

Oyster Mushroom Cultivation

Part II. Oyster Mushrooms

Chapter 4

Spawn

HOW TO MAKE OYSTER MUSHROOM GRAIN SPAWN IN A SIMPLE WAY

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What did you begin with when you started mushroom growing? Some raw beginners might have started with mushroom growing kits or so-called ready-to-fruit bags. From these bags they can harvest mushrooms by just providing the proper conditions for mushroom growing. Others might have started with the organic materials for mushroom growing, i.e. substrate and mushroom seed purchased from their local provider. The former could see only fruiting but the latter could witness the two phases of mushroom life cycle, mycelial or vegetative growth and fruiting, or reproductive growth.

Mushroom seed, commonly called “spawn” in the mushroom industry is a result of mycelial expansion. Using the following guide, any of you familiar with sterile techniques or having specialized knowledge of mushroom culture can make your own “mushroom starters” under sterile conditions, thereby reducing production costs and even getting spawn of higher quality. The first part discusses the construction of a clean room and the latter investigates spawn production. An understanding of the process will also provide common growers with advanced knowledge of mushroom growing.

How to Build a Clean Room in a Simple Way

A practical description of how to construct temporary building panels from polythene sheets and sawn timber, which can be fixed together to form the basis of a sterile clean room. The clean room utilizes a sterile airflow from a basic bench mounted HEPA filter/fan assembly, or laminar flow bench. The size of the clean room is directly correlated to the cubic airflow capacity of the laminar flow bench.

Introduction

What is a clean room?

A hygienically clean, sterile enclosed air space where mushroom mycelium can be isolated from its normal environment in which it must compete with a host of other organisms to survive.

How does it work?

All air in the clean room is 'cleaned' by passing it through a High Efficiency Particulate (HEPA) filter to remove the vast majority of airborne particles (living and dead), including: skin, pollen, dust, mould, bacteria, and fungal spores.

Why do mushroom growers need a clean room?

Mushroom spawn is expensive and can be difficult to find. It also does not travel over long distances well. A grower who produces his/her mushroom spawn gains a greater knowledge and understanding of the mushroom growing process.

Overview

This simple clean room design owes its success due it being constructed with minimal mould growing, organic biodegradable materials. High sterile airflow is achieved by placing a large cubic fan capacity in a small area; any airborne contaminants that enter the clean room are quickly removed by the laminar flow bench.

The clean room can be used to inoculate agar plates with mushroom mycelium from culture slants or with tissue samples taken from wild specimens. It can be used to conduct agar-to-agar, agar to grain, grain-to-grain, sawdust to grain, and sawdust-to-sawdust transfers, using a standard domestic pressure cooker to sterilize the various mediums.

The design is intended as a place to teach, learn and experiment with mushroom sterile culture. It is not capable of withstanding the demands of commercial mushroom spawn production.

Constructing the clean room

Materials required

- 50 × 2.5m roll clear polythene sheeting (120 gauge, 8 mil, 0.5mm thickness)
- 2 × 1" (50 × 25mm) sawn timber-preferably treated
- 2.5" (60mm) length counter sunk wood screws, tacks or staples
- Silicone caulking sealant

The basic premise is that clear polythene lined wooden frames are made to a standard size. The frames are then joined together to make an enclosed space that is entirely lined by the mould resistant, sterile polythene. Four frames in total, one containing a door constructed in a similar manner. The room is best sited on a concrete base. Other materials that can be used for a sterile floor are linoleum (lino), or butyl rubber sheet.

Constructing the frames

Construct two side frames using 50× 25mm sawn timber to the following dimensions: 2m long × 2m high with a bracing bar positioned at 1m (Fig. 1). The two upright lengths of timber should be positioned with the narrow edge facing outwards.

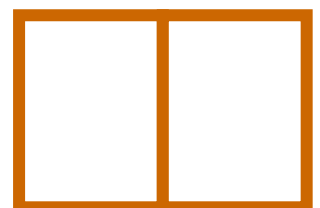


Figure 1. Side frames of clean room

The polythene is cut to length $2.5 \times$ length of the frame. Then folded back and tacked round the rear of one side, thus creating a clear panel. A bead of sealant can be run down the timber to prevent the polythene from ‘flapping’. The two end frames are made to $2 \times 1\text{m}$ in size. An entry must be made in one of the end frames (Fig. 2, 3, 4). The door is best constructed along the same design as the frames. Make sure the door fits well with as few gaps as possible. Household window insulation foam makes a good seal round the doorway.



Figure 2, 3, 4. End frames of clean room

Joining the frames together

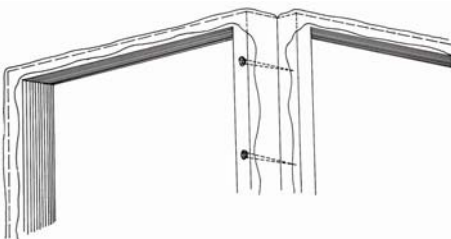


Figure 5. Frame connection

To make an airtight seal between the frames, first run a 10mm wide bead of silicone sealant: 10mm from the edge of each frame, all the way round the edge of each frame. The frames should then be positioned at right angles to each other and secured with screws or nails (Fig. 5).

After securing the four frames, apply another bead of silicon along all the joins, including the floor. Cut a $2.5 \times 1.5\text{m}$ piece of sheeting and secure this over the top of the structure in the same way as the frames were made (a bracing bar may be necessary to add strength).

Equipping the clean room

When creating a sterile environment in the clean room a basic laminar flow bench is required. To be effective at stopping contaminants to mushroom culture the air in the clean room must be passed over a HEPA filter (Fig. 6, 7) once every 60-80 seconds. This is achieved by matching a fan with an output range of 400-500 cubic feet per minute (CFM) with a HEPA filter that is no more than 6 inches (15cm) in depth.

Off the face of the filter, an airflow range of 125-150 CFM will give the desired filtering capacity. The cubic air capacity of the clean room is 141 cubic feet.

Work surfaces should be made from scratch resistant non-degradable materials such as steel, and positioned at waist height.



Figure 6, 7. Simply made HEPA filter

Operation, cleaning and maintenance

The laminar flow bench should be left in continual operation. This will keep the room dry and free from mould growth and other contamination. Cleaning is best carried out one hour before use. A 5% solution of domestic bleach (sodium hydroxide) is used to clean all surfaces. All operatives should wear newly laundered clothes when entering the clean room.

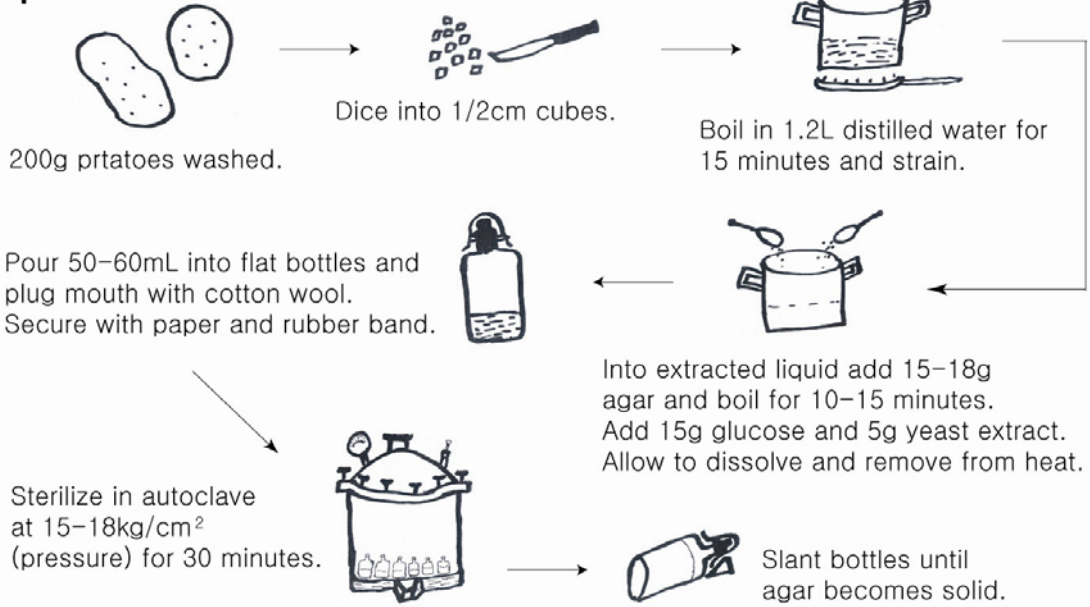
General maintenance should be carried out as wear and tear occurs. Any holes in the polythene should be immediately sealed with adhesive tape, and the silicon sealant should be checked regularly (monthly) and remove and replace as necessary. It is recommended that the HEPA filter be changed after 12 months of operation.

Production of Oyster Mushroom Grain Spawn

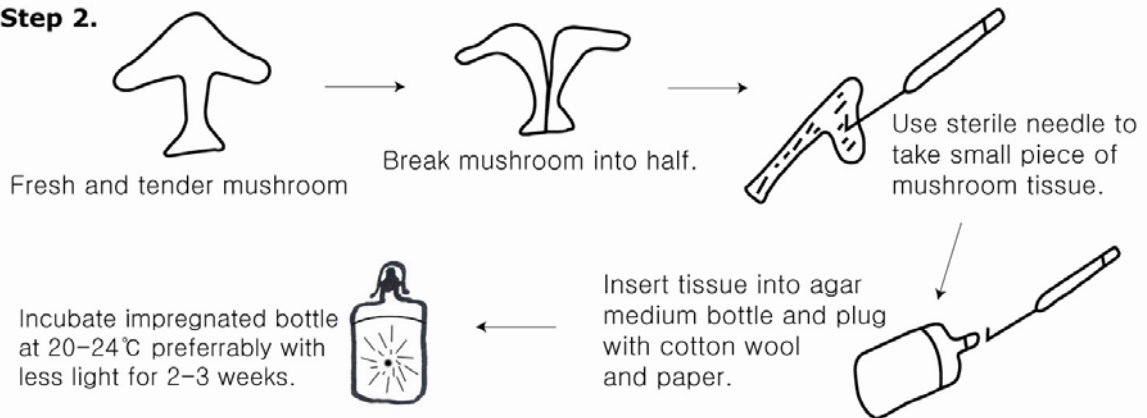
Introduction

Mushroom growing using home grown spawn is a process of cellular expansion. Mushroom mycelium is initially grown on a nutrified agar media. This is then used to make grain spawn. The grain spawn is subsequently used to make the final fruiting substrate.

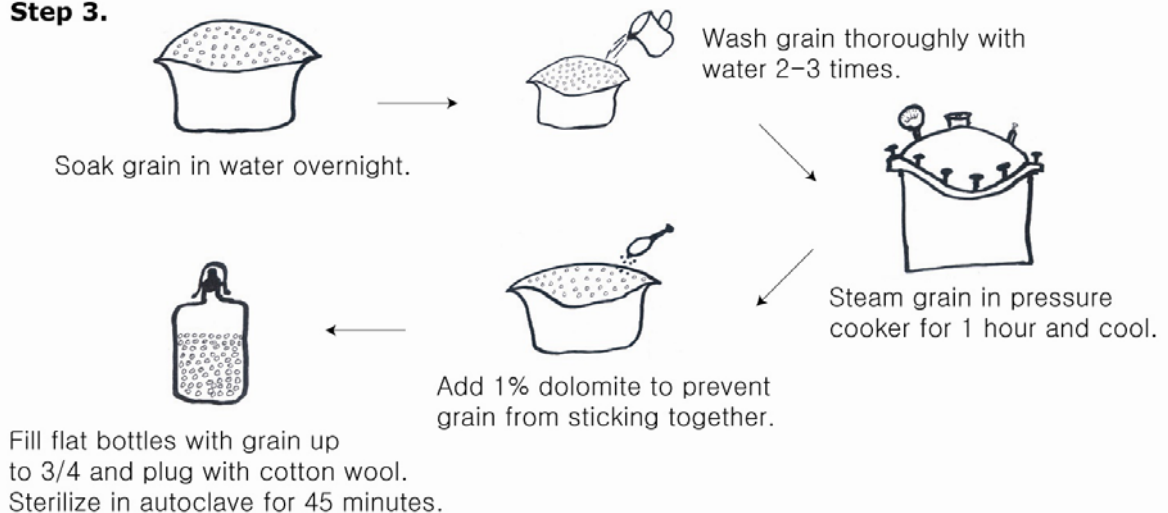
Step 1.



Step 2.



Step 3.



Preparing agar media

The starting point is a mushroom culture (usually in a test tube). This will either be from a clone that you have taken yourself from a mushroom specimen, or purchased from a culture laboratory. Until you have gained experience in cloning and assessing wild mushroom specimens, it is best to use a proven, productive strain from a laboratory.

Growing out your culture

Mushroom mycelium needs nutrition to grow. In other words, it needs something to feed on. Agar agar (a seaweed) contains almost no nutrition, but acts as a gelling agent when mixed with water, so that the mycelium has a flat, solid surface to grow across. A combination of agar agar, water and one or more nutritional substances gives a satisfactory method for growing out healthy mycelium.



Figure 8, 9 Tissue culture from a mushroom specimen and transfer to agar culture

There are various grades of agar agar: food-grade for cooking and higher grades for the purpose of culturing. If possible, it is best to use one of the higher grades, as these will contain fewer impurities and probably have better gelling properties than the food-grade. It comes in dry powder form.

There are many different sources of nutrition that can be used in agar media, but probably the most commonly used formula is Malt Extract Agar (MEA). The MEA formula that we use is as follows:

- 1 liter water
- 20 grams agar agar
- 30 grams barley malt extract*
- 2 grams nutritional yeast

It is actually called MEYA because of the addition of the yeast.

In addition, commercially prepared nutrified agar media can be purchased from mushroom cultivation suppliers; this needs only water adding to it.

Preparing and pouring media

You will need scales and a liter flask that can be used in a pressure cooker. A domestic pressure cooker is ok, but will not form a vacuum when cooled (i.e. it will draw in contaminated air), and therefore should be placed in the clean room environment for cooling. If possible, a proper pressure sterilizer with a pressure gauge is best to use. For pouring the sterilized media, you will also need a supply of isopropyl alcohol and disposable paper towels.

1. Weigh out your ingredients, add the correct amount of water (preferably non-chlorinated, sterile) and mix together in the flask (Fig. 10).
2. Use non-absorbent cotton or aluminium foil to stop up the flask. Do not plug it tightly with a stopper, because pressure built up inside the flask while sterilizing will cause it to fly off and the media boil too furiously (Fig. 11).



Figure 10. Add distilled water so that the potato starch extract reaches 1L



Figure 11. Aluminum foil-stopped jar



Figure 12. Put the jars in the autoclave and sterilize them at 121 °C for 45 minutes.

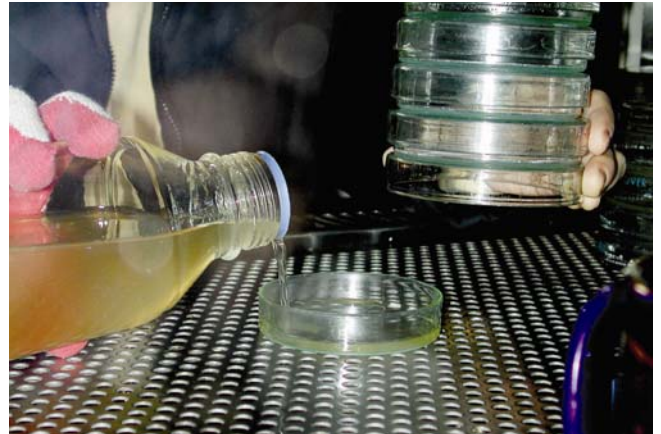


Figure 13. Pour the solution cooled at 50 °C into sterile petri dishes in clean bench.



Figure 14. Early signs of contamination

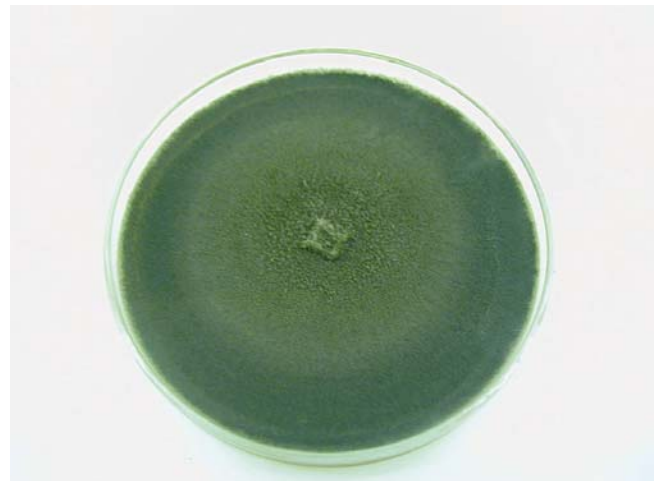


Figure 15. Petri-dish contaminated with green mold

3. Fill your pressure cooker/sterilizer with the correct amount of water (this will vary according to what model you are using-refer to manufacturer's instructions).
4. Place the flask in the cooker. If you are using autoclavable glass petri dishes, these should be sterilized in the cooker at the same time. If you are using disposable dishes, these are usually sterilized already, and the packet should only be opened in the clean room.
5. Place cooker on heat source, bring it up to the correct temperature/pressure-250°F /15 psi and sterilize for 45 minutes. Try to keep the temperature/pressure constant, otherwise the media will not work as well (Fig. 12).
6. Let the pressure reduce to zero-If using a domestic pressure cooker, place in the clean room for this time period.
7. In preparation for pouring your media, shower and put on freshly washed clothes. The aim is to reduce as much as possible the quantities of contaminants, particularly bacteria and lower fungi, that stick to you and your clothes and which could contaminate your dishes. It is impossible to remove them all-after all, humans are naturally 10% bacteria! If you can put on clothes that have not been exposed to the outside air since being washed, so much the better. Hair carries a lot of contamination-either wash it and tie it back (if long), or use a hair net/clean hair covering.
8. As agar media solidifies before pouring, clean the work surface in front of your laminar flow cabinet with isopropyl alcohol. Also, clean your own hands with the alcohol and continue to do this at intervals, until you have finished. Pour the dishes that are closest to the filter surface first. The clean air coming off the filter will pick up bacteria, skin fragments etc that are on you, and blow them away from the dishes. Do not put your hands between the filter surface and the open dish, or the air will blow any contaminants from your hands on to the surface of the media.
9. Lift the dish lid with one hand and pour the media with the other. Do not let your fingers hang over the rim of the dish or its lid, because skin fragments will cling and cause contamination. While the lid is raised, angle its underside towards the filter so that it only has clean air blowing on to it. At all stages, try not to breathe over the open dishes, as breath is laden with bacteria (Fig. 13).
10. When each dish has been poured, leave to cool and solidify. Don't hang around in the clean room for longer than you have to, as you are just potentially spreading contamination (Fig. 14, 15)!

Inoculating petri dishes

You will need an alcohol lamp for sterilizing the scalpel. If laboratory film (e.g. parafilm) is available for sealing the dishes, there is less chance of contamination. However, if the dishes are left in front of the laminar flow filters until they are colonized with mycelium, film is not essential.

1. Heat the scalpel tip in the alcohol lamp until it is glowing red (Fig. 16). Cool it by dipping it into the centre of the dish that you are about to inoculate.
2. Take your culture (having already loosened the lid) and open it without letting your fingers hang inside (Fig. 17). With the scalpel, tease out a wedge of mycelium, lift the lid of the dish and quickly transfer the wedge to the agar, immediately replacing the lid. This is more difficult than it sounds-the mycelial wedges have a huge tendency to cling desperately to the sides of the test tube, while refusing to be picked up by your scalpel! So don't worry if it's a bit of a mess to begin with, it just takes a bit of practice. The mycelial wedge is placed in the centre of the dish so that growth can radiate away from it.



Figure 16. Sterilizing transfer tools and the mouth of test tube by alcohol lamp.



Figure 17. Remove a loopful of agar culture from the test tube

3. If you are using laboratory film, immediately seal up the dish that you have just inoculated. If not, place it away from the immediate area where you are working, but still in front of the filter.
4. Repeat the above steps until there is no mycelium left in the test tube. A standard test tube culture can usually inoculate 2-3 dishes.
5. The dishes should be incubated at 24°C, or as close as possible. Oyster mushroom mycelium is usually quick to start growing; you should see fuzziness, the first sign of growth, within 2-3 days. The mycelium is usually white, and most strains should colonize the dish within 10 days (Fig. 18). If the uncolonized parts of the dish develop areas of different coloring, this most likely indicates some form of contamination, perhaps blue-green mould or yellow slime stuff. In this event, the affected dish should be discarded. If throwing it away is not an option, you can try to leave the contaminant behind, by transferring squares of healthy mushroom mycelium from the affected dish to a new, clean dish. But be warned that moulds in particular produce millions of spores, and any disturbance can cause these to become air-borne and contaminate your healthy mycelium and clean dish, or in a worst-case scenario, your whole clean-room. If this last happens, the only option is to empty the whole room and clean every inch. Clean rooms with a high air flow are less susceptible to this, as any spores are quickly drawn into the filters before they have a chance to settle.



Figure 18. Fuzzy growth on agar culture



Figure 19. Fully colonized pertri dish

When your dishes are fully colonized (Fig. 19), they can be used for 3 purposes :

- To inoculate further dishes of agar media. Follow the above procedures for making the media and pouring the

dishes, but instead of using a test tube culture, cut squares of roughly 1cm out of a healthy colonized dish and transfer one square to each new dish. 1 petri dish can easily inoculate at least 15 more dishes.

- To inoculate test tubes of agar media so that you have back ups of your strain
- To inoculate sterilized grain, resulting in grain spawn

Preparing Grain Spawn

Grain

Many different types of grain can be used. We use rye grain, which is a relatively soft grain that cooks easily without becoming too sticky and clump forming.

Spawn containers

Containers can be jars (glass or plastic) or plastic bags. It is essential that they are capable of withstanding pressure sterilization. It is easier to thoroughly mix mycelial wedges from your dishes through jars. Jars should be fairly wide-mouthed, for ease of inoculation, and have tight-fitting lids with a 8mm hole drilled through them. If filter discs are available, these should be fitted over the holes, on the underside of the lids. If not, you can cut out a layer of cardboard the same diameter as the lid, and fit this into the lid. Filter discs should be soaked for at least 1 hour in a 5% household bleach solution; cardboard discs should also be soaked, but for not as long - otherwise they will disintegrate! Soaking helps to dislodge hidden contaminants, which are then killed by the sterilization process.



Figure 20. Fully colonized grain spawn in the bag

Bags, if used, should be custom-made with filter patches (Fig. 20). If these are not available, it is best to use jars of some sort. Containers without a filter do not work very well, for reasons explained below, and it is difficult to improvise an effective filter for a plastic bag. Also, bags need to be sealed in some way after inoculation - if you have access to a proper heat sealer this is no problem, otherwise something else such as packing tape will have to be used, which is less effective.

The purpose of the filter discs/patches is to allow a low level of gaseous exchange. Mushroom mycelium needs a supply of fresh oxygen while it is growing, otherwise it will quickly become anaerobic (without oxygen) and contaminate, regardless of how clean the environment and materials. The filters allow oxygen to enter, without contaminants being drawn in. If filtration is a big problem for you, the only other option (that we can think of!) is to put a small hole in the top of whichever container you are using, and place it over a heat source. Hopefully, convection will keep gases moving upwards and carry potential contaminants away, whilst giving the mycelium access to oxygen.

Preparing the grain

1. Weigh out the amount of grain you require. The optimum moisture content of rye grain is roughly 50%, i.e. it will double in weight when cooked. 500g or less of dry grain per container is a good quantity to use when you are transferring mycelium from petri dishes to grain. Any more than this and it becomes difficult to mix the mycelial wedges through properly.



Figure 21. Sorghum seed



Figure 22. Grain pasteurization in a rice cooker

2. Grain will always contain hidden contaminants, no matter how fresh it is. Therefore, it needs to be pre-cooked, to release these contaminants that are then destroyed by sterilization (Fig. 22). Bring water to the boil in a large pan, and then add the dry grain. Simmer for around 30 minutes. It is properly cooked when still firm, but soft enough to squash. Any longer than this and the grain kernels will swell so much that they will ‘explode’, which makes spawn more susceptible to contamination. Once you have done this a couple of times, it becomes very easy to determine the correct consistency.
3. Drain the grain in colanders or something similar. If you are making large quantities of grain spawn, it is fairly easy to improvise a draining container—we have used plastic dustbins with holes drilled in the bottom and sides, and also, sheets of metal mesh rolled into a bin shape and attached on to a base. As long as the water can drain off to some extent rather than collecting at the bottom of the container and forming a big grain mush, there should not be a problem, as a lot of excess water is lost quickly to evaporation. Mixing the grain around at intervals helps this evaporation process. If the grain starts to shrivel, it is drying out too much!
4. If you have access to calcium sulfate (gypsum), the addition of this will help to stop grain kernels clumping together after sterilization. Mix through at a rate of approximately 4g of gypsum to 1kg of dry grain.
5. Fill your chosen containers with the grain. If using jars, only fill to 3/4 of their capacity. If using bags, fill to no more than 2/3 of their capacity (Fig. 23). Animal feed scoops are useful for filling containers. You can either use scales to make sure each container is receiving the same amount of grain, or you can use volumetric scoops.
6. Close each container. If using bags fold the excess plastic over the bag. Load the containers into the pressure cookers. If you can space them apart to some extent, sterilization will be more even and efficient. If you have to pack them tightly, remember that it is more difficult for the steam to penetrate the centre of a cooker full of densely-packed containers, and sterilization time may have to be increased.
7. Fill the pressure cooker(s) with the correct amount of water (refer to manufacturer’s instructions) and bring up to sterilization point—250°F/15 psi. We use jars or bags that are filled with 1kg each of cooked grain, and sterilize these for 105 minutes. Smaller quantities of grain in jars will require shorter sterilization times, e.g. 1L jars with 400g of cooked grain will probably only need 1 hour. If you are using bags and they are very tightly packed, they may need 3 hours. As this depends to some extent on your pressure cooker, you will have to experiment to find the optimum sterilization time.
8. When sterilization is complete, let the cooker return to zero pressure (if it does not form a vacuum on cooling,



Figure 23. Jar filled with the sorghum seed

place in the clean room for this). Leave for at least 2 hours so that the jars/bags are cool enough to handle, then unload and place in front of the laminar flow cabinet. It is very important to make sure that they are sufficiently cool, as overly-hot grain will kill the mycelium you introduce. If in doubt, wait until they are cold.

Inoculating the grain

Each colonized petri dish can inoculate up to 20 cups of grain. The more you add to each container, the greater the speed of colonization. We like to use 1/2 to 1 whole dish to 1 jar containing 1kg grain, as this results in very fast colonization. The quicker the grain becomes fully colonized, the less chance there is of it becoming contaminated.

1. Clean the work surface in front of the laminar flow cabinet with alcohol. As always when working in the clean room, you should have showered and be wearing clean clothes.
2. Take a petri dish and remove sealing film (if used). Heat scalpel tip in the alcohol lamp until it is glowing red. Lift the dish's lid and cut a criss-cross pattern of 9 or more wedges of mycelium. If you are not using laboratory film to seal the dishes, it is a good idea to leave a 5mm strip of mycelium running round the edge of the dish-this is in case contaminants have entered the dish from round the edge and fallen on to the outermost mycelium.
3. Replace the dish's lid and reheat your scalpel.
4. Get your container(s) ready for inoculation-if jars then loosen the lids, if bags then open out the mouth. Be careful at all times not to let your fingers hang inside the container.
5. Remove the dish's lid again, and, with the hot scalpel, pick up 1 or 2 wedges of mycelium. Quickly drop into the waiting container and repeat until the required amount of mycelium has been transferred.
6. Reheat the scalpel between each dish-if one of your dishes is contaminated, this will prevent the contamination from spreading across all your containers.
7. If you are using jars, then they need to be thoroughly shaken, so that the mycelium travels through the grain, resulting in even colonization. We find that a rolling and 'see-sawing' motion works well. Try not to let too many wedges stick to the sides of the jar; this again improves with practice.
8. If you are using bags, try to trap some clean air in each bag before sealing. Do this by holding the bag open with its mouth pointing toward the laminar filter, and then sealing. The trapped air helps the mycelium to colonize the bags quickly and healthily. Once sealed, each bag should be agitated so that the mycelial wedges move through the grain.
9. Incubate the containers at 24°C/75°F, or as close as possible. You should see some signs of growth within 3 days. After 7 days, shake the containers to distribute the colonized grain kernels evenly. The grain is fully colonized when it is completely white or off-white. This is the time when it is at its most vigorous, so use it as soon as possible! If it is left to grow further, it will quickly over-incubate, forming a solid lump that is incredibly hard to break up (Fig. 24).



Figure 24. Sorghum seed-based spawn

Your grain spawn can now be used for 2 purposes.

- To inoculate further containers of grain.

1 jar of grain can inoculate 10 times its own mass, so it is very easy to quickly turn small amounts of spawn into large amounts. As always, the more spawn you add, the faster the grain will colonize. (However, when inoculating a container of grain, beware of adding more than 20% of its mass, as growing mycelium produces heat and this will cause the grain to heat up too much and contaminate). Note that when using grain spawn

from a jar, it must be broken up first. If it is a glass jar, do not bang it against your hand in case of breakage and injury-use something springy or semi-hard such as a thick padding of towels on a solid surface. Spawn in bags must also be broken up, but this is easily done by squeezing and shaking.

- To inoculate the final bulk substrate, from which mushrooms will eventually be fruited.



Figure 25. Grain spawn colonizing sawdust



Figure 26. Grain spawn ready to colonize wheat straw



Figure 27. Oyster mushroom on heat straw column



Figure 28. Harvested oyster mushroom