### MUSHROOM CULTIVATION

### **Production of Mushrooms from Sawdust**

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This work was aimed at determining how effectively sawdust and other cellulosic wastes might serve as a medium for mushroom production. The commercial mushroom, Agaricus campestris, was cultivated on fortified, composted gum wood sawdust. The yields on a weight basis were considerably higher than commercial yields from horse manure compost. The wild, wood-rotting mushroom, *Pleurotus ostreatus*, was grown in 2 weeks on sterile sawdust medium fortified with oatmeal. On nonsterile, composted balsa wood sawdust, the unusually high yield of 1.21 pounds of fresh mushrooms per pound of dry sawdust was obtained. When fortified with soybean meal, the yield was 1.41 pounds. In all experiments, the yields on a per square foot basis were low, because of the low bulk density of the sawdust.

**F**UNGI belonging to the basidiomycetes, many of which produce fruit known as mushrooms, are unique in nature in being able to degrade a wide variety of natural polymeric substances. In the 18th century, in Europe, this ability was utilized by the cultivation, on horse manure, of *Agaricus campestris*, the common mushroom of commerce. In Japan, for several centuries, logs have been artificially infected with a wood-rotting fungus, *Cortinellus shiitake*, which produces a strongly flav red mushroom. In many countries, different species of wild mushrooms are gathered and marketed.

The substitution of machines for draft horses has considerably reduced this source of manure and racing stables are now the major suppliers. Synthetic manures have been developed, but as they utilize ingredients which are also employed in animal feeds, they generally cannot compete economically with manure (9, 13, 14, 16, 19).

Woody tissue, the world's most abundant natural waste product, is readily available in such forms as wood, sawdust, bark, bagasse, rice hulls, and straw. In the United States the wood industries produced manufacturing waste amounting to 53,000,000 tons, according to a 1944 report (20). The part of these manufacturing wastes not used for fuel amounted to 20,000,000 tons.

Many basidiomycetes are able to digest wood directly to satisfy their carbohydrate requirements ( $\hat{o}$ ). Some of these produce very tasty fruiting bodies (mushrooms or sporophores). To identify wood-rotting fungi, Badcock experimented with flask cultures of fortified sawdust and succeeded in obtaining sporophores of 81 species (2, 3). European reports (11, 12, 18) demonstrated that A. campestris can be produced successfully on composted sawdust. This paper enlarges on an earlier report ( $\hat{J}$ ).



Figure 1. The common, commercial mushrooms, Agaricus campestris, growing on composted sawdust

### **Experiments with Agaricus Campestris**

In the experiments with Agaricus campestris, the media were prepared by composting, according to standard practice with manures. Not being a wood-rotting fungus, A. campestris is unable to utilize wood directly, but only if it has been subjected to prior partial digestion by composting. Gum wood sawdust was used, because it was available locally and was readily composted. Cubical piles containing about 250 pounds each were prepared. The amounts indicated in Table I are the quantities of the compost ingredients contained in each of the trays used later for growing the mushrooms.

Three piles were assembled. The first was essentially sawdust fortified with mineral salts and organic nitrogen in the form of soybean meal. This pile did not heat up as rapidly as expected. Corncob meal was added to the pile to stimulate fermentation and produce rapid heating. This procedure might not have been necessary had the pile been larger and had it been given a longer time to compost. Because composted hay is a rich source of nutrient for A. campestris, a second pile was prepared with half hay and half sawdust. Nitrogen in the form of chicken manure, rather than soybean meal, was employed in this pile, because protein nitrogen was supplied by the hay. The third pile consisted of hay and corncob meal, a synthetic compost sometimes employed for mushroom growing. Corncob meal was used instead of cracked corncobs to reduce porosity and retain heat in the small pile.

The piles were turned and reformed on the third, sixth, and ninth days from the start of the experiment. On the twelfth day, the compost was put into trays and transferred to a small room heated to 130° F. for continued fermentation and some pasteurization. On the fourteenth day, the trays were removed to the high-humidity growing room and 24 hours later were inoculated with mushroom spawn. Each tray had an area of 2 square feet and was filled with compost to a depth of 4 inches. The 22nd day after spawning, the trays were covered (cased) with a 1-inch layer of soil. Mushrooms first appeared about a month and a half after spawning and continued to appear in some cases until the experiment was ended 3.5 months later (Figure 1). A temperature of 68° F. and a relative humidity of approximately 85% were maintained in the growing room throughout the experiment.

The yield data in Table I show that the sawdust produced about one third less mushrooms per tray than the corncob-hay compost. The sawdust-hay gave a very high yield in one tray, but a much lower yield in the duplicate tray. On the basis of the weight of ingredients employed, however, the vield of mushrooms was greater for the sawdust compost than for the corncob-hay, because the latter weighed almost twice as much per tray. On a weight basis the average yield from the sawdust-hay trays was considerably greater than the other composts, inasmuch as it had the least bulk density. The yield was greatest the first month of bearing and then progressively decreased. With the sawdust compost, about 85% of the mushrooms were produced in the first 2 months of picking, whereas the corncobhay compost yielded only about 65%of the total in the same period, but continued to bear well for several months following.

### **Experiments with Pleurotus Ostreatus**

Growth observations of the mycelia of the nine species of wild, wood-rotting mushrooms on different sawdust media are given in Table II. Pleurotus ostreatus, commonly known as the oyster mushroom, grew more vigorously on most of the media and produced fruit more readily than the other species (Figure 2). For these reasons. and because of its known edibility and growth at high temperatures, Pleurotus osireatus was selected for further experiments.

Table III gives the results of the production of P. ostreatus mushrooms on pine and gum wood sawdusts. Onegallon cans were partly filled with 120 grams of sawdust fortified with 6 grams of oatmeal and moistened with 250 grams of water. The sawdust medium was sterilized, in an autoclave, for 30 minutes at 15 pounds' pressure. Upon sterilization the cans were covered with polyethylene sheets to prevent contamination and to retain moisture. When the medium had cooled, it was inoculated with 21 grams of spawn per can. The cans were kept in a room maintained at 78° F. and 85% relative humidity. In 10 days to 2 weeks, fruiting bodies began to appear and the first flush (crop) was picked 15 to 17 days from the start of the experiment. After 47 days, the experiment was terminated. The yield from gum wood sawdust was twice that from pine.

The results of a similar experiment with rice hulls are found in Table IV. The mushroom mycelium grew well on the unfortified rice hulls, but the vield was only half of that obtained when the rice hulls were fortified with 5% oatmeal. A mixture of rice hulls and gum wood sawdust supplemented with oatmeal gave about the same yield as either the gum wood or rice hulls when similarly supplemented. Where the better yields were obtained, there were three flushes rather than two

### Table I. Production of A. campestris Mushrooms from Composted Gum Wood Sawdust

Ingredients	Grams						
Gum wood sawdust Corncob meal	1	1410 282		605 		1505	5
Hay (timothy)		141		605		1505	5
Urea		21		18.3		450	5.4
Potassium chloride		35.4 17.8		30.6 14.6		30	5
Calcium superphosphate Composting		/		5.8		1:	
Time, days Temperature,° F.		14 140–170		14 14017	0	1 152-	4 -162
Harvesting					- 20		
Time, spawning to casing, days	22 44	22 44	22 46	22 44	22 46	22 43	22 44
Duration of fruiting, days	116	121	144	98	150	148	150
No. of pickings 1st month	12	11	14	12	19	11	15
Wt. of crop 1st month, grams	806	802	778	520	1401	861	1029
Wt of crop 2nd month grams	452	336	278	147	567	382	ر 487
No. of pickings 3rd month	4	3	5	4	4	6	6
Wt. of crop 3rd month, grams	128	67	116	87	119	217	234
No. of pickings 4th month	4	5	5	1	8	6	10
Wt. of crop 4th month, grams	56	60	144	32	320	281	203
No. of pickings 5th month	0	0	4	0	6	7	6
Wt. of crop 5th month, grams	0	0	46	0	90	168	182
Total yield, grams Wt. fresh mushrooms/wt. dry in-	1442	1265	1362	786	2497	1909	2135
gredients Pounds fresh mushrooms/sq. t.	0.75	0.66 1.39	$0.71 \\ 1.50$	0.54 0.87	$1.71 \\ 2.75$	$\begin{array}{c} 0.53\\ 2.11 \end{array}$	0.59 2.35
				-			

### Table II. Growth of Mycelium of Wood-Rotting Mushroom Fungi on Sawdust Media

	Growth of Fungi (Scale 0-3)									
Medium	Cv	Po	Ц	Аb	Ps	Cł	Pf	Am	Hc	
А	1	1	1		_	+	+	_	+	
в	1	2	2	1	1	1	1	1	1	
С	2	2	1	1	1	1	1	1	1	
D	2	3	2	2	2	1	1	1	1	
E	2	3	2	1	2	1	1	1	1	
F	_	2	1	1	1	+	_		_	
G	2	34	2	3	3		3	_	2	
Н	+	1	1	+	—			+	-	
Ι	2	2	2	+	3	2	2	+	2	
J	1	1	2	_	+		-	_	1	
К	3	3ª	2	1	3	+	2	+	1	
<sup>a</sup> Fruit	ting occu	ırs.								

### Key to Table II

Abbreviations	Organisms	Abbrevia	tion <b>s</b>	Media
$\mathbf{C}\mathbf{v}$	Collybia velutipes	A	20 grai	ns pine sawdust + 40 ml
Po	Pleuorotus ostreatus		wate	r
Ll	Lentinus lepideus	В	20 gra	ms pine sawdust + 40
Ab	Agaricus blazei		ml, (	Czapek solution <sup>4</sup>
Ps	Polyporus sulphurous	C	20 gran	ns pine sawdust + 1 gram
Ct	Clitocybe tabescens		accel	$erator^a + 42 ml. water$
Pf	Polyporus frondosus	D	16 grai	ns pine sawdust $+$ 8 grams
Am	Armillaria mellea		oatm	eal $+$ 8 grams CSMA <sup><math>a</math></sup> $+$
Hc	Hydnum coralloides		64 n	nl. water
	- dilute valution of min	E	16 gran	ns pine sawdust $+8$ grams
Czapek sol	n., dilute solution of min-		oatg	rains + 8  grams CSMA
eral salts (17).	autoient minture of corp		+ 64	4 ml. water
Accelerator,	and similar motorials (2)	F F	16 grai	ms bagasse + 32 ml. water
meal, bone mea	lium containing bron and	G G	-20 gra	ams pine sawdust + 6
CSMA, mec	num containing brain and	i	gram	is oatmeal + 52 ml. water
grains used in	rearing mes, according to	[ Н	-20 gra	ms oak sawdust + 40 ml
Chemical Sp	beclatties Manufacturer's	_	wate	r
Assoc.		I	20 grai	ns gum sawdust + 40 ml
			wate	r
		J	20 grai	ms oak sawdust $+ 4$ grams
			oatn	neal $+$ 48 ml. water
		I K	20 gra	ms gum sawdust + 4 grams
		1	oatri	neal + 48 ml water

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Figure 2. Large specimens of *Pleurotus ostreatus*, the "oyster" mushroom, grown on sawdust

Top and underside views shown

This white mushroom grows open, has no button structure



Figure 3. Clumps of *Pleurotus* ostreatus growing on sterile sawdust fortified with oatmeal

Sawdust surface is covered with a layer of soil containing Vermiculite

(Tables III and IV). Table V represents an experiment employing fortified gum wood sawdust where five out of the seven replicates gave three flushes. The yields in this experiment were appreciably higher than in the other runs.

These runs with P. ostreatus differed from those described with A. campestris because sterile, uncomposted media were employed. In scaling up from the gallon cans to trays, there were some successful runs (Figure 3), but more often the medium became contaminated with molds to the detriment of the mushroom fungus and the run was destroyed. Four compost combinations were tested (Table VI) in special, insulated 5gallon cans built to permit aeration, but retain heat and moisture. Plain balsa sawdust was fortified only with mineral salts (see Table I for composition of salts), with soybean meal, with corncob meal, and with black muck from the Florida Everglades. In three of the four trays no attempt was made to case the beds; the mushrooms grew abun-

# Table III. Growth of Pleurotus ostreatus Mushrooms on Pine and Gum Wood Sawdusts Fortified with 5% Oatmeal

First Flush		Seco	Second Flush		d Flush		
Replicate	Days	Weight, grams	Days	Weight, grams	Days	Weight, grams	Yield, grams
1 2 3 4 5	15 15 15 17	16.5 12.0 15.0 13.0	31 27 19 31 32	$9.0 \\ 7.0 \\ 8.5 \\ 11.0 \\ 18.0$	47 47 	6.0 6.0  	31.5 25.0 23.5 24.0 18.0
			Wt. fres Gum	sh mushroom Sawdust	s/wt. dry	ingredients	Av. 24.4 0.18
1 2 3 4 5	15 15 15 15 15	36.5 33.0 30.0 31.5 34.5	32 31 32 32 32	12.518.010.514.011.0	44 44 44	9.0 6.0	49.0 60.0 40.5 51.5 45.5
			Wt. fre	sh mushroon	ns/wt. dry	ingredients	Av. 49.3 0.37

### Table IV. Growth of Pleurotus ostreatus Mushrooms on Rice Hulls

First Flus		t Flush	Seco	nd Flush			
eplicate	Time, days	Weight, grams	Time, days	Weight, grams	Yield, Grams		
		Rice	Hulls				
1	14	22.0	31	13.0	35.0		
2	14	19.0			19.0		
3	15	24.0	a secondary	Part Bar . C. TERMIN	24.0		
				A	v. 26.0		
		Wt. fresh	mushrooms/w	t. dry ingredients	0.20		
		Rice Hulls -	+ 5% Oatmea	al			
1	14	30.0	31	14.0	44.0		
2	19	29.5	35	31.5	61.0		
3	19	26.0	35	20.0	46.0		
				А	v. 50.0		
		Wt. fresh	mushrooms/v	vt. dry ingredients	0.38		
	Ric	e Hulls + Gum	Wood $+ 5\%$	Oatmeal			
1	14	35.0	35	11.0	46.0		
2	14	33.5	36	19.5	53.0		
3	14	23.5	29	16.0	39.5		
				А	v. 46.2		
		Wt. fresh	mushrooms/v	wt. dry ingredients	0.35		

## Table V. Yield of Pleurotus ostreatus Mushrooms Grown on Fortified Gum

First Flush		Second Flush		Thi			
Replicate	Time, days	Weight, grams	Time, days	Weight, grams	Time, days	Weight, grams	Yield, Grams
. 1	18	19	37	37			54
2	18	37.5	37	7	50	23.5	68
3	18	36.5	37	10.5	55	18.5 *	65.5
4	18	34	38	14	55	21	69
5	19	26.5	45	23	63	15	64.5
6	19	33	40	34	63	9.5	76.5
7	21	31.5	46	19		·	50.5
Total		218		144.5		87.5	448
						A	Av. 64

dantly without casing, the top layer of the sawdust substituting for the casing soil (Figure 4).

There was insufficient compost to make replicate trays. However, the plain balsa, and especially the balsa with soybean meal, produced very highweight yields. The yield of mushrooms per unit area was low because of the small weight of balsa compost per square foot, 4 inches deep. However, the high efficiency of its conversion to mushroom tissue is demonstrated in the case of the balsa with soybean, where the residue was weighed and the weight loss computed.

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#### Discussion

The data demonstrate that good yields of mushrooms can be produced from sawdust as compared with the yields obtained in the commercial production of Agaricus campestris from horse manure compost. Three pounds of mushrooms per square foot of bed space in a commercial plant is considered a very high yield. In reported experiments (15), Sinden and Hauser give yields of 400 pounds of mushrooms from a ton of manure containing 50% moisture. This is equivalent to 0.4 pounds of mushrooms per pound of dry manure. Probably the highest yields found in the literature are those of Lambert and Ayers (10) whose best trays in controlled, smallscale experiments gave better than 6 pounds of mushrooms per square foot or, on a weight basis, 0.9 pound per pound of dry manure. Table I shows that sawdust fortified with corncob and soybean meal gave an average of 0.71 pound of A. campestris per pound of sawdust compost and an average of 1.13 pounds of mushrooms per pound of sawdust-hay compost.

With *P. ostreatus*, balsa wood fortified only with inorganic salts gave a yield equivalent to 1.21 pounds of mushrooms per pound of sawdust, whereas balsa fortified with soybean meal and mineral salts gave 1.41 pounds per pound. Recognizing that these are merely small scale, exploratory experiments requiring confirmation, they nevertheless show the favorable possibilities for the use of sawdust in mushroom production.

In experiments with sawdust, Rempe (12) found hardwood, such as beech, preferable to conifers, such as pine and spruce. Results of isolated experiments in this laboratory with oak and magnolia sawdusts show that mushroom production is not limited to the wood species already mentioned.

The yields from sterile sawdust were comparable with those obtained in commercial practice. The highest yield (76.5 grams equivalent to 0.58 pound of mushrooms per pound of sawdust, Table V), however, was considerably lower than those obtained with composted sawdust (Tables I and VI). A possible explanation for the superior results with the composted sawdust is the partial degradation of wood fibers which occurs during composting and makes the sawdust more easily digested by the mushroom. While composting permits higher yields and makes the sawdust resistant to competitive microorganisms, compositing conditions are reproduced so rarely that two composts are practically never alike. The changes which occur during composting are extremely complex and therefore difficult to control. The small laboratory composting can, developed and employed in these experiments, may help to provide more



Figure 4. Pleurotus ostreatus growing in a tray containing balsa wood sawdust

Bal	sa Wood S	atus Mushra awdust	ooms from	Composted
	1	2	3	4
Ingredients, grams				
Balsa wood sawdust	250	270	125	190
Soybean meal		30		
Corncob meal			125	
Muck				95
Mineral salts	14.6	17.3	14.6	16.4
Rice hulls inoculum	20.0	20.0	20.0	20.0
Composting				
Time, days	17	17	17	17
Temperature, ° F.	120-174	108-170	116-175	120-184
Harvesting				
Time, spawning to casing, days	Not cased	Not cased	Not cased	32
Time, spawning to first flush, days	s 30	34	40	35
Wt. of first flush, grams	127	228	70	65
Time, first to second flush, days	10	15	24	36
Wt. of second flush, grams	96	97	41	60
Time, second to third flush, days	19	15		14
Wt. of third flush, grams	123	110		9
Time, third to fourth flush, days		13		
Wt. of fourth flush, grams		40		
Yield Data				
Total vield, grams	346	475	110	134
Wt. of fresh mushrooms/wt. dry				
ingredients	1.21	1.41	0.39	0.42
Pounds fresh mushrooms/sq. ft.	0.57	0.78	0.18	0.22
Dry wt. of compost residue, grams		215		
Wt. lost, grams		122	11 1 2 3 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	a to the set
Efficiency, <sup>a</sup> %		39	dia.d.	a sainterration
<sup>a</sup> Dry wt. of product based on dry	wt. lost.			

uniform composts which will give reproducible results.

Although the yields from sawdust on a weight basis appear most encouraging a practical grower is more impressed with the amount of mushrooms obtained from a bed or a house. Because of the light weight of sawdust, as compared with that of manure, and of the shallow trays employed, the yields on an area basis were low. Rempe's work indicates, however, that deeper bads will permit higher per area yields. He obtained 2.5 pounds per square foot with 8-inchdeep beds (12). As noted in Table VI, production of mushrooms from the sawdust was fairly rapid, thus making possible frequent turnover and multiple utilization of bed space during the year.

Significance. From an economic standpoint, sawdust has certain advantages over manure. It is available in more localities and easier to handle, mix, and turn. It has been estimated (12) that it will permit a saving of at least 50% on transportation and labor costs for compost materials. If it costs 22 cents to produce a pound of mushrooms, about 10 cents of this is made up of transportation and labor costs, 5 cents is overhead, and 7 cents represents the cost of manure. Thus,

sawdust offers a potential saving of 23%over manure, if material costs, overhead, and yields are the same. Where the sawdust would be available at no cost, the saving would be 55%.

As shown in the photographs, P. ostreatus is very different from A. campestris. Pleurotus does not grow in the familiar button form characteristic of young Agaricus. It does not have the thick "meaty" tissue of the Agaricus buttens. In shape and growth it resembles more closely the Japanese shiitake mushroom. Like the latter, it has a tough, inedible stem. The flavor, however, is more like that of A. campestris. Pleurotus would appear to have its greatest value as a flavoring ingredient for soups, sauces, and gravies rather than as a fresh mushroom for table use

Mushrooms are a source of essential amino acids and of the B vitamins (1, 4, 7, 8). They can be "factory-produced" the year around from waste products which originate from non-

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depleting sources and may be visualized as an important food source for the future. Full realization of the market potential, however, awaits more efficient production and lower cost.

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# **Morphine Extraction from Domestically Grown Opium Poppy**

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To meet the needs of our nation in an emergency, a process was developed to recover crude morphine liquor from mature, domestically grown, opium poppy plants. This liquor can be used by licensed refiners as a substitute for opium to produce medicinal morphine, if U.S. opium imports should be severely limited or cut off. Over 95% of the morphine was extracted from meal with water-saturated isobutyl alcohol in a continuous countercurrent extractor. In preparing the meal, stems and seed were separated from capsular material by a four-step size-reduction procedure with a 6% loss of morphine Morphine concentration in the meal was increased from 0.5 to 0.7%. An aqueous ammonia treatment of the meal prior to extraction liberated the alkaloids from their naturally occurring salts. Poppy meal or straw keeps in dry storage without any morphine loss.

MPORTED OPIUM is the source of medicinal morphine in the United States. To ensure adequate supplies of morphine in a national emergency, work was undertaken to develop a method for recovering crule morphine liquor from the mature capsules of opium poppy plants grown in the United States.

Opium is the dried juice or latex of unripe capsules of a particular poppy species, Papaver somniferum. Although the opium poppy has been grown for thousands of years, opium is still obtained through much tedious manual work, both in cultivating the plant and in collecting the latex (2). Some 22

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alkalcids are found in opium, but only three-morphine, codeine, and papaverine-are of commercial importance. The amount of morphine varies over wide limits, but in a good grade of opium the content averages 10%, and the codeine and papaverine are present in much smaller amounts.

Interest in cheaper production methods led to the development of more modern processes for recovering opium alkaloids directly from the ripened poppy plant, and some of these processes were put into commercial operation on a very limited scale in Germany, Switzerland, and Hungary in the 1930's (5, 6, 9). From information given in the literature for these and other processes (1), each appears to have disadvantages.

Morphine Analyses. All of the samples were analyzed by the Matchett and Levine method (11, 16), but for a rapid estimate, a modification of Hanes' ferricyanide micromethod for determining reducing sugars (4) was employed.

Most analysts believe the Matchett and Levine method is insufficiently specific for morphine (16); therefore, a commercial morphine refiner checked duplicate samples from one of the extraction tests. The extract liquor was examined for its morphine content by a modified U. S. Pharmacopoeia method and a special method was used for the poppy meal (8).

Preparation of Poppy Meal. The opium poppy was grown under irrigation by the Field Crops Research Branch of the U. S. Department of