Spawning to Casing in Commercial Mushroom Production

Daniel J. Royse, Professor, Department of Plant Pathology, College of Agricultural Sciences, The Pennsylvania State University, University Park, PA 16802

Definition of terms

Casing
An overlay of neutralized (limestone) peat moss applied to colonized compost to stimulate pinning and fruiting of mushrooms.

Casing inoculum (CI)
An organic material, usually consisting of a mixture of peat moss, wheat bran, vermiculite and limestone that has been sterilized and then colonized by mushroom mycelium.

Compost
A mixture of decomposed organic and inorganic substances with nutrient composition selective for the growth of the common cultivated mushroom.

Phase III compost
Mushroom compost fully colonized by mushroom mycelium and ready for casing application.

Spawn
A steam-sterilized grain colonized by the mycelium of the mushroom and used to "seed" mushroom compost.

Spawning
The processing of broadcasting onto and digging spawn into the compost.

Spawn growth or spawn run
The growth of mushroom mycelium into compost. This period usually lasts about 2 weeks.

Supplementation at spawning
The addition of delayed release nutrients at time of spawning.

Supplementation at casing
The addition of nutrients to colonized compost at time of casing application.

Top dressing
An application of CI to the surface of compost to hasten colonization of compost.

Brown crimini

The information supplied in this publication is an updated revision from a chapter originally published in the Penn State Handbook for Commercial Mushroom Growers (Wuest & Bengston 1982). It is intended as a review and resource for potential and commercial mushroom producers interested in the cultivation of *Agaricus bisporus*.

Spawn

To understand spawn, spawning, and spawn growth, the mushroom itself must be understood. Mushrooms are fruits of the fungus *A. bisporus*, and consist of two main parts - the cap and the stem. As the mushroom matures the cap opens and the gills are exposed. Mushroom spores are produced in the gills. Spores are microscopic spheres roughly comparable to the seeds of higher plants. These spores are produced in large numbers in the gills. An 8-cm mushroom produces as many as 40 million spores an hour. Since spores germinate and grow into mycelium rather unpredictably, they are not used to 'seed' mushroom compost. Spores will germinate and grow into thread-like mycelium that is used by laboratories to produce spawn commercially.

In the spawn-production process, mycelium from a mycelial culture is placed onto steam-sterilized grain, and in time the mycelium completely grows through the grain. This grain/mycelium mixture is called spawn, and spawn is used to "seed" mushroom compost. 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. Spawn making is an asexual method of propagation similar to dicing a potato so an "eye" can be planted. Spawn making is a rather complex task and is not practicable for the average mushroom grower. Spawn may be purchased from a number of commercial spawn manufactures, and most mushroom farmers purchase spawn from these sources.

Millet spawn

Spawning

Immediately after cool-down at the end of Phase II (peak heat) the compost is ready for spawning. If the spawn is stored in a refrigerator, time can be gained by moving it to a warmer place the day before spawning is to be done. The mycelium will resume growing, and time will be saved.

1. Methods

a. *Broadcast* - Spawn is broadcast over the surface of the beds and allowed to grow into the compost. Chief advantages of the broadcast method are convenience, labor saving, and time saving. Its disadvantages include exposure of grains to drying conditions, a slow start due to dry surface conditions, and the time required for mycelium to grow throughout the full depth of compost and establish total colonization -thereby allowing competitors more time to gain access to the compost in the lower portion of the bed. Also, the nutritional value of the grain is lost to the crop. Broadcast spawning is rarely practiced in the mushroom industry today.

b. *Ruffling-in* - Spawn is broadcast over the surface of the bed and ruffled-in as the compost is being manipulated into a flat-surfaced bed. The depth of ruffling-in varies from 1 to 7 cm. This method eliminates most of the disadvantages of

broadcast spawning, except that the mushroom mycelium must still grow down through the compost from wherever the deepest spawn grains happen to end up. This method is more widely used than broadcast spawning, especially at farms where mechanized spawning equipment is not available.

c. *Mixed spawning* - This method was first used in the tray system of growing and now its use has expanded to a greater and greater extent in the bed system. Spawn is mixed throughout the compost, usually by the tines of a specially designed machine.

After the machine has passed through the compost in the beds, the compost is tamped with a flat trowel-like device. There are several advantages to mixed spawning, i.e., it reduces the spawn-growing period and increases mushroom yield. The spawn is evenly distributed in the compost, impregnating it (depending on the amount of spawn used) with one or two grains per cubic inch. All grains become initial points of growth. The mycelium does not have to grow any great distance before the compost is completely colonized, and this saves time. Time is not the only advantage; uncased beds are open to various kinds of infestations during this period, so a shorter period of spawn growth can be an important advantage. In addition, the spawn grains themselves become a supplemental nutrient. Mixed spawning is not without its disadvantages. All the compost in a bed destined for mixed spawning must be cooled to below 27°C at spawning time, since the spawn is mixed throughout the bed as well as near its cooler outer surface. However, mixed-spawning is a recommended practice, since the advantages far outweigh its disadvantages.

2. Rates

In practice, spawning rates vary among commercial mushroom farms. The basic unit of spawn is the quart, since grain spawn was originally made in quart-sized milk bottles and bagged in quart-sized units. Though almost no spawn is made in quart-sized units today, spawning rates are still designated in terms of quarts per square feet of compost surface. Some growers spawn at rates as low as 1 quart for each 60 ft^2 of bed surface. Others will spawn at a rate as great as 1 quart for each 2 ft^2 of surface area. Increasing the spawning rate increases mushroom production and reduces the time needed for spawn growth; this is especially true when the spawn is mixed-spawned. The increased mushroom yield is due to more efficient utilization of compost nutrients that, in turn, is related to more initial growing points and quicker colonization of the compost, as well as to the nutrient value of the spawn grain itself. In effect, by adding more spawn one is adding more nutrients. There are practical limits to the amount of spawn that may be added and still get increased yields. When spawn is ruffled-in the maximum beneficial rate is about 1 unit per m^2 but with mixed spawning, rates as high as 6 units per m^2 have proven advantageous. On the other hand, increased spawning rates are not without disadvantages. Increased heat is generated during spawn growth and cooling equipment must be able to cope with a tendency towards excessive compost heating. If cooling equipment isn't available, higher spawning rates should be used only during the time of year when outside air is cool and readily available to control compost temperatures.

3. Sanitation

Spawning should be one of the most sanitary operations in mushroom growing. Spawning tools, baskets, and equipment should be thoroughly cleaned with a strong stream of water, and disinfected before use. Spawning should be the first operation of the workday to insure that the workers, their outer garments, and their shoes are free of debris or contaminants from less sanitary operations.

4. Treatment of spawn with fungicide to control green mold

In 1985, a devastating form of green mold disease caused by *Trichoderma harzianum* (now known as *T. aggressivum* f. *europaeum*) was discovered on mushroom farms in Northern Ireland. This disease later appeared on farms in nearly all countries producing *A. bisporus*.

In severe cases, green mold disease may cause nearly complete loss of mushroom production by invading compost and preventing the crop from emerging.

Green mold is best controlled by sanitation. Spores are sticky so they attach to tools and equipment, workers clothes and hands, flies, dust particles, etc. If spores come in contact with spawn, the disease may be especially severe.

In the 1990's, researchers in Europe discovered that if a fungicide was used to protect the spawn from germinating spores, the disease could be more easily controlled. Thus, treatment of spawn with benzimidazole fungicides (benomyl, thiophanate methyl and carbendazim) became widespread in the industry. In the United States, routine treatment of spawn with benomyl and thiophanate methyl eventually led to development of resistance in the *T. aggressivum* population on several farms in Pennsylvania and Delaware. Today, growers still use thiophanate methyl to treat spawn (and supplement) but a replacement fungicide (imazalil) is being sought to combat thiophanate methyl-resistant strains (Royse & Romaine 2003, Romaine et al. 2007).

Supplementation at spawning

1. Developing the technique

In the early 1960s yield increases were observed when compost was supplemented with protein and/or lipid-rich materials at spawning, at casing, and later (Schisler & Sinden 1962, Sinden & Schisler 1962). A 10 percent increase in crop yield was obtained when small amounts of protein supplements were added to the compost at spawning. However, excessive heating and strong

stimulation of competitor molds in the compost drastically reduced the rate at which supplements could be used and, concurrently, their corresponding benefit. Overheating of spawned compost can damage or injure the mycelial fruiting capacity - thereby reducing productivity.

In 1976, delayed-release nutrient supplementation was developed (Carroll & Schisler). The disadvantages associated with the supplementation of non-composted nutrients to mushroom compost at spawning were largely overcome by encapsulating micro-droplets of vegetable oil within a protein coat that was denatured with formaldehyde. Increases in mushroom yields of 60 percent were obtained. Many commercial delayed release nutrients are now available on the market.

2. Incorporating delayed-release supplements into compost

The key factor in using a delayed-release supplement successfully is uniform through-mixing. A delayed-release nutrient, usually applied at 4 to 6 percent of dry compost weight, should not be used unless mechanical through-mixing is performed. In the bed or shelf system a supplement should not be applied closer than 5 to 7.5 cm from the sideboards, as this region normally is not as well through-mixed. Where concentrations of supplement exist, at or near the surface, or adjacent to the sideboards, they may become a nutrient source for airborne molds.

On tray lines with a tipper mechanism, the supplement should be evenly applied after the compost has been dumped from the tray. It should be applied prior to the application and mixing of spawn. This practice will assure a more thorough and even distribution of the nutrients. It cannot be overemphasized that thorough and even mixing of the supplement is a key factor in successfully using a delayed-release supplement.

3. Anticipatory control of compost temperature

The addition of supplement to compost at spawning encourages more vigorous mycelial growth. This increased mycelial activity releases more heat into the compost than mycelium growing in non-supplemented compost. As a result, depending on the condition and ability of the compost to dissipate heat, compost bed temperatures may rise 2° to 5° C above non-supplemented compost. The normal peak temperature during spawn run also may be advanced by as much as 48 hours and the response of the compost to lower air temperatures will be delayed when compared to non-supplemented compost. These events are controllable if anticipated. The grower must be able to determine the number of days after spawning that temperature increases will occur. In this way, the air temperature and/or the volume of air can be adjusted in advance of the heat surge, thereby maintaining optimum compost temperature levels during spawn run.

Mushroom cultivars differ in their response to spawn growth temperatures. However, as a general rule, most cultivars have an optimal growth temperature range of 23° to 26° C. Temperatures higher than 27° C may inhibit mycelial growth and cause injury and/or damage to the fruiting capacity of hyphae.

Spawn growth

Many factors can determine the proper spawn-growing period. These factors include, mushroom cultivar used, various compost factors, sanitation and compost top dressing.

1. Mushroom strain

Strains of A. *bisporus* vary in their inherent capacity for rapid or slower growth. This is a genetic characteristic associated with the particular mushroom strain used. Hybrid mushroom strains are widely used in the industry today. A grower must become familiar with each strain used, and spawn makers can often provide helpful information in this regard.

2. Compost factors

a. *pH* - There seems to be little or no correlation between compost pH, subsequent mycelial growth, and ultimate yield. Determinations of pH made at the time of spawning seem to be acceptable all the way from a pH of 6.5 to 8.2. The compost pH during a crop decreases from 7.5 at spawning to 6.0 after cropping. Attempts to correlate pH of the compost with spawn growth and ultimate yield have been unsuccessful. Composts have been found with relatively high pH, containing no ammonia and supporting good mycelial growth. On the other hand, composts with a relatively low pH, 6.7, containing considerable ammonia and supporting little or no spawn growth have been observed.

b. *Ammonia* - There appears to be a direct correlation between ammonia content, subsequent mycelial growth, and ultimate yield. The ammonia content at spawning time should be less than 0.05 percent. If ammonia content exceeds this value, mycelial growth is retarded. Ammonia content of compost is best determined by a modified Kjeldahl method. Most people can detect ammonia contents of about 0.1 percent by smell; this level of ammonia will severely restrict spawn growth. A concentration of ammonia in excess of 0.2 percent usually kills the mycelium.

c. *Nitrogen content* - The nitrogen content should be 2.0 to 2.5 percent at spawning. There is a positive correlation between nitrogen (N) content and yield. The greater the N content provided there is no ammonia present, the better the yield. This correlation, however, does not necessarily hold true with rate of spawn growth. It is possible to have very rapid spawn growth in compost with a low N content, as well as very slow spawn growth. Generally speaking, the

Hybrid white strain

spawn growth may be slower in composts with a high N content but "fills out," becomes denser, as it grows. Of course, slow spawn growth does not necessarily denote good compost. In summary/the rate of spawn growth is not necessarily related to either yield or the N content of compost.

d. *Lipid content* — The amount of lipid in compost will affect both the rate and quantity of spawn growth. Data from oil supplementation of mushroom compost experiments show that linoleic acid is a stimulatory lipid. Crude cottonseed oil added at a rate of 10 ml/lb of wet compost prior to Phase II of composting can result in a 15 to 20 percent increase in mushroom yield. Oil addition also increases the biological activity during Phase II, which results in more heat being given off by the compost. Increases in mushroom yield associated with lipid additions are due to a combination of more microbial protein produced during Phase II, and a direct stimulation of mushroom mycelial growth and fructification during spawn run and production. However, supplementation of the compost with oil at fill poses practical problems such as uniform distribution of the oil throughout the compost and increased thermogenesis during Phase II composting and spawn run. The grower must be able to handle the increased heat load to obtain the benefits of oil supplementation.

e. *Temperature* - The optimum temperature for the growth of the mycelium of the cultivated mushroom is 23° C to 26° C. Practical experience with growing spawn in compost shows that maintaining bed temperatures within the range of 24° to 26° C results in a more rapid growth rate and, consequently, a shorter spawn-growing period. Growers should not permit compost temperatures to exceed 30°C at any time during spawn growth. The mycelium is not killed until temperatures reach 40° C, but growth is severely restricted and permanent damage can be done to the fruiting mechanism if the mycelium is incubated above 30° C.

Mushroom mycelium exhibits a rhythmic pattern of growth so compost temperatures should be monitored 4 or more times a day. One of the merits of maintaining compost temperatures between 23° C and 25° C rather than 24° C to 26° C is obvious considering this rhythmic growth pattern, i.e. the tendency of the compost temperatures to rise above 26° C during peak mycelial activity of the rhythmic growth pattern.

f. *Moisture* - The moisture content of compost at spawning should be at 65 to 70 percent for horse manure compost or 65 to 75 percent for synthetic compost. These ranges are generally accurate for optimum spawn growth and assume a resilient compost structure. However, exceptions do occur where spawn growth, and eventual yield, will be optimum outside the ranges given. Too much water or too little water, depending on the texture of the compost, may cause difficulty in properly "cooking out" the compost. This effect of compost moisture outside the desired range on spawn growth is generally not a direct effect, but an indirect one. Example: Too wet = anaerobic compost; too dry = too little moisture for thermophiles. One direct effect that is generally accepted is that moisture can influence size of mushrooms, i.e., dry compost = small mushrooms. Also, "weepers" - mushrooms from which water exudes - occur with very dry compost.

g. *Ventilation and environment (humidity)* - The ventilation requirements during the spawn-growing period are not fully understood. No precise data exist on the effect of ventilation during spawn growth on mushroom yield. It is generally assumed that little oxygen is required during spawn run. Growers are advised to keep the house shut tightly with no ventilation except that necessary to maintain a desired compost temperature. Withholding ventilation makes it easier to maintain high humidity and denies access of mushroom pests to the house at a time when the mycelium is most vulnerable. Some data indicate certain levels of carbon dioxide (CO_2) accumulation enhance mycelial growth. Long and Jacobs (1968) reported that mycelial growth rate increases with increasing CO_2 levels up to about 10,000 ppm. San Antonio (1972), doing laboratory studies on mycelial growth, suggested the optimum CO_2 concentration for an increased growth rate is between 1000 and 5000 ppm. The humidity should be maintained at 95 percent R.H. or higher to preserve as much compost moisture as possible.

h. *Salt concentration.* The concentration of soluble salts in the compost is believed to have no effect on spawn growth and eventual yield. There appears to be an adverse effect when salts are high in the casing soil, but not in the compost as far as is known.

Sanitation

Beds may be covered with a plastic film to protect compost surfaces from air-borne contaminants and pests. The film also prevents moisture losses from the compost surfaces. Covering beds with plastic is not without its disadvantages, however. Less evaporative cooling occurs, and keeping bed temperatures within the desired range during warmer weather may be a problem. Also, if contaminants are present prior to covering, the moist environment on the compost surface provides ideal conditions for germination and growth of pathogens or pests.

Shortening the crop cycle with CI top dressing

Top dressing of spawned compost with casing inoculum (CI) prior to casing allows shortening of the crop cycle by up to 1 week (Spear 1998). This technique was developed in Australia and is now adopted by some growers in the U.S. Top dressing does not have an appreciable effect on mushroom yield or cull rate.

The use of CI (250 g CI/m^2 compost) allows casing of Phase II compost after 6-9 days of spawn growth rather than the standard 13-day interval. Several attempts have been made over the years

to case early but failed because the mycelium in the CI material in the casing starved before it could connect to enough mycelium in the compost. Applying a layer of CI to the compost circumvents mycelial starvation by allowing a more immediate mycelial connection between the compost and CI in the casing layer. One-problem growers must deal with, however, when casing early is the potential of compost to overheat when casing is applied before spawn run temperatures have peaked and begun to decline.

In summary, under a given set of conditions, length of spawn growth can affect both yield and pattern of yield. In general, a shorter spawn-growing period means fewer pounds per ft^2 in early breaks, consisting of fewer mushrooms of larger size. However, total yield can be generally reduced. The longer the spawn-growing period, the larger the early breaks and, generally, the greater the total yield. Of course, too long a spawn-growing period can cause a reduction in yield.

Supplementation at casing

Increases in mushroom yields of 100 percent have been observed when soybean meal or cottonseed meal were added to fully colonized compost at the rate of 10 percent of the dry compost weight just prior to casing. One additional step to the preparation of the compost for mushroom growing must be added when any of these supplements are used for supplementation at casing. The compost must be fragmented to insure a thorough mixing of the supplement into the compost. Once the compost is supplemented at casing, the compost is leveled, pressed, and cased.

Most of the yield increase obtained by supplementation at casing occurs in the first 23 days of production; this period corresponds roughly with the first two to three breaks (cyclic pattern of mushroom production) of mushrooms. Thereafter, production falls rapidly to about the same level as that of non-supplemented compost.

Certain problems, however, must be overcome at commercial operations before supplementation at casing can be routinely used. First, the mycelium must be grown through the compost thoroughly before it is disturbed by the fragmentation that precedes supplementation at casing. The exact time for supplementation at casing cannot be specified due to its relationship to the time needed for the spawn to fully colonize the compost. The spawn-growing period is a function of the compost, the strain, and atmospheric conditions of the room in which the spawned compost is kept.

Second, the induction of fruiting on casing atop supplemented compost can be a problem. The casing layer plays an important role in limiting the increase in yield obtainable by supplementation at casing. A border break or the development of a thick overgrowth of non-fruiting mycelium (stroma) may occur when a certain type or depth of casing is used.

Third, weed molds, mites, pathogens, and nematodes must not be present in compost to be supplemented at casing. If present, they will be distributed throughout the compost when it is fragmented and can then multiply in advance of mushroom production. Nematode infestations can be an especially troublesome problem with this practice.

Finally, increased mycelial activity in the supplemented compost produces heat in amounts far above that associated with mycelial activity in undisturbed compost. Unless the air around the compost exchanges the heat as rapidly as it is produced, the temperature can rise rapidly above 27° C - high enough to cause possible injury to mycelial fruiting capacity. Maximum thermogenesis (heat production) usually occurs between the third and fourth day after casing. Many commercial mushroom growing operations are now equipped with air handling facilities sufficient to exchange the heat produced.

Bulk spawn run in Phase III tunnels

The search for improved efficiencies in materials handling during the 1980s led to the development of compost preparation and spawn run in bulk. While spawn run in bulk is more efficient from a labor and energy perspective, it has been slower to gain acceptance among growers than bulk phase II composting because of the increased threat from competitor molds and diseases. Although computers have reduced many of the risks of maintaining precise climate control and filtration for large quantities of compost in a single tunnel, many growers still are not willing to adopt this technology (Samp 2007).

One of the most compelling reasons to consider bulk composting and spawn run is the reduction in the cropping cycle

Phase II/III tunnels

to about 5 to 6 weeks from a typical cycle of 11 to 12 weeks. Thus, up to twice as many crops may be passed through the production facility each year.

Most phase III problems have occurred because of poorly prepared phase I or phase II compost. If secondary fermentation occurs during spawn run, hot spots can develop that are difficult or impossible to control. These hot spots can damage or kill the mushroom mycelium resulting in major losses (Samp 2007).

Contamination of the phase II compost during spawning of compost and filling of the phase III tunnel can also result in substantial problems. Severe losses have occurred on farms due to contamination of the compost with *Trichoderma* spp., *Aspergillus* spp. and *Penicillium* spp. However, one of the most troublesome problems that growers have encountered with bulk spawn run is La France disease (Samp 2007).

La France disease, caused by a virus, can be one of the most serious maladies of the mushroom. Infections may result in reduced yield and the development of premature, malformed mushrooms that are prone to accelerated postharvest deterioration (Romaine & Schlagnhaufer 1995). Infected spores or mycelium contaminating the phase II compost at spawning spread the virus. When the tunnel is emptied the infected mycelium may be spread throughout the compost, the working area, and much of the production area. Since it is nearly impossible to kill all of the infected mycelium, compost in all subsequently filled tunnels may become contaminated (Samp 2007).

Attempts to mitigate virus infection have included isolating phase II/III tunnels from the production facilities, redesign of the phase II/III tunnel complex, and implementation of excruciating sanitation programs.

The advantages of bulk spawn run would appear to outweigh the disadvantages. It is anticipated that most mushroom production in the somewhat-distant future will come from bulk-processed compost. Bulk handling of compost provides improved efficiencies through ease of use and by shortening the cropping cycle thereby increasing throughput on each farm.

Summary

Successful mushroom production involves a variety of interrelated factors. Production of compost that is selective for mushroom growth and that contains those nutritional substances necessary to optimize mushroom yields is the first step. Care in compost preparation during Phase I and Phase II will minimize problems encountered during the spawn-growing period from spawning time through casing. Attention to the environmental, physical, and nutritional factors discussed will yield increased quantity and quality of harvested mushrooms.

References

Carroll AD, Schisler LC (1976) Delayed release nutrient supplement for mushroom culture. Appl Environ Microbiol 31:499-503.

Long PE, Jacobs L (1968) Some observations on CO_2 and sporophore initiation in the cultivated mushroom. Mushroom Science 7:373-384.

Romaine CP, Royse DJ, Schlagnhaufer C (2007) Imazalil sulfate as an alternative spawn and supplement treatment for green mold disease. Mushroom News 55(4):4-7.

Romaine CP, Schlagnhaufer B (1995) PCR analysis of the viral complex associated with La France disease of *Agaricus bisporus*. Appl Envrion Microbiol 61:2322-2325.

Royse DJ, Romaine CP (2003) Evaluation of fungicide applications on spawn for control of mushroom green mold, 2002. Fungicide & Nematicide Tests 58:V018.

San Antonio JP, Thomas RL (1972) Carbon dioxide stimulation of hyphal growth of the cultivated mushroom, *Agaricus bisporus*. Mushroom Science 8:623-629.

Schisler LC, Sinden JW (1962) Nutrient supplementation of mushroom compost at spawning. Mushroom Science 5:150-164.

Sinden JW, Schisler LC (1962) Nutrient supplementation of mushroom compost at casing. Mushroom Science 5:267-280.

Samp R (2007) The development of *Agaricus* mushroom compost processing systems. In: Sanchez JE et al (eds) Cultivation, marketing and food safety of *Agaricus bisporus*, Mexico City (In press).

Spear M (1998) Sandwich technique. Mushroom News 46(7):24-29.

Wuest PJ, Bengston GD (1982) Penn State Handbook for Commercial Mushroom Growers. The Pennsylvania State University, University Park, PA