1669-1671.

Cochran, K.W. 1978. *Medical Effects, Edible Mushrooms*. Academic Press, Inc., New York. pp. 169-187.

Croan, S.C. 2000. Conversion of woodwaste into value-added products by edible and medicinal *Pleurotus* (Fr.) P. Karst. Species (*Agaricala* s.l., basidiomycetes). *Inter. J. of Medical Mushrooms* 2:73-80.

Dabbour, I.R. and H.R. Takruri. 2002. Protein quality of four types of edible mushrooms found in Jordan. *Plant Foods for Human Nutrition* 57:1-11.

Effland, M.J. 1977. Modified procedure to determine acid-insoluble lignin in wood and pulp. *Tappi* 60:143-144.

Eggen, T. 1999. Application of fungal substrate from commercial mushroom production. *Pleurotus ostreatus* for bioremediation of creosote contaminated soil. *Inter. J. of Biodeterioration and Biodegradation* 44: 117-126.

_____ and A. Majchercryk. 1998. Removal of polycyclic aromatic hydrocarbons (PAH) in contaminated soil by white-rot fungus *Pleurotus ostreatus*. *Inter. J. of Biodeterioration and Biodegradation* 41:111-117.

_____ and P. Sveum. 1999. Decontamination of aged creosote polluted soil: The influence of temperature, white-rot fungus *Pleurotus ostreatus* and pretreatment. *Inter. J. of Biodeterioration and Biodegradation* 43:125-133.

Gao, Y. and C. Breuil. 1995. Wood extractives as carbon sources for staining fungi in the sapwood of lodgepole pine and trembling aspen. *IRG/WP 95-10098*, Inter. Research Group on Wood Preservation, Stockholm, Sweden.

Gunde-Cimerman, N. 1999. Medicinal value of the genus *Pleurotus* (Fr.) P. Karst. (*Agaricales* s.l., Basidiomycetes). *Inter. J. of Medical Mushrooms* 1:69-80.

Table 5. – Determination of Klason and acid-soluble lignin in southern yellow pine and spent substrates.

Substrate	Klason lignin	Acid-soluble lignin
	----- (%) -----	
Southern yellow pine	31.1	1.0
<i>P. ostreatus</i> , SC23		
Spent substrate	12.11 to 16.14	0.54 to 0.72
Removed lignin	51.90 to 61.06	46 to 28

Hoadley, R.B. 1990. Identifying Wood: Accurate Results With Simple Tools. The Taunton Press, Inc., Newton, CT. 223 pp.

Ishisuka, T., Y. Yaoit, and M. Kikuchi, 1997. Sterol constituents from the fruit bodies of *Grifola frondosa* (Fr.) S.F. Gray, Chemical Pharmaceutical Bull. 45: 1756-1760.

Kawamura, Y., M. Manabe, and K. Kitta, 2000. Antitumor protein (AP) from a mushroom induced apoptosis to transformed human keratinocyte by controlling the status of pRb, c-MYC, cyclin E-cdk2, and p21^{WAF1} in the G1/S transition. *BioFactors* 12, 1-4 BIO381.

Kirk, T.K., R.T. Lamar, and J.A. Glaser. 1992. The potential of white-rot fungi in bioremediation. *In: Biotechnology and Environmental Science: Molecular Approaches*. Mongkolsuk, S. et al., eds. Plenum Press, New York.

Koch, P. 1972. Utilization of the Southern Pines. Vol. 1, Processing. Agricultural Handbook 420. USDA Forest Serv., Washington, DC.

Lamar, R.T., M.W. Davis, D.M. Dietrich, and J.A. Glaser, 1994 Treatment of a pentachlorophenol- and creosote-contaminated soil using the lignin-degrading fungus *Phanerochaete sordida*: A field demonstration. *Soil Biology and Biochemistry* 26:1603-1611.

Manzi, P., L. Gambelli, S. Marcani, V. Vivanti, and L. Pizzoferrato. 1999. Nutrients in edible mushrooms: An inter-species comparative study. *Food Chemistry* 65:477-482.

Marquez-Rocha, F.J., V.Z. Hernandez-Rodriguez, and R. Vazquez-Duhalt. 2000. Biodegradation of soil-adsorbed polycyclic aromatic hydrocarbons by white-rot fungus *Pleurotus ostreatus*. *Biotech. Letters* 22:469-472.

Martinez-Inigo, M.J., P. Immerzeel, A. Gutierrez, J. Carlos del Rio, and J. Sierra-Alvarez. 1999. Biodegradability of extractives in sapwood and heartwood from Scots pine by sapstain and white-rot fungi. *Holzforschung* 53:247-252.

Mattila, P. and L. Pizzoferrato. 2000. Beta-glucans in edible mushrooms. *Food Chemistry* 68:315-318.

_____, K. Konko, M. Eurola, J. Pihlava, J. Astola, L. Vahteristo, V. Hietaniemi, J. Kumpulainen, M. Valtonen, and V. Piironen V. 2001. Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *J. of Agricultural and Food Chemistry* 49:2343-2348,

Miller, O.K. 1969. A new species of *Pleurotus* with a coremioid imperfect stage. *Mycologia* 61:887-893.

Moyson, E. and H. Verachtert. 1991. Growth of higher fungi on wheat straw and their impact on the digestibility of the substrate. *Applied Microbiology and Biotech.* 36: 421-424.

Ogundana, S.K. and O. Okogbo. 1981. The nutritive value of some Nigerian edible mushrooms. *In: Mushroom Science XI. In: Proc. 11th Inter. Scientific Congress on Cultivation of Edible Fungi*. Australia. pp. 123-131.

Payne, C., S. Woodward, and J.A. Petty. 2001. Modification of the growth habit of the softwood disfiguring fungus *Ophiostoma piceae* by monoterpene vapors. *Forest Prod. J.* 51(7/8):89-92.

Raffa, K.F. and E.B. Smalley. 1995. Interaction of pre-attack and induced monoterpene concentrations in host conifer defense against bark beetle fungal complexes. *Oecologia* 102(3):285-292.

Rinker, D.L. 2002. Handling and using spent mushroom substrate around the world. *In: Mushroom Biology and Mushroom Products*. Sanchez et al., eds., Proc. of 4th Inter. Conference, Cuernavaca, Mexico, pp. 41-60.

Racheleau, M.J., B.B. Sithole, L.H. Allen, and Y. Noel. 1999. Fungal treatment of aspen for wood resin reduction: Effect on aged aspen wood chips at room temperature and at 5°C. *Holzforschung* 53(1):16-20,

_____, _____, _____, S. Iverson, R. Farrell, and Y. Noel. 1998 Fungal treatment of aspen for wood resin reduction: A laboratory evaluation. *J. of Pulp and Paper Sci.* 24(2):37-42.

Royse, D.J. 1997. Specialty mushrooms and their cultivation. *In: Horticultural Reviews*. J. Janick, ed, John Wiley and Sons, New York. 19:59-97.

Semple, K.T., N.U. Watts, and T.R. Fermor. 1998. Factors affecting the mineralization or (U-14C) benzene in spent mushroom substrate. *FEMS Microbiology Letters* 164:

She, Q.B., T.B. Ng, and W.K. Liu. 1998. A novel lectin with potent immunomodulatory activity isolated from both fruiting bodies and cultural mycelia of the edible mushroom *Volvvariella volvacea*. *Biochemical and Biophysical Research Communications* 247: 106-111

Sjostrom, E. 1981. *Wood Chemistry, Fundamentals and Applications*. The Academic Press, Inc., New York. 223 pp.

Stewart, D.P.C., K.C. Cameron, and I.S. Beare. 1998. Inorganic-N release from spent mushroom compost under laboratory and field conditions. *Soil Biology and Biochemistry* 30: 1689-1699,

TAPPI. 1997. Solvent extractives of wood and pulp. Test Method T 204 cm-97. TAPPI Press, Atlanta, GA.

_____. 1988. Test Method T 222om-88, TAPPI Press, Atlanta, GA.

Wasser, S.P. and A.L. Weis, 1999. Medicinal properties of substances occurring in higher basidiomycetes mushrooms: Current perspectives (review). *Inter. J. of Medical Mushrooms* 1(1):31-62,

Yang, H.H., M.J. Effland, and T.K. Kirk. 1980. Factors influencing fungal degradation of lignin in a representative lignocellulosic, thermomechanical pulp. *Biotechnology and Bioengineering* 22:65-77.

Zabel, R.A. and J.J. Morrell. 1992. *Wood Microbiology: Decay and Its Prevention*. Academic Press, New York.

Zhang, C.K., F. Gong, and D.S. Li. 1995. A note on the utilization of spent mushroom composts in animal feed. *Bioresource Tech.* 52: 89-91.

Zhang, J., G. Wang, H. Li, C. Zhuang, T. Minuno, H. Ito, C. Suzuki, H. Okamoto, and J. Li. 1994. Antitumor polysaccharides from a Chinese mushroom, Yuhuangmo, the fruiting body of *Pleurotus citrinopileatus* Bioscience Biotechnology Biochemistry 58:1195-1201,

Zhang, M., P.C.K. Cheung, and L. Zhang. 2001. Evaluation of mushroom dietary fiber (non-starch polysaccharides) from sclerotia of *Pleurotus tuber-regium* (Fries) Singer as potential anti-tumor agent. *J. of Agricultural and Food Chemistry* 49:5059-5062