

MAKING SPORE PRINTS

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Spore prints are a fundamental tool for the mycologist interested in larger fungi. The shape and size of prints is almost like a photograph of the original fungus with pores or gills, degree of crowding and numbers of intermediates all clearly visible. Colour of spore print is a very useful guide to the group of fungi to which your specimen belongs, as is any colour change with Iodine. Prints also provide a clean sample of ripe spores for microscopy or culturing. Given their usefulness, it is no surprise that spore prints are often retained with macrofungus specimens in herbaria.

But we're getting ahead of ourselves: what's the best way to make a spore print? The basic principle is to place the fruitbody in its original orientation (ie gills, spines or pores downwards) over a suitable surface to catch the spores, and to cover it over to protect it from just drying out instead of sporulating.

The fungus fruitbody has evolved solely to produce spores and is remarkably effective. A visible print will often appear in less than an hour and by the following morning its thickness can be discernible. After a day or two there will be noticeable ridges under the gills-spaces of a large healthy toadstool, but we won't usually want prints as thick as that.

It's best to select a cap or caps that are fully expanded and in good fresh condition - neither dried up nor waterlogged. Obviously the gills or other spore producing structures need to be healthy and not pressed together by distortion of the cap. For inconveniently large specimens, small pieces (either sectors or segments) may be cut off. Tests have shown that young caps often give slightly smaller spores but can be used if no others are available, as long as the gills are exposed. Old caps may give no spores or very few (especially if the cap is contorted), or be overrun by maggots overnight. Some people cut off the stalk, others insist it should remain, or even propose elaborate arrangements with the end of the stalk in water. Personally I've always removed the stalk and generally got perfectly good spore prints, even

after hygrophanous caps have reached the "dry" state. Other people place a droplet of water on the cap but - in my experience, dried-up toadstools never produce spores however much you wet them, apart from a few desiccation-tolerant genera, notably *Marasmius*.

The whole arrangement must be covered to exclude draughts which will otherwise blow away the spores as they fall. To prevent cross-contamination, it's best to cover each fungus separately. Use small covers (especially for small fungi) to minimise evaporation, but not so small as to encourage condensation.

Ideally, fruitbodies should be freshly collected. Opinions differ on the effect of refrigeration (eg McAdam, 2004; Henrici, 2004) but it probably depends on the fridge temperature (and the species of fungus). Many years ago, I found that specimens kept overnight in the fridge didn't drop spores whereas those kept in the garage did - having found a system which worked there was no reason to go back and check it.

So much for general principles; the rest depends on what you want the print for: record of gross morphology, colour of print, microscopic examination, herbarium or culturing.

To display

A collection of prints, to show print colour and gross morphology of toadstools, can be made using coloured paper. Choose a colour to contrast with the anticipated colour of the spores. The spore print should be dried for 15mins in a warm room and sprayed with hairspray or fixative spray for pastel and charcoal (try your local arts shop). The pages can be kept in an album after adding the name of the fungus and collection data. You can scrape a few spores off for more detailed examination whenever needed, but see below.

To check the colour

Although you can ascertain print colour from prints on coloured paper, it's better to use glass microscope slides. Leave the print to dry for ten minutes then scrape the spores into a heap using a

single-sided razor blade or a second slide. Check the colour in natural daylight (or under a daylight simulation bulb from your local art shop) against a recognised mycological colour chart. (Beware that the *Russula* spore colours, A to H, in the British Fungus Flora colour chart are too pale - subtract one so that "B" becomes "A" and "H" becomes "G". The true "G" is pale "Sienna" or midway between "H" and "Orange")

If you want to get technical you can scan spore prints on a flatbed scanner and measure the colour on the computer. This is the source of the numbers below the spore prints in Breitenbach & Kränzlin vol 4 1995 "B&K" (see p13-14), but they dropped it for vol 5. In reality, it's unlikely to be meaningful unless you have a properly calibrated scanner with colour management software (if you don't know what that means, then you don't have it!)

Some people preserve spore prints on slides by sealing a square coverslip with four strips of sticky tape ("exhibition tape" lasts longer than cheap sticky tape). Such a collection of *Russula* spore prints is more precise than any printed colour chart and can be very useful as the prints of many species are of subtly different shades. The colours will fade a little in a few years but can easily be replaced with new samples.

Spore prints on glass have the added advantage that a drop of Melzer's Iodine can be added to test for an "amyloid" reaction when the spores turn deep blue, or a "dextrinoid" (or "pseudamyloid") reaction when they turn chestnut brown (although the latter is more easily seen under the microscope). N.B. If you put a drop of iodine onto a paper tissue it will turn blue-black so you can't do the amyloid reaction on a spore print on paper, or spores scraped off paper.

Why "amyloid" and "dextrinoid"? Starch and dextrin are two long-chain carbohydrate molecules which both react with iodine: starch turns deep blue and dextrin turns chestnut brown. Mycologists look for this colour change with Melzer's Iodine, but because other compounds may show the same colour changes, we say "amyloid" ("amylum" is Latin for starch) and "dextrinoid" - the "oid" suffix indicating the similarity of the reaction.

Of course, you can also do Melzer's reaction under the microscope by mounting the spores in Melzer's Iodine. Dextrinoid spores are easier to recognise this way than with a spore print as there

is less confusion with the brown colour of the iodine solution. Amyloidity, on the other hand can be very faint in individual thin-walled spores and is then more easily recognised in a thick spore print. Amyloid ornamentation as in *Melanoleuca* and *Russulaceae*, is easily seen in single spores.

Finally, if you intend preserving spore prints, make separate throw-away prints for testing with Melzer's reagent (or scrape a few spores onto a separate slide) - iodine is volatile and will spread through your collection.

To show gross fruitbody morphology or spore print colour, a good thick deposit is needed and this will take some hours - eg overnight, but check it during the evening. Paper is better than a glass slide if the fruitbody is waterlogged, otherwise the print will be wet.

Microscopic examination

For microscopic examination of spores a thin deposit on a microscope slide is best. The print should be only just visible to the naked eye - an hour or two usually suffices. Naturally deposited spores tend to stick to the glass. If a drop of water and the coverslip are applied with reasonable care, the spores stay still when examined, even under oil immersion, which makes measuring easier. A spore print is always preferred for microscopy as all the spores are mature whereas gill preparations necessitate subjective selection of mature spores.

If you are in doubt whether your fungus has dropped any spores at all, tilt the slide to catch the light. It may be useful to mark around the area with the spores using a glass-writing (or laundry) pen so you know where to put the coverslip.

Spore prints in a herbarium allow future researchers to examine mature spores, so they need to be reasonably thick if possible. After air drying for half an hour, they should be protected against being rubbed off, eg wrapped in aluminium kitchen foil. It's a good idea to label both the slide and the foil.

Culturing

Spore prints for culturing require a slightly different approach. The important thing is to keep everything sterile: spores produced from a healthy fungal fruitbody are usually uncontaminated. Generally the print will be made onto a sterile nutritive medium. This is often a petri dish containing a nutrient solution stabilised in the seaweed extract: agar - an "agar plate". Unless the

spores are known to be hard to germinate, only the lightest of spore prints will be required.

The above outlines the general rules, but there are always special cases:

The colour of black spores should be assessed without scraping, eg *Psathyrella* (Kits van Waveren, p15) where the print often has a beautiful reddish tinge which disappears when scraped.

Some white-spored fungi (especially some *Tricholoma* and *Clitocybe*) tend to produce thin prints with spores strongly glued to the slide. Hard scraping is needed to get the spores into a heap and the spores are often damaged in the process so that they appear darker and greasier than they should really be.

Wet fungi do not generally produce good spore prints. If the print gets wet, then colours can darken and, again, the spores may become glued down.

Corticoid ("whitewash") fungi, if in good enough condition to identify, will usually give spore prints adequate for microscopy. If you have a sliver of bark with a corticoid on it and simply lay this directly on a slide to sporulate you will frequently end up with a mix of the intended basidiospores and sundry hyphomycete conidia. If the sliver is supported a few millimetres above the slide only the forcibly discharged basidiospores should appear.

Brackets rarely give good spore prints, even when propped up so that the tubes are vertical. They will continue to grow indoors and after a few days may become featureless lumps!

Most fungal fruitbodies will give spore prints

composed of a single type of spore. Watch out however for parasitic heterobasidiomycetes within the toadstool or (more often) corticioid which can contribute to the spore print. It is often very difficult to trace such mystery spores back to their original.

In conclusion, spore prints provide much useful information. At the end of the foray, when unknown fungi are brought home for identification, the first task should be to lay them out for spore prints - ideally before stopping for refreshment, (do as I say, not as I do!) I prefer to make three spore prints onto slides from each collection - a light one for microscopy, and two thicker ones: one for spore colour and Melzer's reaction and the other for the herbarium. For small toadstools like *Conocybe* or *Galerina*, two or three caps are best, but be sure they are the same species! The same cap or caps can be moved from the microscopy slide to the print and herbarium slides, to generate each spore-print in turn.

References

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Preserving spore prints using clear plastic book covering

Making spore prints is an essential activity for field mycologists. The spores can be easily gathered up to determine their colour and for microscopic examination. If need be the entire print can be preserved using the clear adhesive film intended for backing books.

The spore print should be made in the usual manner:

1. Cut a square of film larger than the fungus cap, peel off the backing and place sticky side up on a flat surface.
2. Cut the stipe off the fungus and gently place the cap, gill-side down, on the film.
3. Cover with an upturned bowl and leave for the spores to drop out. The time should be deter-

mined by experiment, as if the spore print is too thick it will not be well preserved.

Remove the cap from the film. Stick the film onto paper of a contrasting colour to the spores.

The spore prints are permanent. The illustration shows a spore print from *Gymnopilus junonius* made in 1988 which still retains its colour. The spores can be sampled but this entails a degree of destruction.

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