



ROOTLAB

Mycology made easy

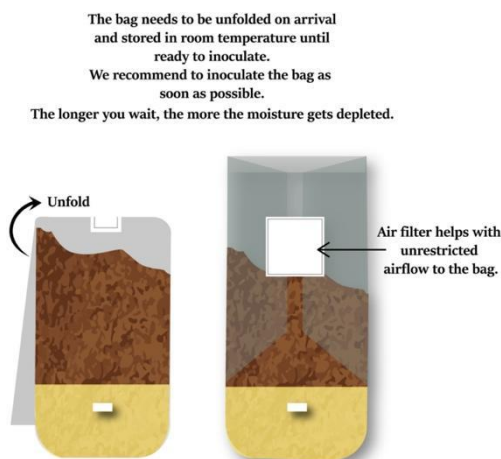
Version 1.4

If you are a beginner or unsure how to use this product, please read the detailed information sheet below.

Firstly, it is essential to check the bag you received for damage and contamination that may have occurred during shipping due to extreme heat/cold temperatures, excessive handling or exposure to the sun.

If your bag is bubble-wrapped, remove the packaging to check for breakage or contamination. Contamination can be a variety of colours, but the most common are green or grey. Please notify us immediately, including clear photographs, if you suspect your product is contaminated.

Note that the contents of the bag may get mixed up during shipping. This will not affect yields.



Storage prior to inoculation:

Take the bags out of their packaging as soon as possible, unfold them and store, them unfolded, in a well-ventilated space (dark or light). This allows the grains access to fresh air through the air vent filter, preventing anaerobic conditions that result in mould or bacteria

thriving inside the bag. If the air vent filter is stuck or pressed against plastic, loosen it so that the grains can access fresh air.

Please ensure that you have all equipment required (such as Liquid Culture/spore syringe etc.) before ordering the bags.

Summary - storage before inoculation

- Wash your hands.
- Remove bags from the packaging, keeping them upright.
- Loosen the bag by removing the tape. (This tape secures the substrate during shipping.)
- Ensure that air is entering the bag through the air filter.

Store the bag at room temperature in a well-ventilated light or dark place. It will stay fresh for at least 1-2 weeks. No need to refrigerate.

Please report any contamination within two weeks of receipt of the bag, including photographs, to info@rootlab.com.au. Note that the product will not be replaced if contamination originates from the injection filter.

It is recommended to inoculate bags immediately to allow the mycelium to grow in 1-2 weeks and build immunity to ward off any contamination. Inoculating immediately also ensures the moisture does not evaporate and mycelium has enough water to last through the full colonisation.

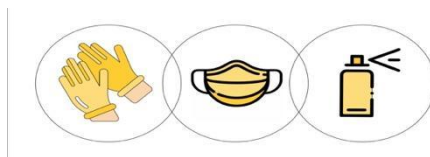
MUSHROOM GROWING STEPS

IMPORTANT



Maintain a sanitised environment.

To successfully grow kit mushrooms a very sterile environment is required. It is impossible to simulate the mushroom's natural ecosystem therefore a sterile environment is required to support growth. Sanitise the environment with 70% isopropyl alcohol (available at hardware stores) or a multipurpose cleaner.



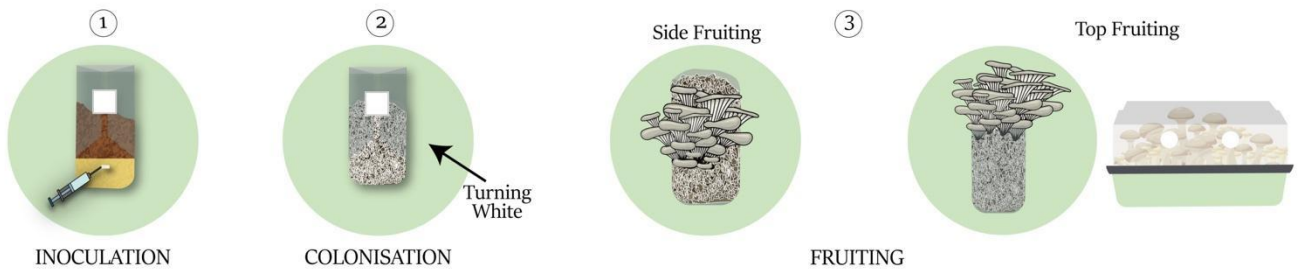
Wear mask and gloves.



Inoculate mushrooms in an area with minimal air flow.

LET'S GET STARTED!

Stages of growing



1) Inoculation (*introducing the mycelium to the nutrient medium*):

What should I inoculate the bag with?

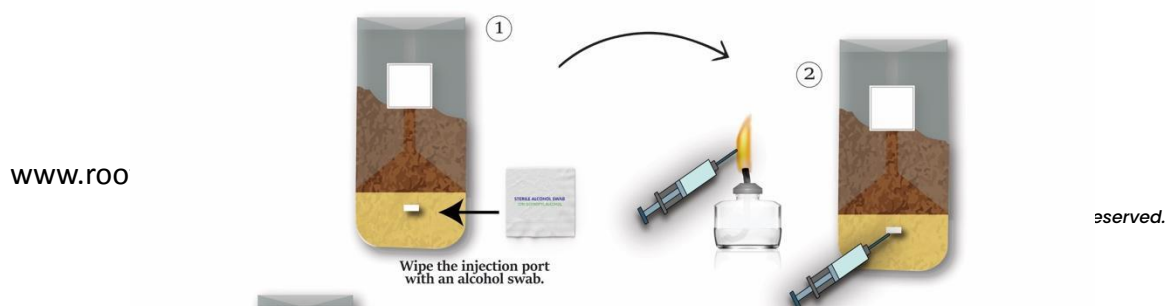
Liquid culture is recommended to reduce the risk of contamination, as it colonises the mycelium at least a week faster than a spore syringe.

If a spore syringe is used instead, ensure that it is checked for contamination using an agar dish. Put a drop from the syringe in an agar plate and wait for 5 days. If contamination starts to grow (green, red, blue, dark brown, or black or yellow slime mould) it means that the spore syringe is not sterile.

The presence of any colour other than white indicates contamination (in most cases).

- Before inoculation, check for any contamination, leakages or holes in the bag.
- Wipe gloves and injection port with an isopropyl swab.
- Shake the syringe vigorously to break up the mycelium in the syringe.
- Sterilise the syringe by holding in a flame for a few seconds until it gets red hot.
- Allow the needle to cool for ten seconds OR cool the needle by gently depressing the syringe to allow a couple of drops of liquid out of the syringe. You will hear a soft hissing sound.
- Insert the cooled needle into the injection port (grey port) and slowly release ALL the contents of the syringe into the bag by depressing the syringe. The injection port is covered by a thin sheet of plastic, do not peel this plastic.
- **DO NOT INSERT A HOT NEEDLE INTO THE BAG.**
- Remove the needle and gently rotate the bag in all directions to distribute the mycelium evenly into the nutrient medium. DO NOT SHAKE VIGOROUSLY or mix the substrate with grains at this stage.

Steps of Inoculation

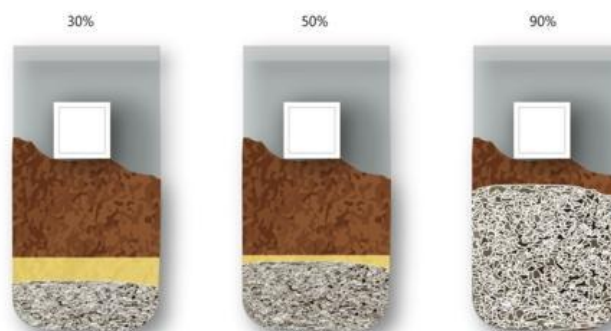


2) Colonisation (*mycelium growth - no mushrooms present*):

NOTE: All in one bags take longer to colonise than grain bags (millet/rye) as there is a substrate in the mix.

- Immediately after inoculation, incubate the bag at approximately 24°C in a dark spot for approximately two weeks.
- When the bag is 30% colonised break the bag with clean hands to distribute the mycelium evenly around the entire substrate block.
- Taking your time, gently shake the bag. This process mixes the grains and substrate evenly. Be careful of the heat sealed top part of the bag as it may tear if too much pressure is applied. We shake the bag three times during the colonising process to assist the mycelium to colonise evenly.
- Return bag to dark spot for another 2-3 weeks (until fully colonised).
- **Do not place bag on heat mat to keep it warm as this will cause all moisture in the bag to evaporate and mycelium would die.**
- When fully colonised gently shake the bag for the final time.

Shake bags at these stages of colonisation:



Keep in mind

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After 2-4 weeks generally, the substrate will be completely white with very few to no spots or brown patches left. It is a good idea to take note of what the average storage temperature was and how long colonisation took, for future reference.

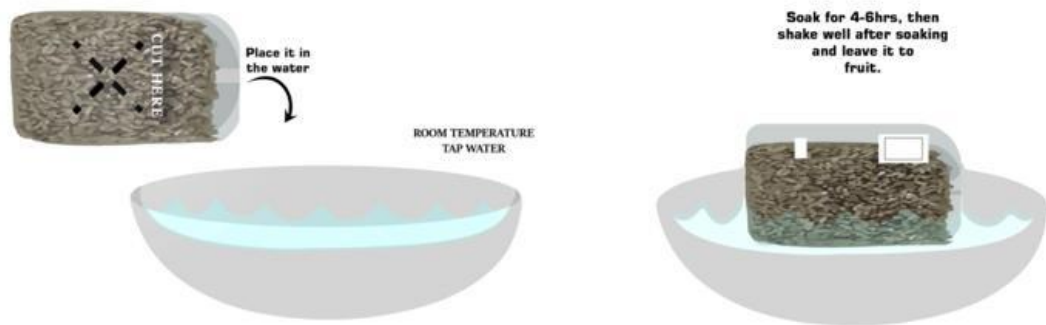
If a fully colonized bag is not used (ie. put into fruiting) within two weeks, the vigour of mycelium will start to diminish and die off from day 14. If not fruiting immediately we recommend storage of the bag in the fridge.

As the colonised bag ages a yellow liquid will be produced. This is called mushroom pee and it is a sign that the mycelium is stressed or old. These natural metabolites will not harm your produce but it is a signal that the mycelium is no longer at its optimum health and could be susceptible to contamination.

FRUITING YOUR BAG – THE FUN PART!

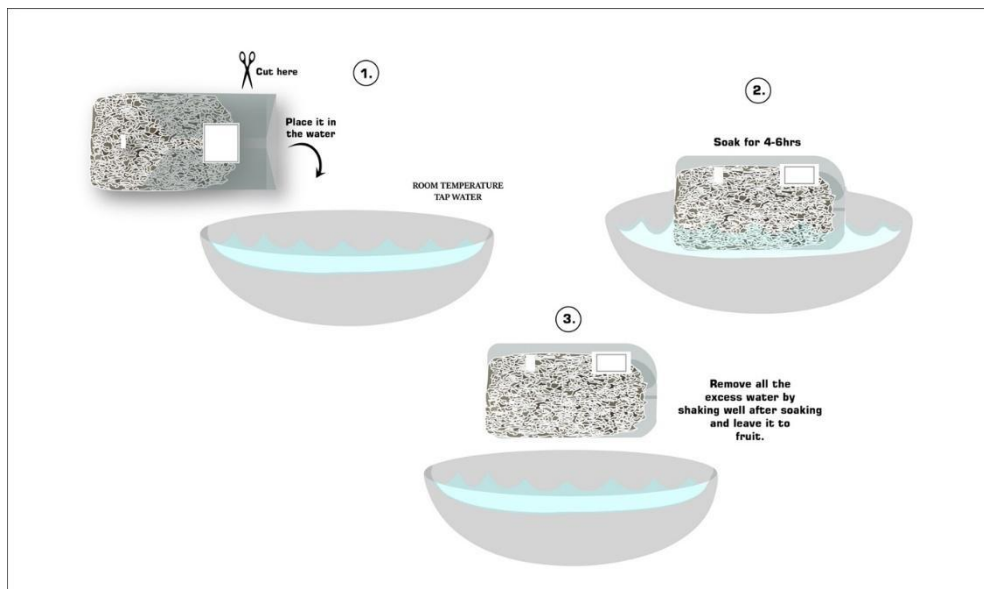
Mushrooms are 90% water. During colonisation, the mycelium consumes all the water in the bag. We need to give the mycelium another good soak before they will fruit.

Soaking for side fruiting:



Soak bag in room temperature water for 4-6 hours. The mycelium will soak up all the water it needs.

Soaking for top fruiting:

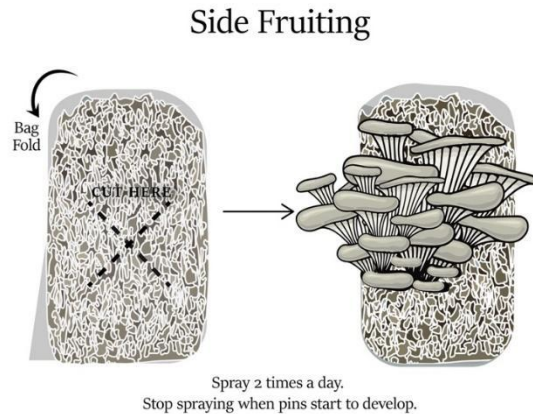


3) Fruiting (*mushrooms start to grow from the mycelium block*):

- Mycelium dies when it meets a dry surface, therefore it is essential to maintain humidity.
- Humidity induces pin formation (baby mushrooms) which is a key factor for mushroom growing success.
- Mist twice a day, stop spraying when the baby mushroom form.
- Depending on the mushroom variety fruiting will occur in one of two ways - “Side Fruiting” or “Top Fruiting”.

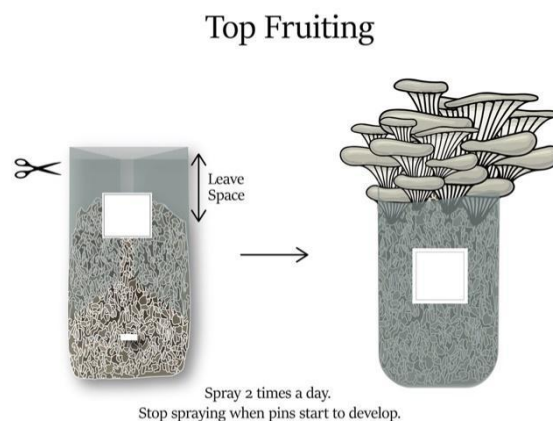
For side fruiting varieties:

- Side fruiting varieties can be fruited directly from the bag by cutting an “X” in the middle of the bag and then hydrated in water.
- Ensure proper humidity of 80-85% is maintained by misting twice daily.
- If in doubt about the level of humidity, it is recommended to place the bag in a shotgun fruiting chamber. Instructions on how to make a fruiting chamber can be found on YouTube.
- You can also place side fruiting varieties in a small greenhouse tent with a small humidifier to help with fruiting.



For top fruiting varieties:

- Top fruiting varieties can be fruited directly from the bag by cutting the top part of the bag and leaving some space as shown in the picture below. We also need to soak the bag to get back the humidity.
- Ensure proper humidity of 80-85% is maintained by misting twice daily on the surface. Make sure it's not too moist but has small droplets of water. Spraying from a distance or use of a fine mister is recommended.
- Discontinue misting when you see some pinset or baby mushrooms developing.
- If in doubt about the level of humidity, it is recommended to place them in a shotgun fruiting chamber. Instructions on how to make a fruiting chamber can be found on YouTube.
- You can also place top fruiting varieties in a small greenhouse tent with a small humidifier to help with fruiting.



Greenhouse Setup for fruiting:-



For fruiting in a monotub (highly recommended) for top fruiting species:

The guide below indicates how to grow mushrooms that do well in monotub, such as Swordbelt and King Oyster.

Need to order:

- Monotub
- Monotub filter or micropore tape
- Peat moss for casing (optional)

Order these items while your bags is colonising and do some research on how to optimise mushroom growth in a monotub.

- 1) Purchase a tall, clear monotub, preferably 10-21 litres to allow height for the mushrooms to grow. Do not purchase a larger monotub as colonisation will take longer, increasing the risk of contamination.
- 2) Line the bottom of the monotub with bin liner to prevent side pinning. You might like to search *“How to Make an Easy Liner for Any Size Monotub by earth cap mushroom”* on Youtube. As the substrate in the monotub gets consumed by the mycelium, it shrinks. As mycelium shrinks, space is created between the substrate and the inside wall of the monotub. This space is very humid, creating a perfect microclimate for mushroom to grow, resulting in side fruiting. The bin liner prevents side growth by sticking to the block of mycelium and preventing side growth. Top fruiting mushrooms are more abundant and easier to harvest.

Fact:

Contrary to popular belief, it is not necessary to paint the bottom half of your tub black to prevent light causing side pinning. Light plays only a secondary factor in pinning and makes

mushrooms slightly healthier, providing direction for growth. Painting the lower half with black paint serves no purpose.

- 3) Spray the bin liner liberally with Isopropyl alcohol before and after placing it in the monotub. Replace the lid and allow the alcohol to do its job.
- 4) Pour the contents of the colonised all in one bag into the monotub. Select a clean environment and we recommend you wear a mask. You may need 2-3 bags if using a bigger monotub. Pack the content tightly but not too compact. (If you grow plants you know not to pack the soil tightly as it restricts airflow to the roots. With mycelium slightly tighter packing is required.)

Alternatively, you could also buy some sterilised substrate from our store to increase the quantity of growing media. However, pasteurising your own substrate is also a great choice.

To make your own substrate:

- 8 gms of calcium hydroxide.
 - 1800ml boiling water
 - 225gm coco coir
 - 125gm vermiculite
- 1) Mix calcium hydroxide with boiling water in a food-safe container.
 - 2) Add coco coir and then vermiculite.

Makes 2kg of substrate.

Note: It is essential to follow the steps in the order above.

Adjust the ratio if you wish to make more or less substrate.

28 gms Gypsum can also be added to this mix to facilitate air exchange but is optional.

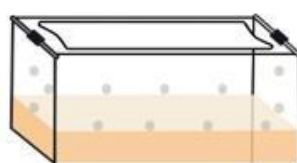
Making holes in your Monotub:

Holes are generally made where the substrate ends to assist air exchange:

Small holes (Use Micropore tape):

A monotub has long and short sides. Holes should be 1cm diameter and inserted to all sides of the monotub.

- Long sides - 4 holes on all the long sides.
- Short sides - 1 to 2 holes on both the short sides.
- The holes on the short side are also required on the TOP of the monotub to remove the hot air, these are made on the short side.
- Bottom holes are made just above the substrate as this is where the gas exchange is needed.



Bigger holes when using a Monotub filter:

- 1) Cut 5cm diameter holes on both short sides of the monotub using a hole saw. When making 5cm holes you do not need more than 2 holes for a tub sized up to 21L.
- 2) Monotub filters can be bought from the Rootlab website (rootlab.com.au) and are reusable. There is no need to replace them.
- 3) Monotub filters maintain humidity while allowing gas exchange. As they are 0.2 microns they do not let contamination in.
- 4) There is no need to make bottom and top holes for this method as the holes are already large.
- 5) Holes are made just above the substrate as this is where the gas exchange is needed.



Colonisation and fruiting

- The monotub needs to go for colonisation once the content is either mixed with more all in one bags or with additional sterilised or pasteurised substrate.
- Colonisation will be much shorter when mixing with a few colonised all-in-one bags together as they have already gone through the colonisation stage.
- During this stage all holes (big and small) will need to be covered with sticky tape. Colonisation is optimised when kept in darkness. Sticky tape helps to block air flow and helps to retain higher CO₂ and higher temperature and humidity that helps to speed up colonisation.
- Once the monotub is completely colonised (when it's all white), go over to the fruiting stage.

Fruiting

- Fruiting is generally a few degrees cooler than the colonisation stage but read specific temperature for your species.
- Replace sticky tape with micropore tape for smaller holes.
- The same process applies for tubs that have monotub filter - simply remove the sticky tape that you added on top of monotub filter while colonising.
- Introduce to indirect light (no direct sunlight).

First 48 hours:

Make sure the surface of the mycelium is always kept moist but not too wet. Spray generously to make sure there is plenty of water for the mycelium, but do not overdo it. Field capacity (the amount of water in the substrate) is an art to master. It needs to be around 60% of its total weight. There should be no pool of water. Don't worry if you spray a lot at first as mycelium will absorb all the water overnight.

After 48 hours.

- The mycelium block is now rehydrated and ready to fruit. Fruiting is induced when the evaporation takes place from the air circulating in the monotub through the holes.
- The water droplets on top of the surface of white fluffy mycelium is always present. The droplets should be small.
- To create small droplets either use a fine mister or spray using normal spray bottle with a longer spraying distance.
- These drops of water help with keeping the substrate cool as when the air circulates in a monotub it touches the water and due to heat exchange, water evaporates, and it pulls a bit of heat with it, leaving the substrate cooler.
- This continuous process of cooled substrate leads to pinning or pin set development.

Useful youtube vidoes

- Basics of How a Monotub Works - Understand Fruiting Conditions, Gas Exchange for Growing Mushrooms (by oneearth mushrooms). Useful for gaining an understanding of how to make holes and the basics of fruiting.
- How to Grow Mushrooms from Start to Finish in a Monotub by boomer shoomer-watch youtube video
- Loading Substrate and Grain Spawn to Colonize a Monotub - Growing Mushrooms at Home - Field Capacity. Useful if you are using an all in one bag with extra pasteurised or sterilised substrate. Use that all in one bag as grain spawn bag as shown in this video.
- Ideal Fruiting and Surface Conditions by Philly Golden teacher. Watch this video to understand the surface conditions required in a monotub for fruiting.

FAQs

Q. Why must I use an all-in-one bag instead of a grain spawn?

- A. To understand the rationale behind using an all-in-one bag for mushroom cultivation, let's first grasp the concept of the "lag period." This period occurs when mycelium encounters an unfamiliar food source and needs time to adapt to it. Growing mushrooms requires two key components: grains (which act as a colonisation point) and substrate (the actual food source). Grains are effective because they can easily spread and colonise the substrate. Traditionally, people cultivate mushrooms by colonising a grain bag, like millet, and then transferring the colonised grains to a sterile or pasteurised substrate in a monotub. However, for beginners who may not fully grasp the importance of maintaining sterile conditions, this process can lead to a lag period during which mycelium must adapt to the substrate before it can effectively digest it. This vulnerability during the lag period can result in contamination issues. Using an all-in-one bag containing grain and substrate simplifies the process for beginners. You can grow mushrooms in a monotube with multiple bags or mix the bag's contents with the additional substrate. The advantage here is that there is no lag period when adding more substrate, reducing the risk of contamination. However, it's worth noting that there is still a lag period within the bag itself. One drawback of the all-in-one bag approach is that it is slower than using grains alone. Nonetheless, the benefit is that the lag period occurs in a relatively sterile environment within the bag, which is an improvement compared to the traditional grain technique, where the lag period occurs in a sanitised monotube (which may not be as sterile).

Q. What is a spore syringe?

- A. A spore syringe is made by placing the mushroom on foil, scraping those prints into distilled water, mixing it, and then adding to the syringe. Most of the work is done in a sterile workspace. However, it has a high likelihood of being contaminated while the spore print was taken, as the mushroom itself could be contaminated. That is why we always recommend to test your spore in agar first before inoculating the all in one bag.

When you see contamination and mycelium in agar plate you do not need to throw the syringe away. You need to isolate the mycelium into a fresh plate and upon successful isolation of mycelium away from contamination you can create your own liquid culture to inoculate the bag. It is important to do all this before you order all in one bag.

Rootlab has half Malt Extract Agar plates (MEA) and half Antibacterial Plates with Gentamycin (MEAG) available to purchase on our website. You need MEA plates to test out the culture and MEAG plate to isolate contamination as bacteria cannot grow on an MEAG plate.

You can't test culture in MEAG as you won't be able to tell if the spore syringe was contaminated with bacteria.

Q. What are common mistakes to avoid:

- Adding liquid culture or spores more than necessary. 10 ml is sufficient.
- Using an unchecked spore syringe (ie not tested in agar).
- Unsterile workspace, needle or hands.
- Not allowing the needle to cool down after sterilization and before inserting into the injection port, leading to damage to the bag and increasing the risk of contamination.
- Not labelling the bag with the variety you are growing.
- Not cleaning the injection port before inoculating with alcohol swab.
- Delayed inoculation of the bag (except if the bag is too moist on arrival).
- Selecting storage area based on light levels (eg a dark wardrobe) over adequate airflow.

Q. What do I do if I suspect contamination?

- A. Many people, including us, have experienced contaminated bags as beginners. It's important to take detailed notes and to find the vector of the contamination to avoid reoccurrence in the future.

If contamination appears to originate at the site of inoculation it is likely that the syringe was contaminated, or that proper sterilisation procedures were not maintained. Wrap the contaminated bag in a plastic bag and dispose of it in the general waste bin. Sanitise your workplace and yourself thoroughly.

This product is susceptible to moisture and temperature fluctuations and is considered perishable. Correct handling and product knowledge is essential.

If you need further assistance contact us on info@rootlab.com.au with photographs and the process you followed.

Additional Research:

It is recommended that you conduct additional research based on the species you are growing:

- Fanning of the tub once or twice a day may be necessary to release all hot air and CO₂. It is suggested that you research this topic further, based on the conditions of your tub's storage.
- Harvesting mushrooms and how to prepare a second harvest.
- Casing

(Optional for most species, essential for a few, research on the specific species you plan to grow):

Casing maintains surface conditions for optimum growth of mushrooms. Growers experiencing trouble maintaining water on the surface of mycelium cake or substrate should case their monotub.

Casing ensures high CO₂ and high humidity which leads to pinning and consistent fruiting. Casing maintains the proper surface conditions for fruiting.

Instructions:

- Clean your hands and wear gloves and mask.
- Open the monotub lid and, for a species that requires casing, add a mix of vermiculite, peat moss and calcium hydroxide (pasteurising agent) or keep it simple with just peat moss. Peat moss prevents contamination however be aware that it can be contaminated with fly eggs, which will eventually lead to flies contaminating the substrate. Therefore it is best to sterilise the peat moss by microwaving or baking it for some time to kill the fly eggs, and then adding hot water. To keep 60% field capacity (hydration) you need to add 60 ml of water to 100 gms of peat
- Only case on a fully colonised substrate or cake.

You can recase after harvesting the first flush. More on harvesting can be read online.

GLOSSARY

Important words to know before you proceed.

Colonisation - The stage in which mushroom mycelium spreads its roots all over the substrate, similar to a plant putting out its roots.

After the substrate is colonised, the mushroom secretes enzymes to break down the substrate so that it can be absorbed by them.

Colonisation requires a warm temperature, around 24°C for most species. Too hot and the contamination thrives; too cold and mycelium metabolism slows down drastically.

Fruiting - Mycelium has fully colonised a substrate and, with nothing else to do, directs its energy towards propagation and keeping its genes alive. They form mushrooms that are fruiting bodies with spores. Every species requires a specific temperature and humidity to fruit.

Incubation - the process where we place the mycelium in the perfect temperature and darkness to colonise.

Inoculation - The stage in which mushroom culture is introduced to a nutrient medium. The culture is usually contained in a nutrient liquid in a syringe or a nutrient agar petri dish.

Mycelium - A root-like structure of fungus that spreads, looking for water and food. Once mycelium gains sufficient energy from the food, it will fruit. This fruit is what we call mushrooms.

Pasteurisation - Different chemicals or steam are applied to a substrate, killing all organisms but not the spores (the majority of which remain alive). Pasteurisation occurs at a temperature of 75°-78°C.

A pasteurised substrate will contaminate in two weeks' time as spores that were not killed off during pasteurisation will grow, mate and compete for food.

When mycelium takes over a substrate introduced via grain spawn they fully colonise the substrate and then secrete enzymes to ward off contamination.

FUN FACT: Penicillin is the most common antibiotic - and it's an enzyme secreted by fungus to ward off bacteria.

Sterilisation - The substrate or grains are put under extreme heat (121°C) and immense pressure (15 psi), killing all organisms and their spores.

Substrate - Food for mycelium. Just as plants need nutritious soil to grow in, mushrooms need wood, manure/compost, straw or insects.

FUN FACT: Mushrooms that naturally grow in insects are called parasitic mushrooms, the most common example of which is Cordyceps.

All the best!

For further information and questions regarding this, please do not hesitate to contact us at info@rootlab.com.au.

We welcome feedback and suggestions from our customers.