HortScience 19(5): 684–686. 1984.

Using Basidiospores of the Oyster Mushroom to Prepare Grain Spawn for Mushroom Cultivation

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Additional index words. sporeprint, spore suspension, serial dilution, spore germination, spawn disk, *Pleurotus ostreatus*

Abstract. **Serial dilution of basidiospores of a spore print to prepare grain spawn of the Oyster mushroom is described. The method, which has a contamination rate of about 5%, requires sterilization of a grain medium and the use of some simple "clean" procedures. The method is especially advantageous for small scale and part-time cultivation of mushrooms on logs. *Pleurotus ostreatus* Kumm. mycelium grew rapidly into the wood of freshly cut logs inoculated with disks of grain spawn prepared using spore inoculum. Unlike pure-culture spawn disks, however, disks prepared from spore-inoculated grain did not become completely tightly bonded to the wood surface.**

During the period 1925–1970, essentially only one mushroom species, *Agaricus brunnescens* Peck [=also *A. bisporus* (Lange) sing.], was cultivated in the United States. Almost all of this cultivation was on a full-time commercial basis. The recent trend toward diversification of cultivated mushrooms (4, 9), however, has promoted widespread interest in small scale and part-time mushroom cultivation. Growing mushrooms on logs, e.g., Shiitake, *Lentinus edodes* (Berk.) Sing. (8) or Oyster mushrooms, *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. (7) is especially well suited for supplementary farm production or for the home garden.

To cultivate mushrooms, a suitable substrate is inoculated with spawn, a planting material which consists of a nutritive substrate (e.g., cereal grain or wood) colonized by a pure culture of the mushroom mycelium (6). Because of the special facilities, cultures, and aseptic techniques required to make spawn, most mushroom growers purchase their planting material (spawn) from spawnmakers. Usually, spawn of *Agaricus spp.* is delivered to the mushroom grower within several hours of the time it is needed for spawning. Because of the highly perishable nature of grain spawn, spawnmakers use refrigerated trucks to deliver spawn to distant mushroom farms.

It is likely that the development of small scale or part-time mushroom cultivation will be affected greatly by the availability of spawn. At present, spawn of recently introduced species of mushrooms, e.g., *L. edodes*, *Pleurotus sp.*, and *Flammulina velutipes* (Fr.) Sing. is not available locally in many areas of the United States. Shipping grain spawn by mail, however, is expensive and unreliable.

Although mushroom spores play a crucial role in nature, they are not used, except in truffle cultivation (1), as a planting material. Duggar (2), (Fig. 1) who developed the pure culture of mushroom mycelium used to make spawn, "believed that in time a method of spawn production from spores without pure-culture precautions will be developed." Contrary to Duggar's expectation, however, there is no evidence that such a method was developed. Although the method would be of little value in the highly developed technology now used for making spawn of *Agaricus spp.*, it may be advantageous for small scale and part-time cultivation of other mushroom species.

The purpose of this report is to describe a method that uses serial dilution of basidiospores to prepare grain spawn of the Oyster mushroom. Although the basic technique of serial dilution has long been used to isolate single spore cultures (3, 10), we are not aware of its direct use for the production of planting material for mushroom cultivation. Although not pure culture, our method requires sterilization of medium and the use of some simple "clean" procedures.

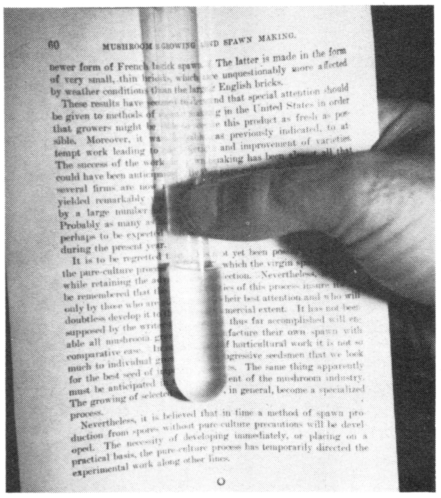

Fig. 1. Method for visual estimation of the density of a spore suspension. The text shown was copied from p. 60 of Duggar's 1905 bulletin (2) on mushroom growing and mushroom spawn making.

Collection of basidiospores. P. ostreatus sporophores were obtained from logs inoculated with Beltsville culture 467 (ATCC 52927). *P. ostreatus*, which occurs commonly throughout the United States, can be collected from nature (Fig. 2). Depending on their condition, Oyster mushrooms bought in food stores also may be used. Young, fresh, firm, and clean mushrooms that had not been rained on were prepared for collection of basidiospores within 2 hr after being picked. After removing the stipe, a mushroom cap was placed gill surface down on a double layer of 30 × 30 cm pieces of fresh wax paper and covered with wax paper to prevent drying. Depending on the fruit body, a dense deposit of white basidiospores (spore print) was obtained within 1–4 hr (Fig. 3). The mushroom cap then was placed on another double layer of fresh wax paper to continue spore collection. Handling only the edges of

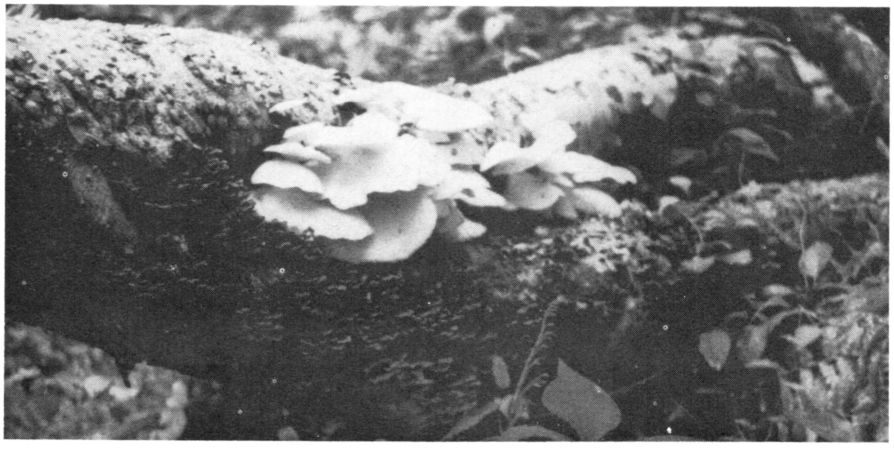

Fig. 2. Oyster mushrooms on forest log.

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Table 1. Frequency of contamination in spore suspension ssII and aliquots of spore suspension ssIII prepared using spore prints of *Pleurotus ostreatus*.

Spore print (month sporophore picked)	Months spore print stored at 4°C	Avg no. of contaminants/ 10^6 basidiospores (min – max no. contaminants)[z]	No. *P. ostreatus* spores in ssIII aliquots	No. contaminated aliquots/40 ssIII aliquots
A (Sept. 16th)	11	2.1(0-12)	10^2	0
			10^3	0
B (Dec.)	8	0.4(0 – 3)	10^2	0
C (Nov.)	9	0.8(0-6)	10^2	0
			10^3	0
D (Sept. 17th)	11	0.2(0 – 3)	10^2	0
			10^3	0
E (Aug.)	13	0.5(0-3)	10^2	0
			10^3	0
F (June)	14	0.3(0 – 3)	10^3	5
G (May)	15	0.6(0 – 6)	10^3	0

[z]Average number of contaminants in each of 20 aliquots of the same spore suspension ssII (range of number of contaminants in each of 20 aliquots of the same spore suspension ssII).

the paper, the wax paper bearing the spore print was carefully folded and the resultant envelope containing the spore print sealed in a 30 × 30 cm polyethylene plastic bag and stored in a refrigerator. Usually, 6–12 spore prints were obtained from a single fruit body.

Preparation of spawn medium. Substrate ingredients (175 g rye grain, 20 g mixed wood shavings and sawdust, 2 g $CaCO_3$, and 225 ml tap H_2O) (7) were placed in a quart-size glass canning jar. The jar opening was covered with a 2.5 cm-thick filter disk of fiber glass (11 cm diameter) securely held in place by the jar lid (Fig. 4, 5). In addition, a cotton-stoppered test tube (18 id × 150 mm) containing 5 ml of tap water and a glass jar with a fiber glass filter containing 500 ml of water were prepared. Jars containing substrate were sterilized in a steam sterilizer by "precooking" at 100°C for 90 min, followed immediately at 121° for 90 min at 1 Kg/cm. The test tube and jar containing water were sterilized for 30 min at 121°. To prevent clumping of the grain after sterilization, the jars of hot substrate were shaken cautiously but thoroughly at several different times during cooling.

Preparation of spore suspensions. To avoid gross contamination, preparation of spore suspensions and inoculation of grain medium were done on a table surface covered by a fresh sheet of paper in a room with little or no air movement. Care was taken to wash the hands and not touch any surface used in preparing spore suspensions or inoculating grain. Disposable sterile 2.5 ml hypodermic syringes were used to add water and to inoculate grain by injecting an aliquot of spore suspension through the fiber glass filter. To insure accuracy, all spore suspensions were agitated continuously and gently as aliquots were taken. The concentration of spores in spore suspensions was determined by using a hemicytometer and a microscope.

A sharp-pointed pair of clean scissors was used to cut out 1–2 cm^2 of spore print. Holding the small piece of wax paper with the tips of the scissors, the cotton plug was removed from the test tube and the spore covered paper dropped into the sterile water. The test tube was carefully shaken to obtain a dense white spore suspension (ssI). Using a hypodermic syringe, sterile water was added to ssI to obtain a concentration of ca. 6.0 × 10^6 spores/ml (ssII). The hypodermic syringe then was used to inject 0.3 ml of ssII into the jar containing 500 ml of sterile water to obtain a concentration of ca. 4 × 10^3 spores/ml (ssIII) (Fig. 5).

Preparation of spore suspensions was facilitated by using a simple method for estimating spore concentration. To prepare ssI, just enough spore print was added to water so that it was not possible to read letters or words when looking through the thoroughly mixed spore suspension when the test tube was held tightly against a printed page (Fig. 1). SsII was obtained by carefully diluting ssI to the point that letters and words could just be recognized when looking through the spore suspension and slightly rolling the test tube against the paper at the same time. In a test with several people (n = 5), ssII prepared by this method was determined to contain an average of 6.6 × 10^6 (n = 22; SD ± 1.4 × 10^6) spores/ml.

Inoculation of grain medium with basidiospores and colonization of the substrate. A new hypodermic syringe was used to inoculate jars of grain medium each with 0.2 ml of ssIII, (ca. 800 basidiospores of the Oyster mushroom). This inoculum was injected through the fiber glass near the periphery of the filter so that all of the ssIII aliquot was deposited on a small area of substrate about 1 cm from the glass container. (Although there are 97 chances out of 100 that the mycelium resulting from the germination of a random sample of only 7 *P. ostreatus* basidiospores will form a dikaryotic culture, i.e., a culture capable of fruiting, 800 basidiospores were used to insure rapid complete domination of the grain substrate by *P. ostreatus* mycelium.) Without shaking or disturbing the grain, each jar of inoculated grain was sealed in a 30 × 30 cm polyethylene plastic bag and incubated at 24°C for 9 to 12 days.

Germination (+90%) of *P. ostreatus* basidiospores and initiation of mycelial growth usually occurred 3 to 4 days after inoculation. When 1 to 6 cm diameter circles of white mycelia were visible on the grain substrate (8 to 12 days), the plastic bag was discarded and the substrate thoroughly mixed by carefully shaking the jar. The culture was reincubated and thoroughly mixed at 3-day intervals during the following 9 days. After the grain substrate was completely colonized, 17 to 21 days (Fig. 6), the jar was shaken to break up all substrate clumps and the substrate used to prepare spawn disks for inoculation of logs to produce mushrooms (7).

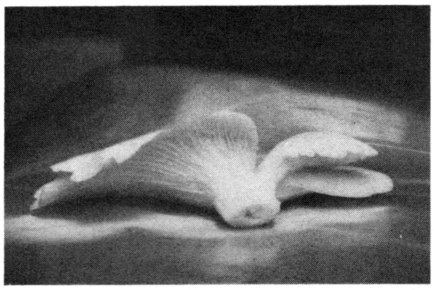

Fig. 3. Collecting basidiospores from *P. ostreatus* sporophore. Note the white spore deposit (spore print) on the wax paper (XI).

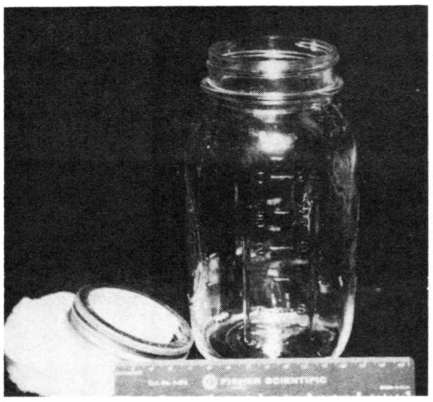

Fig. 4. Parts of spawn container: glass jar, jar lid, and fiber glass filter disk.

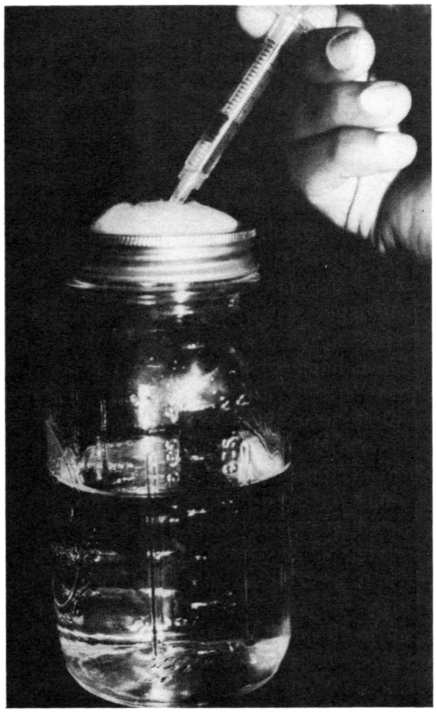

Fig. 5. The use of a hypodermic syringe to inject an aliquot of basidiospore suspension ssII into sterile water.

Presence of other microorganisms in P. ostreatus spore suspensions and aliquots of spore suspensions. Results of tests using aseptic technique and Potato Dextrose Agar (Difco) to determine the frequency of intrinsic contamination in spore suspensions indicated that contaminants were mostly fungi. Bacteriostatic effects of growing *Pleurotus* mycelium (5), however, may have inhibited development of visible colonies of bacteria. Results of these tests indicated that there was approximately 1 contaminant per 10^6 *P. ostreatus* spores in spore suspension ssII (Table 1). Apparently, neither the month that a sporophore was picked nor the number of months (8 to 15) that a spore print was stored had any marked effect on the frequency of occurrence of contaminants in ssII. Eger (3) reported, however, that after 4 years storage at 2° to 4°C the percentage of germination of *P. ostreatus* basidiospores was less than 25%. Results of testing aliquots of spore suspension ssIII illustrated the advantage of serial dilution: only 5 of 240 aliquots each containing 10^3 *P. ostreatus* spores and none of 200 aliquots each containing 10^2 spores were contaminated. Thus, approximately 2% of jars containing inoculated substrate would be contaminated. Because of the possibility of introducing additional contaminants during the preparation of spore suspensions and inoculation of jars, however, a realistic rate of expected contamination would be 5%. In one test, one of 30 jars of inoculated grain became contaminated.

Using spawn prepared with basidiospores. Pleurotus ostreatus mycelium grew rapidly into the wood of freshly cut logs inoculated with disks of grain spawn prepared using spore inoculum. Unlike pure-culture spawn disks (7), however, these disks were not completely tightly bonded to the wood surface 10 days after inoculation. This apparent decreased vigor of mycelial growth may be a result of the growth of bacterial contaminants in spawn prepared using spores. To avoid the consequences of possible subsequent invasion by other microorganisms, such spawn disks probably should be removed from logs 2 to 4 weeks after inoculating the logs. As an added precaution, these spawn disks should be used to inoculate logs before insects become active in the spring.

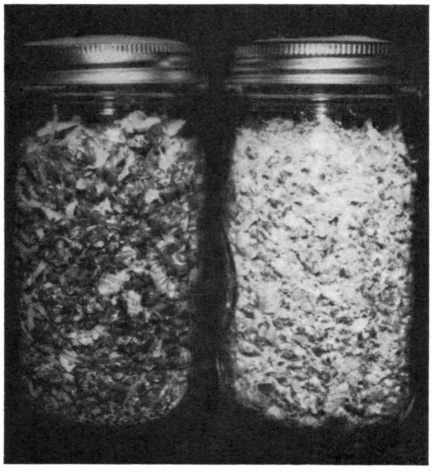

Fig. 6. L. uninoculated grain spawn, R. growth of spawn mycelium in inoculated medium after 17-days of incubation at 24°C.

Oyster mushrooms were produced on composted straw that had been inoculated with disk grain spawn prepared using *P. ostreatus* basidiospores. The spore-spawn method may be easily adapted. The substrate can be modified to provide *P. ostreatus* wood plug inoculum like that used in Shiitake mushroom cultivation (8). It should be possible, also, to use the method to prepare spawn of any mushroom species whose basidiospores (or ascospores) germinate on a grain-wood substrate as rapidly and completely as those of *P. ostreatus* and whose mycelium grows on the grain-wood substrate.

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