The purpose of this fact sheet is to provide basic information on the process of seeding substrate and the management of the vegetative stage of growing the commercial mushroom, *Agaricus bisporus*. The process before this stage, substrate preparation and the processes after, casing and production, are discussed in other publications.

A mushroom is the fruiting structure of the fungus and consists of a cap and stem. The mycelium is the fine "root" system that grows in the composted substrate adsorbing nutrients and water. After fruiting is initiated and the mushroom matures, the cap opens and gills are exposed. These gills produce spores in huge quantities; for example, a three-inch mushroom produces 40 million spores per hour. In nature, spores germinate and grow very poorly; therefore, another method to seed the mushroom substrate is required. The mushroom mycelium (threadlike filaments that become interwoven) is propagated on a base of steam sterilized cereal grain (usually rye or millet). This cereal grain/mycelium mixture is called spawn. Most spawn is made with mycelium from a stored culture, rather than mycelium whose parent was a spore. This is because each spore is likely to yield a new strain and its performance would be unpredictable. Preparing spawn is highly technical task that is not practical for most mushroom growers to perform; therefore, it is mostly produced by specialized companies that supply growers with pure culture spawn. However, in some cases, it may be necessary for the growers to produce their own spawn. In such cases, access to cultures (cultivars) with proven productivity is a key component of a successful enterprise.

History

Spawn, spawning and spawn growth begin with a little fascinating history. Until the 20th century, mushroom growers used mill-track spawn to seed their substrate. Mill-track spawn was obtained from under the horses driven around a pole to provide power to the flour mills. Spawn from this source had uncertain varietal characteristics and was not free from pest and other competitors. Later "Flake Spawn" was developed. Flake spawn was the mushroom tissue or mycelium transferred to special substrate manure piles. When the spawn had thoroughly grown through this "Manure Spawn" was broken up and spread onto substrate in the house. England was the first to develop this "Manure Spawn" and when the Lambert brothers immigrated from Belgium to Minnesota, they brought the process to America. One brother, L. F. Lambert moved to Chester County Pennsylvania and began producing flat bricks of moist horse and cow manure that were stacked together with bits of mushroom mycelium.

Manure spawn was used worldwide until the 1930's, when a scientific breakthrough occurred in France and later in the United States. Researchers learned how to germinate spores on sterilized media. This procedure, which we all take for granted, allowed pure cultures to be maintained and then eventually used to make "Pure Culture" manure spawn. This spawn was usually made in a bottle that was stoppered with wool plug. The grower had to break the bottle to remove spawn for use. A practice that was probably fun for the kids, but messy and probably unsafe.

The next step in spawn history was "Tobacco Spawn", shredded dry stems of tobacco from Lancaster County, Pennsylvania. Tobacco spawn was more consistent than manure spawn, however the process of using reusable, screw top bottles was labor intensive and tedious. Tobacco spawn was used for about 10 years.

In 1930, Dr. James Sinden was assigned to the mushroom research project at Penn State. He needed a more consistent method of inoculating substrate for his research. He developed a method using sterilized grain inoculated with pure cultures of the mushroom mycelium. At first spawn, makers resisted its adoption, but by WWII grain spawn prevailed in the US. After 1950, it was introduced into Europe along with the process of thorough mixing of spawn into substrate, which was developed by Ms. Hauser and Dr. Sinden. Until 1926, cream-colored mushrooms were the only strains grown commercially. It was Chester County grower, named Downing that found a cluster of white mushrooms and realized its significance. He called L. F. Lambert from whom he had bought the spawn, who then made spawn from tissue cultures that grew only white mushrooms, hence the birth of the white strains.

Culture Maintenance

Maintenance of pure mycelial cultures is a necessity fo ultimate spawn preparation and spawn production. Once a culture is obtained, it is advisable to take note of the appearance of the mycelium, to observe the normal growth patterns of the specific lines. These observations are important to do, so that one will be aware of even the subtlest change that may lead to deleterious effects.

A desirable mycelial culture is one that is pure, free of contaminants and of sectoring of other abnormalities. Contaminants include other fungi, bacteria, or insects growing on or infesting the culture media along with the desired mycelial culture. When a culture is first obtained, by whatever means, it should be transferred several times to fresh media, to observe for any from of contamination.

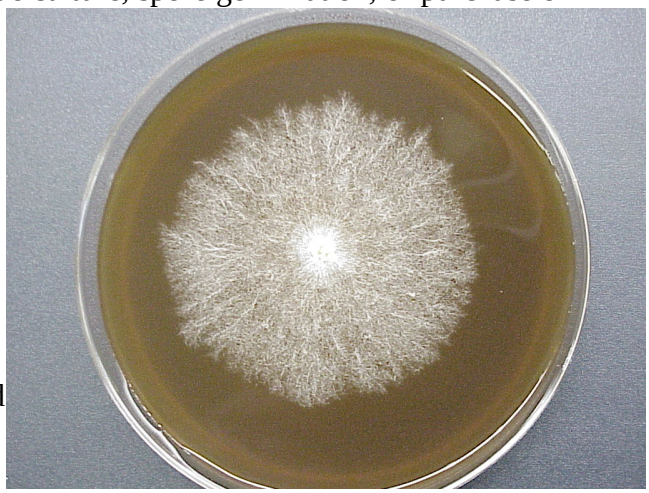
Sectoring is any type of mycelial growth that differs in appearance, growth rate, color, or otherwise from the typical appearance of a given strain. Sectoring is often observed as a more rapidly growing area near the leading edge of growth, exhibiting a different growth habit from the rest of the culture. Other abnormalities that might appear in a culture are fluffy, aerial mycelium, thick or rubbery textures, of color changes such as browning or darkening of the mycelium. Sectors of other change in vegetative growth could affect the productivity of the culture. Therefore, it is very important to recognize and avoid propagation of abnormal mycelium to agar and further spawn production.

Cryogenic Storage

Storage of cultures in liquid nitrogen (LN_2) is superior but expensive. It is the best long term culture storage method because mycelial growth is completely suspended at -196C. This method maintains a very stable mother culture for many years. Only the highest quality mycelial cultures are selected for long term storage -- a culture's qualities will not improve with storage. Unfortunately it is also the most expensive. Initial equipment costs are high and liquid nitrogen is a continual monthly expense. The liquid nitrogen storage unit requires constant monitoring of the liquid nitrogen level in the chamber. The basic method is to fill a cryotube (special plastic tube designed for use in LN_2) with a cryoprotectant and then to submerge a few mycelial plugs or spawn grains into the liquid. Then the filled cryotube is placed in a divided box or cane, then into rack and finally housed in the cryostorage unit. The purpose of the cryoprotectant it to protect the mushroom cells from damage during the freezing and thawing cycles.

Obtaining A Culture

Methods of obtaining a culture are: tissue culture, spore germination, or purchase of culture from a culture collection.

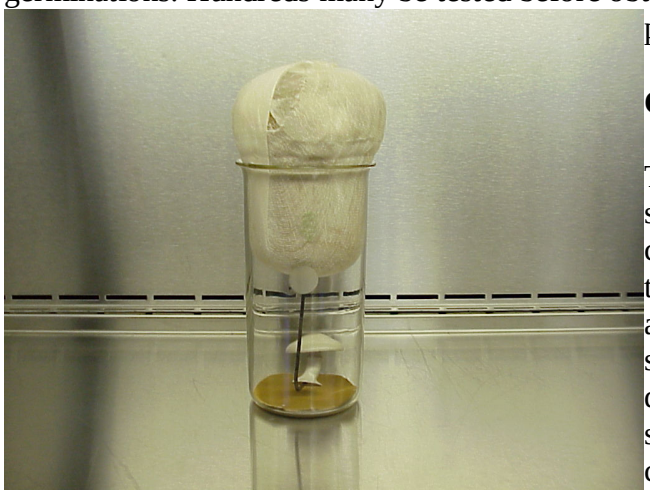


Tissue Cultures

Tissue cultures are the simplest way to obtain a mycelial culture. A tissue culture is essentially a clone of a mushroom. *Clone* is defined as an identical duplicate of an organism. The basic procedure is to sterilely remove a piece of the mushroom cap or stem, and place it on an agar plate. After a week to ten days, mycelium grows from the tissue and colonizes the agar. Great care should be taken to select a fruiting body of the highest quality, size, color, shape or any highly desired characteristic.

Spore Cultures

Mushroom spores are found on basidia lining the gills of the cap. To collect the spore, the cap is hung over sterile filter paper in a spore collecting chamber. Spore cultures differ genetically from tissue cultures. Cultures originating from spore germination are unpredictable. Spores germinate creating many different genetic combinations that are desirable when attempting to develop new strains, but undesirable when trying to maintain a specific strain. There is no way to predict if a culture's fruiting potential is similar to its parent other that to test many different lines obtained from spore germinations. Hundreds many be tested before obtaining a culture with good production potential.



Culture Validation Cropping Trials

There is no in-vitro test to determine a stock culture's validity. A series of cropping trials must be conducted on the mycelial stock culture to determine a culture line's value. Mushroom yield, size, color, cap shape and any other desired quality or growth factors are selected and then compared for each culture line.

Sub Culturing (agar to agar transfer)

Once a culture is obtained by whatever means, it needs to be maintained to preserve its viability. Agar to agar transfer (sub culturing) it the most popular culture maintenance method. Vegetative propagation of a mushroom culture is uncomplicated, but labor intensive. A small amount of mycelium from a mother culture is used to innoculate multiple fresh agar slants (these become the daughter cultures). The daughter cultures, after incubation at room temperature ~74F for 2 weeks. Are visibly screened for traits expressed in the mother culture. Then, one elected culture becomes the "new" mother culture. The new mother culture is stored in a refrigerator incubator at ~5C. After two months this culture is taken from refrigerator incubation and further transferred to freshly prepared agar slants repeating the cycle.

Spawn Production

The process of making spawn remains much the same as what Dr. Sinden first developed. The grain is mixed with a little calcium carbonate, then cooked, sterilized and the cooled. Small pieces of pure culture mycelium are placed in small batches of the grain. Once the small batch is fully colonized, it is used to further inoculate several larger batches of grain. This multiplying of the inoculated grain continues until the commercial size containers, usually a plastic bag with a breathable filter patch, are inoculated. During the colonization of each batch the containers are shaken every few days to further distribute the mycelium around. Temperatures are maintained where the mycelium is growing in a range of 75-76F . Uniformity of the air circulating around the bags is important to insure

that all containers are keep at the desired temperature range. The mycelium is sensitive and its fruiting mechanism can be easily damaged at high temperatures.

Spawning

Before the spawning operation, attempts may be made to improve the substrate moisture. Sometimes water is applied or an pesticide application may be made. Farms with a historical problem of mummy disease may avoid water applications at spawning, because the free water improves conditions for the bacteria to reproduce and spread, thereby increasing the incidence of this disease. On bed farms spawn and supplement is broadcast over the surface of the substrate. Uniformity of the distribution is critical to achieve an even distribution and more even spawn growth and temperatures. On tray or bulk farms, spawn is usually metered into the substrate during the mixing operation. Some growers will bring the spawn brought to room temperature before mixing it into the substrate. Gypsum (1-2% of substrate dry wt.) can be added if substrate that is wet, little greasy and/or not completely conditioned.

Historically, three methods of spawning have been used. However, on most commercial farms the "Through Mixing" method prevails. "Broadcast" and "Ruffling In" spawning were the other two methods. Broadcast spawning consisted of covering the substrate surface with spawn. Whereas "Ruffling In' included the scratching the spawn into the top few inches of substrate.

"Through Mixing" spawning has many advantages over the other methods. Research has shown there are increases in yield. Because the mycelium does not have to grow far to colonize substrate, it reduces spawn-growing time, which is important because spawned substrate is exposed to various types of infection. Another advantage is that spawn grains act as supplemental nutrients for the mushroom mycelium. With through mixing spawning it is important that substrate must be cooled throughout the bed before spawning.

Spawning is the cleanest operation performed on a mushroom farm. All equipment, baskets, tools, etc. should be thoroughly cleaned and disinfected before spawning. Normally it is the first operation of the day when personnel have not been into any of the older houses. After spawning, the beds should be covered with plastic to protect the freshly spawned substrate from air borne contaminants and pest. Unfortunately, for any contaminants present before covering, the moist environment provides ideal conditions for germination and growth. Plastic sheeting is sometimes used to cover the substrate to help maintain substrate moisture.

How Much Spawn?

The amount of spawn used depends on the crop cycle and cost. The spawning rate can be expressed as a unit or quart per so many square feet of bed surface; 1 unit (~ 1 lb or 1 liter) per 6-8 ft. is considered a standard rate on a commercial farm. The rate is sometimes expressed based on spawn weight versus substrate weight; therefore, 2-3

percent spawning rate is equivalent rate. A low spawning rate is about 1 unit for every 12-15 sq. ft. (1-2% Dry Weight), whereas a high rate about 1 unit for every 4-5 sq. ft (>3% of Compost Dry Weight). The more spawn used the better, since it is a cheap supplement, increasing overall production; and the more initial growing points will provide a quicker and more efficient use of substrate nutrients. Both of these factors will improve the colonization of substrate, which also helps insure the mushroom will grow quicker than other fungal competitors. Furthermore, as the spawn rate is increased, more heat is generated and the heat surge occurs earlier during spawn growing period.

Spawn Growth

During the colonization of the substrate by the spawn (spawn run) is a good time to evaluate the crop and substrate. Spawn growth and the presence or absence of other molds helps to indicate how the substrate preparation process has been carried out. Problems with substrate formulation or process and Phase II composting or conditioning may first develop during the spawn-growing period. Weed and indicators molds may tell the grower how the composting process went and what nutrient was lacking or in excess. These molds may grow on compounds that have not been used by the microbes during the Phase II will often suggest a problem occurred.

Although the type of spawn growth depends on many factors, often it may indicate the nutritional and moisture level of the substrate. The spawn strain itself will vary in their inherent capacity for rapid or slow growth. This variation is a genetic characteristic; therefore, growers should be familiar with the characteristics of the different strains and the suppliers of spawn are a good source of this information. The other obvious important factor that determines the type of spawn growth is the substrate. Substrate element analysis is important but does not always correlate with growth or yield but should be monitored to determine trends in substrate preparation. The lab analysis should be used as guidelines and establishing trends from crop to crop. There is a direct correlation between substrate ammonia content and subsequent growth and yield of mushrooms. Substrate should have less that 0.05% ammonia, dry weight, at spawning time. By smelling, most growers can detect 0.1% ammonia levels, which will restrict spawn growth. Ammonia content above 0.2% will kill spawn. The substrate pH has little to no correlation to spawn growth or yield. Spawn can tolerate a pH in the range of 6.5 to 8.2 and normally it will decreases from 7.5 to 6.0 during cropping. When the substrate nitrogen content is analyzed at this time, it should be in a range of 2.0 - 2.5% on a dry weight basis. A positive correlation of substrate nitrogen and yield has been shown. The greater the nitrogen content, with no ammonia, the better the yield. Nitrogen content has no correlation with spawn growth, since rapid spawn growth has been observed in both high and low nitrogen composts. However, generally a high nitrogen substrate has a

slower spawn growth, but fills out and becomes denser. The lipid (fats/oils) content of the substrate will influence both the rate and quantity of spawn growth. More nutritional substrate will support slower and finer texture spawn growth. It is suspected that the thinner strands of spawn are slowly adsorbing nutrients. In less nutritional substrate, spawn growth is more rapid and white with more rhizomorphs, suggesting the spawn is seeking nutrients.

Ideal substrate moisture at spawning varies according to the type of substrate. With horse manure substrate, moisture of 65-72% is normal. With synthetic composts a moisture range of 65-75% is normal. However, there are exceptions to these ranges where spawn growth and yield are better outside these ranges. Mushroom size and quality is affected directly by dry substrate, where dry substrate will produce smaller, off-color mushrooms.

Ventilation and environmental requirements for substrate are not well understood. It is assumed that little oxygen is required within the substrate. Carbon dioxide levels are kept high within the room or at least under the plastic that is used to cover the substrate after spawning. It is known that there is an increased spawn growth rate with increasing CO_2 levels to 10,000 ppm. The desired relative humidity is 95% or more in order to preserve substrate moisture. Relative humidity within the room can be maintained by watering the walls and floors. Some high-pressure misting systems have been developed, but they are expensive to purchase and maintain. Steam can also be used to maintain humidity, however it is a source of heat and would increase energy cost and put more demands on the air conditioning system.

During the spawn-growing period, little outside ventilation is used, unless outside air is used as a supplemental source of cooling. During the warmer months outside air is not used, and the room air is re-circulated through the air conditioning units to be cooled. The higher humidity of the outside air requires more cooling capacity.

Substrate and Spawn Growth Management after Spawning

Substrate may lose about 5 F during spawning. During the summer, substrate may be warmer before spawn and therefore it is important to bring substrate temperature down to optimum temperature within 12-18 hrs after the spawning operation is completed. The mycelium within grain is slightly insulated; and this mycelium can survive in 90 F plus temperatures for a short time. However, with unfinished substrate it is usually better to be cooler than warmer when spawning, insuring the substrate temperature is brought down below the temperature range for mesophilic mold growth (> 85 F). The optimum spawn growth temperature is 75-76 F, however there is some indication that growth is faster at 73-75 F. Growers normally run substrate bed temperatures in the high 70's but most try not to exceed 80 F. At that temperature or higher the spawn growth becomes restricted and permanent damage to fruiting mechanism may occur. Spawn is killed at about 104 F.

Spawn growing temperatures should be maintained at a steady level. It is important to anticipate heat surges in substrate temperatures. Heat surges may occur early if the substrate is not conditioned properly. Spawn heat surges later in the growing period are a

result of the spawn growth and dependent on the spawn and supplement type and rate. The initial first few days substrate activity should be minimal, unless substrate is unfinished and mesophilic microbes become active of the left over food. As tips of the spawn begin to contact each other and then fuse together (anastomose) more metabolic activity occurs. As the metabolic activity increases, more CO_2, water vapor, other gases, and heat are produced. Gradually substrate temperature output will increase 6-9 days after spawning. Growers anticipate a heat surge at this time and will lower air temperatures before this heating occurs.

The removal of metabolic heat from the substrate involves conduction and other methods of heat transfer. Conduction is heat transfer by contact, like a touching a spoon in hot soup; eventually one feels the heat of the soup through the spoon. Metabolic heat is moves from one solid particle to another or water molecule to another. Therefore, tightly packed substrate with good moisture will help remove the heat from the substrate. Dense beds, less air spaces, its is easier for heat to be transfer to the surface and then removed. Loose, fluffy substrate is a harder to control more air spaces where heat transfer is slower. When the heat reaches the surface of the substrate heat it is removed by evaporative cooling and convection, heat transfer by circulation of currents from one region to another. Cooler air moving across the surface of the substrate removes the heat from the substrate. Evaporative cooling is the removal of heat when liquid phase turns to gas phase and occurs when dry air moves across the surface substrate moisture is lost and heat removed with it. Too much evaporative cooling is not good, since substrate will tend to dry out too much.

Measuring and Controlling Temperatures during Spawn Growth

The methods used to monitor and control temperatures vary at each farm. The quantity of thermometers used for a room may vary. One may use glass, dial thermometers, or use remote sensing thermocouples with computer control. At least two thermometers are usually placed into the substrate in most mushroom houses, and these are left in the same location throughout the entire spawn-running period.

Several factors should be considered when determining how to and how often one should monitor substrate temperatures. The cooling and volume capacity of the air handling units, substrate nutrition and dry weight are several factors that will determine how easily the spawn temperatures can be controlled during its most active period. At the beginning of spawn growth, when the substrate temperature is uniform, thermometers are usually left in the same location. One should monitor substrate temperature twice a day and during the accelerated spawn-growing period, more often is advisable.

These stationary thermometers are used to represent the range of temperatures encountered in all areas of a room. Probing of the substrate temperatures should be done to insure that the stationary thermometers represent that area of the substrate or room and provide another tool to monitor and control the crop temperatures. Most growers invest time every few days, if not daily, to probe all areas within each house, both upstairs and down, to monitor differences and changes in temperatures. Hand-held rapid response

thermometers are inserted into as many spots of compost as possible to monitor temperatures. This probing determines the range of temperature within the substrate and areas of the room. However, it is important to have personnel available to spend the time probing the room. Probing can be done by any trained and culturally clean individual, not necessarily a grower. More uniformity in the substrate's moisture, nutrition and dry weight will reduce the need for extensive comprehensive probing. When the grower has enough experience about how the crop will react and can anticipate when and how much of a heat surge may occur, less probing may be required. After spawning, before the spawn heat surge, during heat surge and before casing are the most critical times to probe.

Probing and or stationary thermometer temperatures are usually averaged into a mean temperature for the room. This average substrate temperature is used to make decisions for raising or lowering the air temperature. Decisions on whether to lower the air temperature or to raise it should be based on both the average substrate temperature and the frequency or distribution of higher temperatures. If the majority of spots are a bit warm then air temperature should be lowered a degree or two. Conversely, if the majority of areas are a bit cool 68 - 72 F, either the air temperature or the volume of air should be decreased since both of these factors can affect the substrate temperature.

The varying air volume during spawn growing to control substrate temperature may be a new consideration for some mushroom growers. The use of air temperature to control substrate temperature is normally how substrate temperatures are controlled. By lowering the air temperature, the substrate temperature is decreased. It may also be obvious that the more air moved through a house, the greater the amount of heat removed from the air in the house and, indirectly, the substrate. If the air is dry, substrate moisture will evaporate and evaporation is a form of cooling. The greater air volume moving through a house and the drier that air, the greater the cooling effect on the substrate.

Substrate temperature control is also dependent of the cooling capacity of the air handling system. Most single-zone bed farms design their air conditioning systems on the requirements during spawn run. Tons of cooling that is required to maintain temperatures range from 2 to 5 tons per 1,000 sq. ft. of growing space. Cooling capacity is also dependent on the location of the farm and weather conditions. Cooling for spawn growth also depends on the air volume, movement and distribution within the room. Substrate density also determines to amount of cooling required, the more substrate dry weight the more cooling that is needed. The condition of the substrate will affect the amount of heating that occurs the first few days of spawn growth. When substrate is not conditioned well, other microbes have food to use and will tend to grow and produce a heat surge early is the spawn growing period. Substrate moisture also affects the temperature control during spawn run. The drier substrate the harder it is for the heat to be removed and substrate will tend to heat more rapidly and for longer time. While wetter substrate has less air in the pore spaces, and heat is conducted more easily, making substrate temperatures easier to control. When hot spots are found the plastic covering the bed should be removed. In addition, additional fans may be directed on the hot spots or one may dig a hole in the substrate and allow the heat to escape.

The spawn-growing period is normally 10-18 days. Longer spawn run times can reduce yield and substrate moisture, thus influencing fresh quality. A short growing time will create more heat production during the time after casing. This additional heat will require more cooling and increase the drying of the casing. The cooler air temperatures will slow the spawn growth into the casing, delaying the flush and first harvest. Spawn growing period is considered complete when the spawn has completely colonized the substrate and the metabolic heat surge is subsiding.

Summary

Important points to remember about spawning include the complete and thorough mixing of spawn into substrate. Spawn is a cheap nutrient supplement, therefore if economical; a high spawning rate is desirable. Spawning should be the cleanest operation the farm, often performed early in the workday. During the spawn run, it is a good time to evaluate the crop and substrate. Spawn growth and the presence or absence of other molds helps to indicate how the composting process has been performed. It is important to anticipate temperatures by monitoring temperatures often and by probing establishing crop variation and trends.