Mushroom Cultivation For Remediation

A Radical Mycology Publication

For the planet and its inhabitants
For the fungi

Mushrooom Cultivation For Remediation: A Radical Mycology Publication | Ed. 1.0 | 2013
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Preface

This zine was produced by Radical Mycology, an organization that educates the world on the benefits of the fungal kingdom for personal, social, and ecological well-being. One of the main goals of Radical Mycology is to develop and educate the public on safe and effective protocols for emergency disaster response and environmental restoration. This zine and associated video (see below) are meant to begin achieving this goal by enabling the reader to easily and cheaply grow mushroom mycelium for large-scale fungal remediation and restoration projects.

This zine was designed with the novice mycologist in mind and is written in a highly simplified manner. To keep this introductory text approachable and easy to read we have left out a lot of information on the more complex theories behind fungal cultivation and remediation, focusing instead on core skills and basic concepts. The cultivation strategy given in this zine is one among many possible approaches and, thus, we encourage further study as well as suggestions for improving upon the approach presented.

We realize that despite our best efforts, some of this information will still be somewhat confusing to the beginner cultivator. Thus, this zine is meant to be studied in conjunction with a YouTube video entitled "Radical Mycology's Mushroom Cultivation for Remediation." We also recommend reading through this entire zine at least once before beginning your cultivation efforts.

This zine includes some simple, introductory remediation application ideas. Future publications will cover more in-depth design and application principles. For those seeking to learn how to grow mushrooms for food or medicine or for more in-depth studies on cultivation and remediation, check the recommended resources section of this zine and stay tuned for future publications from Radical Mycology.

Terms to Know

Fruiting body: The above-ground fleshy entity which most people associate with the term "mushroom."

Fungal restoration: The intentional use of fungi to build, stabilize, and "clean" the soil in healthy or damaged ecosystems and to enable forests to rapidly regenerate following a clear cut or other destructive practice. Also known as mycorestoration.

Fungal remediation: The use of fungi to reduce or eliminate chemical and biological contaminants from polluted systems. Also known as mycoremediation.

Mushroom: In this zine, we apply the term "mushroom" not just to fruiting bodies but also to the types of fungi that produce them.

Myceliate: To be overrun/consumed by mycelium. We use this word in place of the more offensive word "colonize," which is commonly used to describe this process.

Mycelium: A network of threadlike cells that comprise the majority of the fungal mass. The analogous tree from which the "fruit" of the mushroom grows.

Mycology: The study of fungi.

Saprotrophic: An organism that derives nourishment from decaying matter.

Spawn: Bulk mycelium growing on any carrier substrate intended to inoculate more massive substrates (e.g. grain spawn, sawdust spawn).

Substrate: Any material on which mushroom mycelium will grow (e.g. grains or sawdust).

Introduction

The study of fungal remediation/restoration is a relatively new field, dating back only a few decades. In that time, scientists around the world have proved that various mushroom species are excellent at reducing, eliminating, or accumulating harmful chemicals, microorganisms and heavy metals in controlled studies. However, very few well-documented experiments have been done outside the lab to test the effectiveness of these discoveries in the real world. This has left the layperson hard pressed to find any protocols that clearly define how to effectively remediate a given disaster scenario, such as an oil spill. Further, techniques of mushroom cultivation (for remediation purposes or otherwise) are often presented as incredibly complex and expensive, again leaving the average person unable to readily approach the topic. This limited access to information has led to a world that is largely uninformed about the potential for cheap and easy rehabilitation of damaged environments using fungi.

In response, the Radical Mycology project started with the goal of ending this problem through free education on these topics and the empowerment of the average person to actually put them to practice. Our goal in writing this zine is not only to teach about the basics of fungal remediation but also to encourage individuals and groups to begin experimenting with these concepts in an effort to help move this science forward. We believe that only when "citizen scientists" begin experimenting with fungal remediation and designing projects that address real world scenarios will this science begin to develop into a tangible reality for addressing environmental crises.

Mycology is a very young and understudied science and the practices of fungal remediation & restoration are even younger. Much has yet to be discovered in regards to the potential ways humans can ally with fungi and much work still needs to be done to begin healing the planet from centuries of environmental destruction. As the years progress, one can more readily see the negative effects of pollution and environmental destruction increasing in the world. Simultaneously, it seems that very little is ever done to effectively change these practices. Healthy forests keep getting clear cut and replaced by tree farms and international oil pipelines (such as the Keystone pipeline) keep getting proposed despite their potential to destroy pristine aquifers and water systems. We at Radical Mycology do not believe that fungal remediation is the solution to such problems. The true solution comes from eliminating these destructive practices and the conditions that enable them to exist (e.g. economic structures that require growth at all costs, poorly designed industrial systems, over-consumptive societies, and political climates that disable the individual from having their concerns truly heard). That said, we do believe fungal remediation is a powerful tool in the fight to save and restore the planet from the ecological problems that it faces. We see fungal remediation as a solution-based approach to countering the destructive practices of government and industry that should be used in conjunction with more front-line tactics of resistance such as projects to educate and increase awareness, direct action campaigns, and even legislation reform.

We see the fungi and their mycelium as great teachers on the road of human evolution. As incredible healers and stewards of their environments, fungi exemplify many of the ways that humans should integrate with each other and the natural world. Through working with the fungi in remediation projects we hope that you not only create positive change in the world but also within yourself. By directly transforming and improving the land, the remediator can begin to purge negative beliefs surrounding their ability to make a difference and become empowered in the process. And through learning about and working with mycelium, one can readily realize the beauty and strength found in decentralized, collaborative systems.

Saprotrophic Basidiomycetes

Fungi are found in every corner of the planet, from the permanently frozen tundra of the north to Sahara desert. This branch on the tree of life includes species that form a variety of complex symbioses with plants, others that facilitate succession in the forest canopy by killing off weakened trees, and still others that recycle the organic matter of the world. Found in our stomachs and on our skin, fungi are integral to essentially all natural lifeforms and their incredible diversity in the world deserves all the respect they are given.

The fungal kingdom is divided into many sub-groups based on variations in lifecycle and ecological niche. In this zine, we will focus on the *saprotrophic basidiomycetes*, a group of fungi that includes the mushrooms most commonly used for remediation (e.g. Oysters, Turkey Tails, and Shiitake). Saprotropic means that the mushroom is a decomposer. Basidiomycete refers to the specific way that the spores develop in the mushroom. It is recommended that you come to understand the saprotrophic basidiomycete lifecycle before beginning your hand at cultivation or remediation so as to best understand what aspects of nature you are trying to mimic throughout your trials.

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We begin with the spore. Spores prolifically develop on a microscopic layer of fertile (spore-producing) tissue known as the hymenium. This tissue develops in mature mushrooms on the surface of structures called gills, teeth, or pores, which themselves are often found underneath the cap of a mushroom. A given mature mushroom can produces millions, or even billions, of spores in a single day, all of which are ejected from the mushroom at an incredibly high force to enter their surrounding environment. When a given spore lands in a habitat with food and water, it quickly germinates, producing a single-cell filament, or hypha (plural hyphae), which begins to grow through its substrate, or food source, in search of a genetic mate. Like the sperm and egg of animals, spores contain only half the genetic information of their parent and thus need to join with the hypha of another spore in order to be genetically whole.

Once the spore's hyphal network encounters a mate, the two fuse into a joined system, which is then referred to as mycelium. This mycelium now has all the genetic information it needs to successfully grow through its environment and ultimately produce mushrooms. As the mycelium grows through its substrate, this thread-like structure continuously branches in all directions, forming an incredibly dense network (imagine a web with clearances smaller than any woven structure humans can produce) in the search for water and food. In the case of the saprotrophs, as the mycelial tips encounter organic matter (such as a fallen tree) they exude a mixture of complex enzymes upon this material in order to break it up into a usable form. These enzymes essentially turn the large molecules of this foodstuff into smaller molecules that the mycelium then sucks up and metabolizes. One of the main energy sources for these fungi is the long chain-like molecule of cellulose (the fibrous stuff that makes up the walls of plant cells). Saprotrophs have developed an array of enzymes that can readily snip this long chain in to simpler, shorter carbohydrates (mostly sugars). Some saprotrophs have even adapted to break down lignin, the highly complex compound that makes wood hard and rigid, something few things on Earth are able to accomplish.

If the fungus runs out of food or a change in environmental conditions arise (e.g. a temperature drop & increase in humidity), the mycelium will be triggered to produce a mushroom and will start to accumulate in to numerous tiny pinheads, or primordia. These primordia will soon develop as 3-dimensional structures of mycelium into what are called fruiting bodies or, more commonly, mushrooms. After just a few days the mushrooms will have matured and will begin to drop millions of spores to continue the lifecycle anew.

Cultivation Overview

Using fungi for remediation purposes requires a large amount of mycelium. And, really, the more the better, as the mycelium (and its digestive enzymes) is what does the bulk of the cleanup work. Fungal remediation is possible because the enzymes that saprotrophic mycelium produce to digest trees and other organic matter can also be used to break apart big toxic molecules into smaller and less toxic chemical structures. This has been shown to be possible with many persistent chemicals such as dioxins, diesel, herbicides, TNT, and DDT. Thus, it is the goal of the DIY remediator to grow large quantities of mycelium for use in installations. Acquiring this mycelium is the greatest challenge to the remediator as an accessible abundance is not readily found in nature. Thus, the choice becomes to either buy commercially cultivated mycelium (a rather expensive route to take) or to learn how to grow your own. The good thing to know is that growing just mycelium is the easier and cheaper part of the mushroom growing business. The harder and more expensive stage comes when attempting to provide the precise conditions needed to induce fruiting in the mushroom. Luckily for remediation purposes, we are not concerned with getting edible mushrooms but with applying large amounts of mycelium to our damaged environment.

Cultivating mycelium is simply a series of feeding cycles. At intervals of roughly 2 weeks, the cultivator provides their mycelium an increasing amount of moist, nutrient-rich foodstuffs (aka substrates) to both increase the size of the mycelial network and to enable the mycelium to successfully survive and adapt to a given remediation installation. The cultivator must work in a clean manner to ward off competitive bacteria and fungi that will readily, and happily, consume the provided substrate. Traditionally this required working in an impeccably clean laboratory-type setting that was not easy or cheap to create. The techniques described in this zine, however, were chosen for their ability to be cheaply done in a non-sterile environment, such as a kitchen, thereby making cultivation much more approachable to the average person.

The key stages to the approach offered in this zine are as follows:

1) A small amount of source mycelium (taken from a fresh mushroom or acquired commercially) is first introduced to a slightly sugary water broth. This broth is then aerated by the cultivator daily as the mycelium begins to grow inside of it.

2) 7-21 days later, once the mycelium has grown into a visible mass, a small amount is transferred to a container filled with sterilized, and nutrient-rich grains.

3) 10-21 days later, the grains will have been consumed by the mycelium and are then transferred to a final sawdust-based substrate to grow.

4) The mycelium then consumes this final substrate over a matter of weeks or months and is then used as the final inoculant for a given remediation/restoration project.

You should realize up front that many people have problems with contamination or slow/no mycelial growth in their first few attempts at cultivation. But if you go into the process knowing this fact and are ready to face that inevitable day, you will be that much more resilient when the cold green face of *Trichoderma* mold stares you in the eyes from your contaminated projects. Only through experience, following the growth parameters and sterility considerations outlined here, and developing a sense of play, will you find what works best for you.

Equipment Checklist

Below are the items needed for the techniques outlined in this zine. Many items can be found used on eBay, craigslist, at garage sales, or home made. Mushroom cultivation doesn't have to be expensive, especially if you start a mushroom group, collaborate on projects with other organizations in town, or even work with a university.

Cheap / Free	Notes
__ 3 Mil Large Trash Bags	
__ 5 Gal Bucket	
__ 70% Isopropyl Alcohol (ETOH)	Found at pharmacies. Isopropyl alcolhol is also called ETOH in this zine.
__ Alcohol Wipes	Can also use cotton balls sprayed with ETOH.
__ Aluminum Foil	Caps for jars made with foil are reusable
__ Burlap Sacks	Found at coffee shops
__ Canning Jars & Lids	Jars need not be official canning jars. Jars from store bought food that can withstand pressure cooking are fine.
__ Colander	
__ Large Pot	
__ Large Tarp	
__ Needle and Heavy Thread	
__ Polyfil	Found at fabric/craft stores
__ RTV / High-Temp Silicone	Found at hardware stores
__ Rubber Bands	
__ Scissors	
__ Spray Bottle	
__ Toilet Paper Tubes	
__ Trash Can	
__ Well or Pond Water	

Media	Notes
__ **Dextrose**	AKA corn sugar. Found at beer brewing shops or health food stores.
__ **Gysum**	Found at beer brewing supply shops.
__ **Wood Chips**	Ideally felled in the winter and chipped within 2 months of use. Hard to find in some areas. Try arborists, power companies, & parks departments.
__ **Hardwood Sawdust**	Try landscaping supply companies or furniture factories.
__ **Light Malt Extract** (LME)	Found at beer brewing supply shops.
__ **Rye, Wheat Berries, Spelt, or Millet**	Organic preferably.
__ **Oragnic Straw**	NOT HAY! Found at feed stores.
__ **Well Water**	This is ideal. If your only main water supply is tap water, leave a pot of it out, uncovered, for 24 hours to allow the chlorine gas to evaporate out.

__ 22 & 16 Gauge (Ga) Luer-Lok Needles	These can be harder to find, depending on local laws. Call around to pharmacies and needle exchanges.
__ 10cc & 60cc Syringes	Syringes can be found cheaply on ebay and at some pharmacies. "Single Use" syringes can actually be re-sterilized via pressure cooking.
__ Digital Scale (optional)	Used when determining moisture content of substrates.
__ Drill & Bits (3/16" & 5/16")	
__ Liquid Culture Syringe	Ordered from mushroom growing websites.
__ Polypropylene Mushroom Grow Bags	For the techniques in this zine you can use cheap bags from the online company Uline. Their 8 x 4 x 18" 2 Mil bags are a good size to start with.
__ Pressure Cooker	A good pressure cooker is crucial to successful cultivation. There are a variety of brands, designs, and sizes available. Used ones can be found at thrift stores and garage sales though all parts should be inspected for cracks or excessive wear. A cracked pressure cooker can explode!

Airport Lid Prep

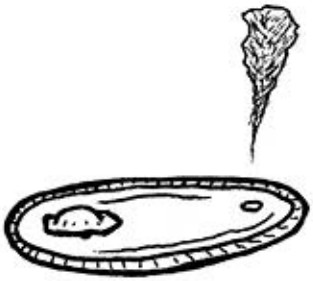
Once materials have been gathered, the first thing you'll need to do is modify your canning jar lids to have an air filter that allows the mycelium to breathe and keep contaminants out. You will also create a silicone "airport injection site" through which mycelium will be introduced and extracted between jars of substrates. This injection port concept was invented by user Hippie3 on the online forum Mycotopia. RIP, Hippie3.

1) Drill two holes in the lid on opposite sides: 3/16" and 5/16". These are suggested hole sizes.

2) Cover the 5/16" hole on both sides with a big blog of high-temperature RTV silicone and position the lid so that the silicone can dry for 24 hours.

3) After 24 hours, take a tuft of Polyfil and twist it through the smaller hole to the point that it fits snuggly and wont fall out.

4) Cut off any excess Polyfil.



Sourcing Mycelium

Before you can begin cultivating you must acquire some amount of initial mycelium. It is from this source mycelium that all your future mycelial lineages will develop. Two easy sources are given here:

Order a liquid culture syringe from an online vendor - For roughly $20 you will receive a 10mL syringe filled with a small "cloud" of mycelium suspended in a sugary liquid. This syringe can then be easily expanded to make a much larger quantity of liquid culture. This is the recommended option for the beginner. **Pros**: reduced risk of contamination, selection of exact species. **Cons**: cost, the genetics of the mycelium may not be well suited to your environment.

Clone a mushroom - As mushrooms are condensed mycelium, it is possible to use a small piece of the inner tissue of a fruitbody as your source mycelium. **Pros**: cheap; if wild harvested, the mushroom is likely to be better adapted to your environment. **Cons**: not super easy to clone, higher risk of contamination, access to species depends on season and climate.

How to Use a Pressure Cooker

Good pressure cooker (PC) usage is essential to effective and safe cultivation. This is the first skill that must be learned before all others. Below are generic instructions for operating a pressure cooker, follow your pressure cooker's manual if it has one.

1) Put 1" of cold water in the PC. Some pressure cooker manuals will tell you to use more water. You need to use enough water so that by the end of your pressure-cooking there is still some water left at the bottom of the pressure cooker. Never run the pressure cooker dry! Place jars on the rack. Fasten the PC lid securely.

2) Leave weight off vent port or open the petcock. Turn the heat on at a setting from 2/3 to the highest setting until steam flows from the petcock or vent port. You don't want the pressure cooker heat up too fast, since this can cause certain types of jars to break. Adjust the heat so that it takes around 15 minutes for the steam to begin to come out of the vent. The bigger the pressure cooker, and the more filled it is, the higher setting you can use.

3) Once steam starts to produce a heavy jet out of the vent, let it jet out for 5 minutes and then place the weight on the vent or close the petcock. The PC will pressurize during the next 5 to 15 minutes, depending on the size of the PC (the bigger the PC, and the more jars are in it, the longer it will take).

4) Start timing the process, depending on the pressure cooker type:
- when the pressure reading on the dial gauge indicates that the recommended pressure has been reached or
- when the weighted gauge begins to jiggle/ rock or
- when the valve comes up to the of second or third (= last) ring or
- when the steam is starting to get released from the pressure setting dial.

5) Adjust heat lower to maintain a slow, steady rocking motion, or the pressure indicator staying at the proper ring, or there is no steam coming from the pressure setting dial, depending on the pressure cooker type.

6) When you've cooked your jars for the recommended time at the right pressure, turn off the heat, and let the PC cool and de-pressurize at room temperature. Do not force-cool the PC under water or force the steam out! Just walk away and let it cool completely (meaning the dial needle moves back to "0" or no steam escapes when weight is gently nudged).

Dealing With Contamination

Contamination happens. It is a sad reality for the cultivator that despite best efforts, projects fall victim to the onslaught of fierce and hungry bacteria and molds. To avoid this as best as possible an awareness of all avenues of entry for competitor bacteria and fungi is key to lowering contamination rates. In general, the main causes of contamination can be categorized in to the following 7 groups.

THE CULTIVATOR
Cleanliness of your body and person is the first step to contaminant avoidance. The cultivator would ideally be freshly bathed and wearing freshly washed clothing. Depending on the stage and scale of work, wearing a facemask, gloves, tyvex arm sleeves, and a hairnet might be pursued.

THE AIR/ENVIRONMENT
The space where fungi are growing and where sterile transfer work is done must be as clean as possible as well. The ideal space would have a HEPA filter employed to filter the air. Failing that, a good misting of the air with 70-80% alcohol, 10% bleach, or Lysol disinfectant 3-5 minutes prior to working will help clean the air of particulates. The room should lack carpeting, be easy to clean, and cleared of all sources of mold, mildew, rodent infestation, or any other nasty elements. In the fruiting space, pockets of stagnant air should be avoided as this encourages mold growth.

THE SUBSTRATE
The substrate you provide must be properly pasteurized of sterilized. If not treated to recommended time guidelines, your substrates will undoubtedly contaminate. For grains, presoaking may help to germinate the endospore form of some bacteria (bringing them out of hibernation), which they might otherwise use to withstand pressure-cooking.

THE INOCULUM
Your fungus can be a source of outside contamination as certain bacteria can reside on the microscopic surface of mycelium. Cloned wild mushrooms may have this issue as can sterile tissue that was transferred and/or stored improperly.

THE TOOLS
All of your tools used to transfer mycelium, the surfaces they touch, and the outside of any vessel should be as clean as possible. Despite best efforts to maintain sterility, all surfaces must be considered covered in bacteria and treated as such.

PESTS
Flies and mites are definite vectors for contamination and can easily spread such problems from one mushroom to another. While fly tape and pet tree frogs are options to help reduce these vectors, the initial source of entry for the pests must be identified and controlled.

TECHNIQUE
The use of conscientious, quick, controlled movements and habits in the practice of sterile mushroom cultivation is essential for high success rates. At every stage the cultivator must be aware of the impacts of each motion in their ability to transfer unseen microorganisms. The concept of technique extends from the care with which the cultvator works to the maintenance of the space worked within.

The above are ideal considerations. If you experience low contamination rates, don't bother worry about making changes to your practice. If the opposite is true, try to follow the above guidelines as closely as possible.

Viruses, bacteria, molds, and sometimes yeasts are your main competitors in the world of cultivation. In general, anything that looks or smells unlike clean and white mycelium should be considered a contaminant. See this link for a breakdown of the most common contaminants:

www.shroomery.org/5276/What-are-common-contaminants-of-the-mushroom-culture

Liquid Culture

Once you've sourced your mycelium and made your airport lids, the first step in the actual cultvation process is to make a large quantity of Liquid Culture (LC) which will be used to inoculate your other substrates. You now need to prepare jars of sterile sugary water to inoculate with your source mycelium.

Preparing Sterile Liquid Culture Broth

1) For every quart jar of LC you wish to make, mix 1 TBSP corn sugar (aka dextrose), 1 TBSP Light Malt Extract (LME), and 1 pint non-chlorinated water together in a pot on the stove top at low heat. Stir until dissolved. Do not boil or caramelize the sugars while heating as this can be toxic to the mycelium.

2) Pour this sugar water into canning jars that have been thoroughly cleaned.

3) Fill the jars half way with the sugar water, cover with an airport lid and an aluminum foil cap.

4) Pressure cook at 15psi for 20 minutes.

5) Allow to cool inside the pressure cooker overnight.

Note: *There are many LC recipes on the internet. The most important points to consider are that the correct types of sugar are used and that the concentration of sugar is no more than 4% (4 grams of sugar per 96 mL water). This is barely sweet to the human tongue but is plenty for the fungus. Any more is toxic to the mycelium. Household table sugar (sucrose) shouldn't be used.*

INOCULATING YOUR LIQUID CULTURE

Now that your media is prepared, it is time to inoculate it. This step of introducing mycelium into the sterile sugar water must be done with the utmost care and cleanliness. All it takes is one spore or a single bacterium to contaminate your entire jar of sugar water.

Inoculating with a purchased LC syringe

This method simply takes the small amount of LC that comes in the commercially acquired syringe and expands it by putting that mycelium into more sugar water to grow thru.

Materials:

- __ LC syringe bought online
- __ 1 canning jar (or more) filled with dextrose/MEA water that has been pressure cooked and cooled.
- __ Airport lid(s)
- __ Aluminum foil
- __ Spray bottle filled with 70% ETOH
- __ Alcohol wipes

Method:

1) Wash hands and spray them with alcohol.

2) Wipe off the injection port on the LC jar and spray with alcohol, then quickly insert the needle from your LC syringe into the injection port and inject 2-3mL of the LC.

3) Repeat if multiple jars are used. It is recommended to spread the 10mL syringe to at least 2-3 jars of new LC to try and ensure that at least one does not become contaminated.

4) Label each jar with species and date before incubating.

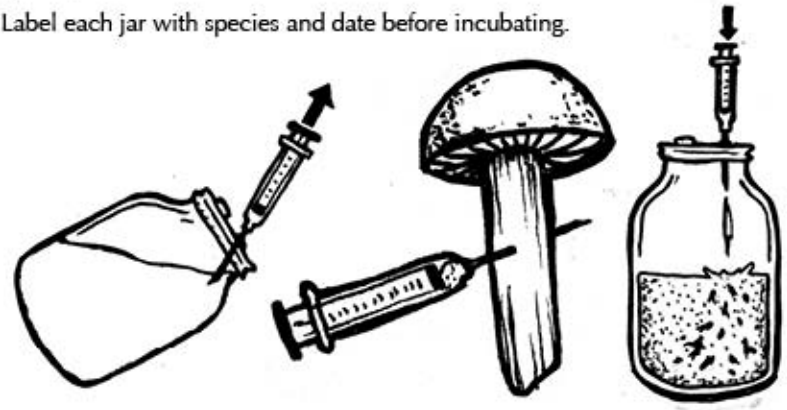
In this approach we will be taking mushroom tissue directly from a fresh wild or store-bought mushroom. If the mushroom is from the wild there is the risk that bacteria may be growing inside of the mushroom, which can subsequently contaminate your LC. So be cautious! This technique is more difficult and only available to people that can correctly identify wild mushrooms.

Materials:

- __ 1 jar of non-chlorinated water & an airport lid
- __ 1 canning jar (or more) filled with dextrose/MEA water.
- __ Airport lid(s)
- __ 1 fresh mushroom
- __ 1 syringe with 16 Ga Luer-Lok needle
- __ Aluminum foil
- __ Spray bottle filled with 70% ETOH
- __ Alcohol wipes

Method:

1) Wrap your syringe and needle in aluminum foil, cover both jars in foil. Pressure cook both jars and the syringe at 15psi for 20 minutes.
2) Once cooled, remove and unwrap your jars and syringe. Swirl/vortex the liquid for 1 minute to oxygenate it.
3) Wipe the injection port on the water jar with an alcohol wipe and spray with alcohol. Insert the syringe into the jar of sterile water and draw up at least 5ccs. This water will help shoot out your mushroom tissue in step 5.
4) Wipe the mushroom stem or cap thoroughly with alcohol and insert the needle. You want to check the needle after stabbing the mushroom to see if you got a small piece of tissue in the needle. If you don't get it the first time, keep trying. This can be kinda tricky.
5) Wipe off the injection port on the LC jar and spray, then insert the needle and inject your mycelium chunk and water into the LC.
6) Label each jar with species and date before incubating.



GROWTH & AERATION

As the mycelium grows through the liquid culture, air will need to be replenished. As well, the mycelium needs to be broken up so that it can later be extracted with the syringe needles. The easiest and cheapest way to do this is to simply swirl the LC, creating a vortex as best you can, once a day for 30 seconds. Be careful not to wet the Polyfil filter as this can enable contaminants to grow from the outside inwards through the filter. The jar should be ready to use in 2-4 weeks. Really, as soon as a visible "cloud" of mycelium is present, it can be extracted and moved on to grains.

Grain Spawn

Once you have amassed some mycelium in your liquid culture, the time has come to transfer the mycelium to a jar of properly cooked and sterilized grains. These grains will provide a large amount of nutrients and minerals to feed the mycelium and, once engulfed by the mycelium, will serve as a jar full of individual "seeds" of inoculum for the next stage in the process.

PREPARATION OF GRAINS

There are a variety of grains used to grow mycelium. Rye, millet, and wheat berries are commonly used in the professional world. But spelt, popcorn, whole birdseed, and milo can also be used as well.

Regardless of the type used, your grains must be properly cooked and sterilized so that the mycelium can readily enter and consume them before competitors can. There are many approaches to preparing grains for cultivation. Some people will simply mix water and grains in their jars and pressure-cook them immediately as such. Others prefer to soak their grains for 12-24 hours beforehand. This pre-soaking helps to germinate the dormant endospores of bacteria inside grains so that they become more susceptible to the heat of the pressure cooker (thereby decreasing chances of contamination). Others rinse their grains several times to rid them of any dirt and debris that may make the grains stick together while also harboring contaminants. However you choose to do it, these factors must be your guiding principles:

Evenness of moisture content between grains (none too wet or dry, all ideally around 50% saturated)

Minimum number of sprouted or burst kernels (which are much more readily consumed by competitors).

Ease of breaking the grains up once consumed by the mycelium (i.e. not making sticky grains)

Sterility!

One preparation process described here is for 10 quart jars of organic rye grains.

Materials:

- __ 10 clean canning jars, airport lids, and aluminum foil caps.
- __ 10-11 cups of dry whole rye grains
- __ 1 TBSP Gypsum
- __ 1 large pot with lid
- __ Colander
- __ Large spoon
- __ Pressure Cooker

Method:

1) Measure out 10-11 cups of dry rye in a large pot.
2) Fill the pot with hot water and swirl the rye around in order to remove the dirt and debris from the mix. Drain off water and repeat until water runs clear.
3) Cover grains with chlorine-free water or well water.
4) Add 1 TBSP of Gypsum (adds beneficial calcium and sulfur), stir, and let sit covered for 12-24 hours.
5) Place pot on the stove and bring to a boil for 5-10 minutes.
6) Drain grains through a colander and stir around until the grains have cooled and all the excess moisture has steamed off the grains.

7) Add roughly 1/10 of this mixture to each quart jar (about 1 pint each).

8) Screw down an airport lid and cover it with an aluminum foil cap.

9) Pressure cook for 60-75 minutes at 15psi.

10) Turn off the stove and let the pressure cooker cool over night.

11) Open the pressure cooker and remove the jars. Inspect each jar for cracks and/or an excessive number of burst kernels. If you get a lot of burst kernels change your grain prep process to reduce water content. A few burst kernels is fine.

12) Shake the jars to break apart the grains and to distribute the wetter and drier kernels.

TESTING MOISTURE CONTENT

If you use a different method of grain preparation, you should consider measuring the moisture content of your grains after preparation. To do this, simply measure out 100 grams of prepared grains and then place them on a baking tray in a 350°F oven for 20 minutes or until bone dry. Then weigh out these grains a second time. The difference in weight will correspond to the moisture content created by your preparation method.

> **Example**: *If the grains come out to be 40 grams after drying, then 60 grams of water was lost and the preparation method you had used created a 60% moisture content in the grains.*

A more subjective test for moisture content is to simply bite through an individual grain. If it is easy to bite through with no hard interior but is not so cooked that the kernel is squishy or falls apart, then it is probably fine.

GRAIN SPAWN INOCULATION

Materials:

- __ LC jar with ample mycelium
- __ 60 mL syringe with 16 Gauge needle (fresh or re-used)
- __ Aluminum foil
- __ Sterilized grain jars with airport lids
- __ Spray bottle filled with 70% ETOH & alcohol wipes
- __ Lighter

Method:

1) If you are not using a fresh syringe and needle, be sure to sterilize these tools by wrapping them in aluminum foil and pressure cooking for 15 minutes at 15psi.

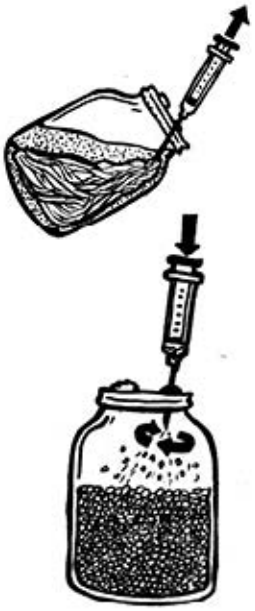
2) Wipe down all injection ports with an alcohol wipe and spray them with alcohol. Uncap your needle and quickly insert it into the LC jar.

3) Draw out 3-10 mL of LC per quart jar of grains. Be sure to actually draw out mycelium and not just the sugar water.

4) Quickly remove the needle and insert into your first grain jar. Inject 3-10 mL per jar in a swirling fashion to distribute the mycelium. Using more LC will result in faster growth.

5) Repeat with each jar. If you run out of LC in your syringe and need to extract more, be sure to flame sterilize the needle before extracting more mycelium. This is to ensure the cleanliness of your master jar.

6) Label each jar before incubating in a warm (70°F-ish) place where they wont be disturbed.



From here check the jars every couple of days for signs of contamination. This will likely come in the form of discoloration (e.g. black or green molds) or greasy bacterial splotches. If this is your first time using your LC you will need to test each LC jar on several grain jars to ensure that there is no contamination in the LC. If all of your grain jars contaminate in the same way, it is likely that your LC was contaminated to begin with and you will need to start over, making fresh LC. If only some of the grain jars contaminate then something went wrong in the inoculation process. This is why it is recommended to make several LC jars with your source mycelium, just in case one or more become contaminated you will hopefully have one that is clean. Once you know that an LC jar is not contaminated it can last you for a very long time. You can use this "master" jar to expand to more LC jars and, in between, it can be stored in the fridge for 6-12 months.

Once the mycelium has started to grow over roughly 1/4 - 1/3 of the grains, give the jar a shake to distribute the myceliated kernels and to speed up the rest of the process. In 2-4 weeks, the grains will be fully consumed by the mycelium and are then ready for their final transfer.

Sawdust Spawn

From grain spawn the cultivator now moves the mycelium on to pasteurized hardwood sawdust. Like the grains, this sawdust must be properly hydrated and cooked prior to inoculation to ensure the best chances of success. Once myceliated, this pasteurized sawdust spawn is what will be used to inoculate your remediation installations.

Pasteurizing means that your substrate is only heated to 140-170°F for an hour, as opposed to the high (250°F) sterilizing temperatures obtained by pressure cooking. Pasteurization kills the mesophilic organisms that normally live in the 68-113°F temperature range. Pasteurization kills many immediate competitors that may either overwhelm your mycelium or slow down its growth while leaving alive beneficial microorganisms (mainly bacteria) that help guard the substrate against other contaminants, such as molds. Some bacteria and molds still survive however, so we recommend using a high inoculation rate (i.e. a lot of myceliated grains) with your grains to ensure rapid sawdust myceliation.

Alder and maple are the most commonly used types of wood. Not only are these trees more easily acquired as a commercial product but they are also softer types of hardwood, which the fungi can break down faster than the harder hardwoods like oak. Most species of cultivated fungi can use these two types of wood with a minority of mushroom species also being cultivatable on softer coniferous wood, such as douglas-fir. Other hardwoods can also be used, depending on the mushroom species. Such specifics are best referenced in the cultivation books listed in the recommended reading at the end of this chapter. Alder sawdust may be available for cheap from a landscaping supply company, wood mill, or furniture manufacturer. Shop around.

PREPARING SAWDUST

Sawdust needs to be first moistened before it is pastuerized and inoculated. The ideal moisture content for sawdust spawn is 60-65%. To achieve this, you will need to spray down the material until it is wet enough to hold its form but will also easily break apart. You want the sawdust to feel something like fresh potting soil: not too dry, not too wet. One way to achieve this is to set up a window screen on two blocks. On this screen, pile a layer of sawdust 2-3 inches thick and mist it with a garden hose, stopping often to mix the water in and check the moisture level. Then wait at least 60 minutes for the water to absorb into the sawdust. You can also wet your sawdust in a bucket if you don't want to set up a screen. Drier sawdust is better than sawdust that is too wet. Sawdust that is too wet leads to standing water which encourages bacteria to grow and also becomes too compacted for the mycelium to easilty travel through. In time, you will quickly learn to feel when your sawdust is sufficiently saturated.

Materials:

__ Polypropylene mushroom bags
__ Dry Hardwood Sawdust
__ Weight
__ Thermometer

Method:

1) Fill the specialty bags ½ full with hydrated sawdust and tie off with a loose knot. Insert a thermometer into the center of one of the bags. Place the bags in a pot of water on the stove and hold them down with a weight on top.

2) Turn on the heat to the stove and bring the temperature of the sawdust up to 120°F (this may take an hour or more).

3) Turn off heat and observe the temperature of the sawdust, which will continue to climb. Once the thermometer reads 140°F, start your timer. You will want to keep the temperature of the sawdust at 140-160°F for 1 hour. If the sawdust temperature drops below 140°F in that hour, turn the heat back on to bring it in range.

4) After an hour, remove the bags from the pot and allow to cool overnight in a clean environment.

An alternate pasteurization method outlined below is somewhat easier than the above technique however it leaches some nutrients from the sawdust and does not easily control for moisture content. For these reasons it is not preferred.

Materials:

__ Freshly laundered pillow case
__ Dry Hardwood Sawdust
__ Large Pot
__ Thermometer
__ Timer

Method:

1) Fill the pillowcase with dry sawdust, insert a thermometer into the center of the sawdust, and submerge the pillow case in a pot full of water pre-heated to 170°F. Place a weight on top of the case.

2) Once stabilized, the sawdust temperature should be in the 140-160°F range. If not, turn on the heat until the thermometer reads within range. Once in the 140-160°F range, set your timer for 1 hour. If the sawdust temperature drops below 140°F in that hour, turn the heat back on to bring it in range.

3) After an hour, withdraw the pillowcase and hang to drip dry and cool overnight.

4) The next day, with clean hands, give the pillowcase a good squeeze to draw out excess moisture. You want the sawdust to be somewhat light and fluffy. It is now ready to use.

SAWDUST SPAWN INOCULATION & MAINTENANCE

Once the sawdust bags have cooled over night you can inoculate them with your grain spawn. This can be done in an open environment, such as a kitchen. Simply take your grain spawn jar and shake it until the myceliated kernels break apart into individual grains. Quickly open the sawdust bag and grain jar and pour ¼ to all of the grains into the bag. Close the bag and shake for a minute to evenly distribute the grains as best as you can. Place the opening of the bag thru a toilet paper tube or similar object, fold the top of the bag back, and stuff the opening with Polyfil. Label the bag with species and date. Place this bag in a warm place where it will not be disturbed. After 1-3 months or so, the mycelium will have grown out and will be ready to use for installation.

Bulk Substrates

Now that you have bulked up your mycelium and actually have something substantial to work with, you can move on to the final stage of giving the mushroom a longer term source of food. With this last stage the fungus will now have something on which it can feed for months or years while it starts taking care of your pollution problem. Whether you are trying to clean up a polluted waterway or dirty soil, one of the most effective and versatile options is to create bunker spawn. This simply refers to burlap sacks filled with a combination of sawdust spawn, wood chips, cardboard, and pasteurized straw.

As with sawdust spawn, the age and species of wood chips you use should be appropriate to the fungus you are working with and will ideally have been pre-soaked in non-chlorinated water for 24 hours to increase their moisture content. The straw you use will need to be treated against contamination, as it is rather susceptible to attack from competitors. Traditionally, this called for the extended submersion of the straw in large vats of heated water to pasteurize the material. However, in recent years, a process known as "Cold Water Fermentation" has been developed to cut out this needless waste of fuel and bring the ease of cultivation one step closer to the average cultivator.

COLD WATER FERMENTATION

This technique kills off competitors to your mycelium by the simple act of submersion of your substrate in water over a period of days. During the submersion process the anaerobic bacteria present on the straw thrives by eating all the aerobic (oxygen loving) bacteria. When the water is removed after a week, the anaerobic bacteria die, leaving "clean" substrates to use for inoculation. Here is the process when using straw.

1) Line a garbage can (or any hard, upright container) with a heavy duty (3 mil) trash bag.

2) Wet the inside of the bag down with a bit of water. Ideally, this would all be non-chlorinated water or, better yet, well water. Alternatively, if you leave tap water out for a day, the chlorine gas will evaporate out of it.

3) Fill the bag with dry straw that has (ideally) been chopped with a weed whacker into 2-3" pieces. The increase in surface area provided by the use of this machine will enable better myceliation and easier handling later on. Simply place the straw in a dry trashcan, insert the weed whacker, and chop away.

4) Place the chopped straw in the can with the trash bag and fill with water, covering the straw.

5) Add a clean weight on top to of the straw to keep it submerged.

6) Put a lid on the can and keep it in a warm place, ideally in the sun.

7) Wait 7-10 days, depending on ambient temperature.

8) At this point the water should be discolored and stinky. This is good, it means that your substrate is ready. You will now want to turn the can upside down and drain the water off. Once empty, twist up the top of the trash bag and turn the can upside down to allow the remaining water to drip off for 2 additional days.

9) At this point the straw is ready to be used in a mix of Bunker Spawn (see next page).

This same process can also be used for woodchips that are not very fresh. Further, experimentation is showing some success using this soaking technique with coniferous woodchips. Normally conifer chips are not favored by the remediative fungi. However, If you soak conifer chips for a week, dump the water, soak for another week, then dump this water you will have leeched out many of the compounds and resins that would nornally have inhibited mycelial growth. This is great news for people that have a hard time accessing hardwood chips.

Bunker Spawn

Now that you have treated your straw, you are ready to create bunker spawn. In its simplest and quickest form, bunker spawn bags can be made by simply mixing hydrated wood chips with sawdust spawn together and then stuffing it all in a burlap sack. The steps below, however, describe a more thorough process that provides the mushroom with a more successful recipe.

1) Soak pieces of clean, ink- and tape-free corrugated cardboard in hot water until saturated. Let sit for now.

2) Wash your hands and follow any cleanliness procedures desired.

3) Lay out a large tarp and clean it off. This could be as simple as spraying it down, though the more sterile conscious will wipe it down with 80% isopropyl alcohol for added cleanliness.

4) Spread fermented straw and wood chips that have been soaked in non-chlorinated water for 24 hours together on a tarp. Depending on your needs you might add more straw than chips, or vice versa. The former provides for faster myceliation, while the latter with a more long-tern food source.

5) Break up the bags of sawdust spawn and dump most of their contents over the straw and chips. Observe a roughly 1:10 (by volume) suggested inoculation ratio, but work with what you got. Reserve a small portion of the spawn for step 7.

6) Mix all of this together thoroughly by rolling it back and forth within the tarp.

7) Take your wet cardboard from step 1 and strip one side, exposing the corrugations. Introduce a small chunk of bulk spawn to these corrugations and roll up the cardboard like a burrito. Make a good number of these. You can optionally add extra nutrients to your burrito, such as a sprinkling of gypsum, coffee grounds, or sawdust to help the mycelium.

8) Stuff the mixture from the tarp into your burlap sacks. As you do so, introduce 2-3 "burritos."

9) Once full, stitch the bags closed with a heavy-duty thread. Pile these bags on the ground or on a pallet outside in the shade.

From here the mycelium will hopefully do rather well. Like building a fire, the mycelium will quickly jump from the cardboard to the straw to the wood chips until, in time, the entire bag becomes consumed in mycelium as the fungus digests the organic material. Once the bags are well covered in mycelium (usually in several months, depending on the species used and inoculation rate) their contents can now be used in a number of ways for your remediation purposes.

Remediation in Practice

You've come this far, the goal is in sight, now is the time to put your fungus to work! Some of the easiest ways to ally with these mycelial networks is through the direct application of your newly created bunker spawn. The techniques below are general guidelines and, while seemingly short, contain numerous possibilities for creative interpretation. Fungal remediation techniques are not set in stone and are constantly being improved upon by people just like you. Play around with these techniques and adapt to your circumstances as needed. Then let us know what worked for you.

WATER FILTRATION

If you have a polluted stream or pond, bunker spawn bags may be submerged in the waterway to act as a living filter, thereby trapping chemical and biological pollutants as the water passes through them. For flowing water, you will want to form a solid barrier that will require the majority of the water to channel into the bags instead of around or over them. In standing ponds, myceliated straw bales or bunker spawn can be introduced to potentially trap pollutants, including heavy metals.

Furrows can be dug in the land and filled with mycelium to act as a standing filter that will treat accumulated surface water before it soaks through the mycelium and into the soil. This approach can take the form of long trenches that follow the contours of the land, of terraces upon a hillside, or as a single large depression / shallow pond. These furrows (or swales as they are called in permacultural practices) can then be filled with mycelium via the layering of bunker spawn bags or by the formation of a *mushroom bed*. A mushroom bed is made by layering plain cardboard; 2-3 inches of suitable woodchips; an even layer of sawdust or bunker spawn; followed by 2-3 more inches of woodchips. A second layer of spawn and a third layer of chips can additionally be added if desired. Try to not lay a solid layer of spawn. Dispersal encourages the mycelium to grow faster by maximizing points of inoculation. The bed should be thoroughly watered and covered with 6" of straw or leaf mulch to prevent drying out. This bed will last for years as long as fresh chips and water are introduced as needed.

SOIL REMEDIATION

If you have a problem with polluted soil, this dirty earth could be dug up, piled, and then layered with the bunker spawn to begin treatment. By forming a lasagna effect with the spawn and contaminated soil you may be able to provide a suitable habitat for remediation. This method has proven successful in some instances but may require experimentation to enhance results. The introduction of treated straw or fresh wood chips might also help the mycelium survive better in the face of heavily contaminated soil.

Alternately, your bags of bunker spawn could be expanded in one more generation to more bags, this time with the addition of the polluted soil as well as more treated straw and wood chips. This is where the sense of play must be emphasized as experimentation with different ratios of contaminant to fresh substrate as well as fungal species or strains may be necessary to find what is most effective. The Amazon Mycorenewal Project performed similar experiments and determined that a 1:4 ratio of polluted soil to clean substrate yielded the best results in their work with using Oyster species to remediate oil-saturated jungle soil. Ultimately, however, we encourage researching what species and techniques are known to be effective against any given pollutant. While experimentation is critical for the advancement of understanding, there is a wealth of research that has been done thus far to save you the time of repeating experiments that are known to fail.

EROSION CONTROL

In areas where erosion issues may present a problem the laying of a mushroom bed may help to stabilize the soil structure. The selection of species here should cater to native species that are adapted to the environment and climate of the area as the bed will need to survive for years in order to be most effective. As opposed to the beds described in the remediation section, those laid for erosion control might benefit from direct contact with the ground (i.e. no cardboard bottom) and from the introduction of native grasses on top of the straw layer to further enhance the soil structure.

Short Species List

Below are recommended species to start growing for specific contaminants. All are relatively easy to work with as a beginning cultivator. Future publications will address the best ways to actually implement these species in remediation & restoration projects.

Shaggy Mane (*Coprinus comatus*)
Useful for metal polluted soils as it hyper-accumulates arsenic, cadmium, and mercury. Studies have also shown it to inhibit *Aspergillus niger*, *Bacillus* species, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Tends to grow in disturbed areas.

Elm Oyster (*Hypsizygus ulmarius*)
Incredibly easy to cultivate on many substrates. Not well studied in the labs but well suited to experimentation based on its strong ability as a decomposer.

Shiitake (*Lentinula edodes*)
Shown to break down polycylic aromatic hydrocarbons (PAHs), polychlorinated biphenols (PCBs), and pentachlorophenols (PCPs). Spent sawdust blocks (loaded with powerful degestive enzymes) from mushrooom farms can be packed in burlap sacks and used as water filters.

King Oyster (*Pleurotus eryngii*)
Known to breakdown a variety of toxins (including the base agent in Agent Orange, 2,4-dichorophenol). It is recommended for soil remediation and filtration applications.

Pearl Oyster (*Pleurotus ostreatus*)
An aggressive decomposer of a range of pollutants, notably the PCBs and PAHs found in all kinds of petroleum products, pesticides, and many other toxins. It is also known to hyper-accumulate cadmium and large amounts of mercury. This is one of the easiest mushrooms to cultivate.

Pheonix Oyster (*Pleurotus pulmonarius*)
Aggressive and highly adaptive, this species has been shown effective against dioxins and TNT and is known to sequester cadmium, mercury, and copper. Like the other Oysters, it is very easy to cultivate.

Wavy Caps (*Psilocybe cyanescens*)
A phosphorus scavenger with the ability to decompose munitions similar to TNT, organophosphates, and chemical weapons such as VX and sarin. Also a potent entheogen with a clean high. LC syringes not commonly sold as its illegal to grow in some countries.

King Stropharia (*Stropharia rugosoannulata*)
Tenacious and highly adaptive to many kinds of substrates, this species has a natural affinity with bacteria and seems to thrive in their presence. A great candidate for filtering water contaminated with fecal coliform.

Turkey Tail (*Trametes versicolor*)
Known to sequester mercury and filter *Escherichia coli*, *Listeria monocytogenes*, *Candida albicans*, and *Aspergillus* species. Has also been shown to effectively break down many PAHs including pyrenes, fluorine, and styrene, as well as pentochlorophenols, TNT, CCA, dioxins, anthracenes, and persistent organophosphates. Also good for chemical dyes. An easy and aggressive species to cultivate.

Recommended Resources

Books

Mycelium Running – Stamets
Fungi in Remediation - Gadd
Mycoremediation - Singh

Websites & Online Forums

Scholar.Google.com
Radicalmycology.com
Shroomery.org
Mycotopia.net

Contact Us!

We would love to hear about fungal remediation projects going on in the world. We encourage all comments, questions, submissions, mycoexperiment reports, guerilla mushrooming stories, etc. Contact us via email or the blogosphere.

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Notes

Spread Your Spores

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