Working with spores for restoration

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Spore restoration basics

Most larger (macrofungi) that are ectomycorrhizal tend to partner with trees and shrubs (woody plants). We will develop suggested restoration methods overtime starting with some Puffballs.

- You need landholders permission to collect fungi
- On public land you need permits to collect fungi.
- Take care to not breathe in spores, these are fine particulates that maybe harmful if inhaled.

Collecting spores for indigenous restoration

We still don't know how the species distributions work across Australia so you should work within your local bioregion and the local vegetation, habitat and communities.

Take care to avoid exotic species that are likely to be weedy these are commonly found in disturbed sites. Remnants and more intact bushlands are the best place to collect fungal spores.

Mature spores are needed. To maximise genetic diversity choose spore bodies that are further apart. Sporebodies within meters are likely to be the same genetic individual, sporebodies 30-100 meters apart are more likely to be different genetic individuals.

Things to note with collecting spores (WWF-2):

- Date (at least month and year)
- Location – suggest geocoordinates
- Habitat & vegetation notes
- Ecological community or if ecotone the types of communities
- Maturity of host plants at location
- Time since last disturbances like fire, landclearing etc
- The likely plant associates, the trees & shrubs nearby

Keeping spores

Spores should be kept dry until germination is likely to occur. Wetting spores or even just humid conditions can cause spores to germinate. If germination starts where these fungi can not find food, then they will die. Sealed plastic containers with colour change silca should be used to make sure spores are kept dry.

Dilution and bulking out spores with dry sand or other inert fine material is useful. Coloured spores should just be changing the colour of the sand if pale.

Keeping things dry:

Knowing when moisture is around can be tricky. Using colour change silica is useful to monitor that spore collection stay dry (WWF-1). Silica colour changes as moisture is absorbed are typically from dry:

- blue becoming purples to pink
- orange becoming yellow to greens

Cheaper uncoloured silica is sometimes mixed through. But there is no way of guaging how much moisture these have absorbed. Double bagging collections with some colour change silica in the outer bag will warn you that moisture is starting to get in.

WWF-1 Colour change silica is useful to monitor if materials stay dry. Dry silica are typically blue becoming purples to pink changing with moisture or orange becoming yellow to greens. (SMF)

Procedures for adding spores

Spore treatments (inoculation) can be added in two main methods wet or dry. For both methods if coloured spores are used the darker the sand or water slurry mixes are the more concentrated the spores. Pale spored fungi are harder to gauge the concentration of.

Spore treatments spore mix to plantings or to nursery seedlings. When planting out add inoculations into the holes. Or if already planted lightly lift the top layer of litter or mulch within 3-15 cm from plant and sprinkle sand-spore mix and then return the litter layer to protect the spores from ultraviolet (UV) damage.

Dry method

Mix with clean sand (or other fine inert material) to dilute the spores. Sprinkle ¼ teaspoon per plant.

Wet method

Make a 'spore slurry' by mixing the spores in approximately 1 tablespoon of spores per 1 Litres of water, if spores are being are not mixing well because they are hydrophobic add a small amount of washing detergent 1 drop per 5 Litres should be enough we don't want to damage the spores with potassium hydroxide (KOH). Agitate again before dispensing 1/2 cup 3-15 cm from the plant stem.

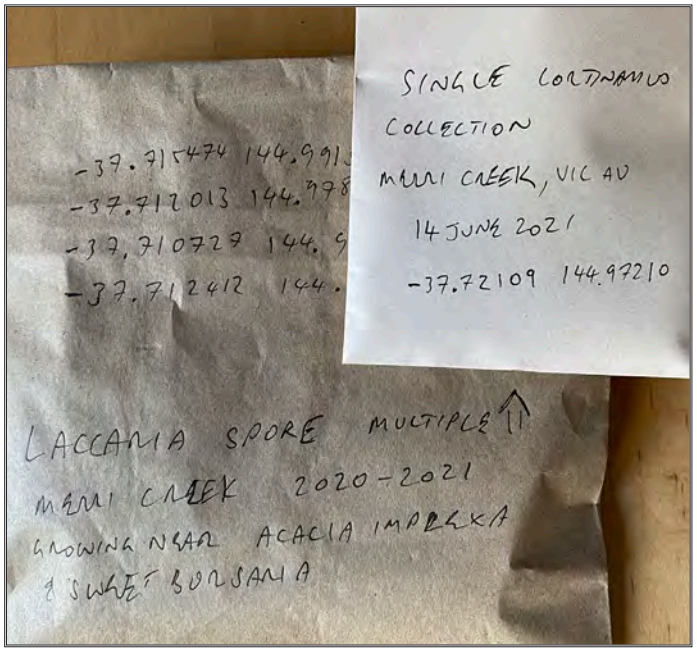
Monitoring for success

Record details of what spores were used under which circumstances so you can gauge success over time. Where possible have a few 'sacrificial' or control plants that are not treated and are separated by at least 10 m from treated plants. Mark the plants you want to monitor and take notes and images over time.

To have comprehensive monitoring of success when planting, choose 6-10 replicates of each species with 3-5 that get the spore treatment and 3-5 that don't get treatment. If there are several different types of treatments you need 3-5 of each species and treatment combination and 3-5 controls. Controls can be seen as 'sacrificial' plants that show you what happens if the treatments are not added. Any replicates are better spread across the site and be at least 10 meters apart from other monitored plants.

If there is just a single treatment and control another way to monitor is in pairs. Where a 'pair' of the same species are planted (with 10 meters between them) so both can be monitored both for a few of years (even just staking photos of each from the same spot at the start and end of wet (growing) season. Ideally these plantings should be marked and monitored at months 1, 3, 6, 12 and then annually.

Mycorrhiza may not show their true value until harsher periods. Please share your experinces with Fungi4Land so together we can improve our land restoration knowledge.



WWF-2 Spores in labelled paper bags & envelopes, these are kept sealed in cool, dry & dark conditions with some colour change silica to allow monitoring to prevent moisture.

Puffballs in restoration

Puffballs are a broad group of unrelated fungi that share the common trait of dispersing their spores from a ball. Some are mycorrhizal and others are recyclers. Most puffballs have hydrophobic spores as rain splash helps their dispersal.

The easiest mycorrhizal restoration genus to learn is Pisolithus. Several names have been mistakenly used in Australia but research has shown neither of these northern hemisphere species have definitely been recorded from Australia. Identification of species is tricky and needs microscopic and sometimes DNA work. Luckily identification to genus is relatively easy if you cut them longways and expose the 'pea' shaped structures which are where the spores develop. So mark the patch and come back to collect them when they are powdery. Sporebodies that still look round and ball like will contain mostly immature spores. When mature these produce lots of spores so 1-5 should give plenty of spores (see spore note).

These often are triggered to reproduce by compaction so are commonly found along paths or roads, less busy roads and paths through local bushland are the best place to collect. Identify one by cutting it and note the area - you should collect spore bodies when they have started to break down and have powdery tops. Take care to not breathe in spores. Best to collect into paper bags as this helps contain the spores. These need to be actively dried as they can contain lots of moisture and go mouldy if not dried properly.

Caution in using other puffballs

Puffballs may be mycorrhizal and others are recyclers. Instructions about how to collect Dyeball ***(Pisolithus)*** **spores for restoration** follow in the next section. Earthballs *(Scleroderma*,WW-3) are also recognisable genus, however we do not recommend using this genus if you are trying to encourage

WWF-3 (SMF) Earthballs *(Scleroderma)* are common along paths & other disturbed areas, these are likely to be exotic weedy species. (SMF)

indigenous species restoration. Most commonly seen Scleroderma are likely exotic and potentially weedy. There are indigenous species but it would take taxonomic investigations to be sure. If Scleroderma are harvested for spores, this should be once the sporebodies have already begun to crack open naturally, this is the sign that spores are mature.

Future resources will suggest other recognisable puffballs for restoration like local Prettymouths ***(Calostoma)*** species.

WWF-4 Dyeballs *(Pisolithus)* are common across Australia with at least 15-20 species, some found across Australasia, many still need scientific definition.

The low nutrient sandy soils of the Cooloola support several species including **A** *Pisolithus albus;* **B** *P. croceorrhizus*; **C** *marmoratus; P. microcarpus;* and other enviroments across Australia like **D** *Pisolithus* sp. from Cooloola, QLD and two from WA **E** *Pisolithus* sp. Tarin Rock Reserve, west of Lake Grace, WA; **F** *Pisolithus* sp. Wyadup, near Yallingup, WA (**A-B, D** SMF; **C** M Prance; ; **E-F** R Hart)

Dyeballs *(Pisolithus) for restoration*

The easiest restoration genus to learn is *Pisolithus*. Their Genus name comes from Pea ***pisum*** and stone ***lithos.*** These are sometimes called Dyeballs, as they have been used for dying fibers.

Recognising this genus is easy (WWF-4) but identification to species is hard. Luckily, to utilise local spores you only need to identify them to genus. Two names have been often but mistakenly used in Australia (WWF-4) with research has shown neither of these northern hemisphere species *(P. arhizus* nor *P. tinctorius)* have definitely been recorded from Australia (Lebel *et al.* 2018). With 10-12 local species, some are still needing to be named. So it is tricky to get identifications to species and may need microscopic and sometimes DNA work.

Pisolithus are often triggered to fruit by compaction so are commonly found along paths or roads. Less disturbed areas including roads and paths through local bushland are the best place to collect. Identify one by cutting it lenthways (WWF-5), identification to genus is relatively easy if you cut them longways and expose the 'pea' shaped structures which are where the spores develop. Take note of the spot - you should collect spore bodies when they have started to break down and have powdery tops. Take care to not breathe in spores. Sporebodies that still look round and ball like will contain mostly immature spores. When mature these produce lots of spores so collecting 2-5 should give you plenty of spores.

It is best to collect into paper bags, as this helps contain the spores. Chop off the upper part of the spore body. You are trying to collect the dry powdery spores but leave behind the firmer, often damp, spores where the 'pea' structures are still forming, these spores are not yet mature. By leaving behind the lower part of the spore body will leave some spores for the local environment, reducing the impact of collection. The spores collected need to be actively dried as these can contain lots of moisture and go mouldy if not dried properly.

Procedure for Dyeballs (*Pisolithus,* WWF-5):

Step 1 Identifying the fungus

- Cut them longways and expose the 'pea' shaped structures which are where the spores develop.
- Mark the patch to come back to when they are mature, checking about weekly depending on the weather.

Step 2 Are the sporebodies mature?

- Sporebodies that still look round & ball like, with entact skin, may contain mostly immature spores.
- Cut longways to check as needed.
- If surface of the sporebodies are starting to break down and have powdery tops, these powery spores are mature.

Step 3 Collecting the sporebodies

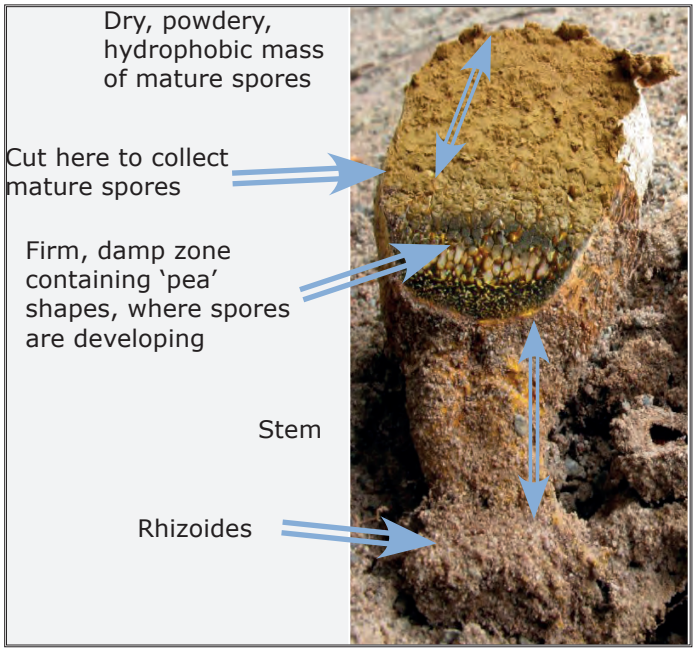
- Collect 1-5 (note how many) sporebodies into paper bags· To aid drying, chop spore bodies into quarters or for larger ones sections that are <1 cm thick.
- Dry the sporebodies at lower temperatures, for example in a food dehydrator set to its lowest setting 35-40 degrees Celsius.

Step 4 Storing the spores

- Store the fully dried sporebodies somewhere cool, dry and out of direct sunlight.
- In order to keep moisture out it may be necessary to place spores in a sealed container with silica beads packets or similar. Note use at least some silica that indicates colour change so you can redry if necessary.

Step 5 Spreading the spores

- Wait till the beginning of wet season or until after there has been a good ground-soaking rain.
- Create a spore mix using either of the following dry or wet methods from 'Procedures for adding spores'.



WWF-5 *Pisolithus albus* cut vertically to expose developing spores. (SMF)

Want to help by making culture collections?

- We need to maintain culture collections to help research
- If you use this spore method & have access to patches of Pisolithus that you used as a source of spores.
- Let us know about it by emailing us with 'Pisolithus reserach' in the subject. We can put you in touch with researchers who are keeping vouchered culture collections.

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WWF-6 Dyeballs *(Pisolithus)* are renown for pushing up through hard ground, even tarmac roadbase like this. (R Hart)

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