SOME PRACTICAL SUGGESTIONS FOR FUNGAL STUDIES

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These procedures have been accumulated over a number of years—mostly from trial and error (large amounts of error it always seems in retrospect) when working either in the field or with either fresh or dried material under the microscope. Admittedly, since my field of study is the agarics, the suggestions here are derived from those studies, but they can generally be easily extrapolated to any mycologist's work. Some, if not all of these ideas will be obvious to and already being used by the workers who have been dealing with the fungi for many years and already I can hear the statement: 'I know that—it's an oldie'. But I ask those people to bear with me; I can only say that once upon a time it wasn't an 'oldie' to them either and all of us forget the frustration we often faced as we struggled to obtain results when everything we tried to do seemed to go wrong.

The problem is that for people just starting to traverse the 'minefields of mycology', there is no 'compendium' of tricks of the trade that can be appealed to when things just *don't* go right and nothing that is tried seems to help. Sooner or later, something is done which allows the work to proceed—but the method is then stored in the mind of the worker and never committed to print—or at best disseminated by word of mouth. This little script tries to collate a few tips. Not all of the tricks will be covered and if the reader knows of others, please let me know so that for a later edition they can be included.

Field work

Dealing with those 'Very Slight Odour' Fungi

Smells are notoriously difficult to describe because everyone's sense of smell is different: I love the smell of jasmine for example, but my wife detests it and even finds the odour painful to breathe. Fungi are often described as having various smells, including a 'fungousy' odour that we all recognise but find it difficult to put in words. Some fungi are described as having an odour but in the field the worker often cannot detect it. A classic case is that of *Cantharellus concinnus* (= *C. cibarius* var. *australiensis*). This definitely does have a distinct apricot smell that is immediately detectable by some (including myself) but others struggle in vain to notice anything at all. A very good way of enhancing the odour is to put the collection in a very small, clean plastic container for half an hour, then carefully open the lid a fraction and sniff the air at the opening. Any odour is concentrated and will be rapidly detected. This has proven an excellent method of proving the apricot odour of the above species even to 'non-believers'.

Detecting Viscidity of the Pileus and/or Stipe Using the 'Lip Test'

Many species are obviously viscid or sticky with a layer of gluten over the pileus and stipe, or they have gelatinised surface hyphae which also create this state. Unfortunately, many species rapidly lose this viscidity as they mature, sometimes because the humid conditions that were present at first have vanished and the pileus and stipe are now dry and sometimes because that is how the particular species develops. Often the pilei of these species have a varnished appearance that gives them away, but equally as often there is nothing to suggest the original viscidity. This becomes very irritating as many keys quite happily ask the user to choose whether the pileus (or stipe) was viscid and if there is no way to tell if the viscidity was originally there, the key user comes to a full stop.

Most people use their finger to test fresh material for the stickiness of the viscid pileus. However, for these difficult species, the finger is not sensitive enough. The best method to detect, in the field, the presence of a viscid cuticle is to use the 'lip test'. Touch the fungus gently onto the very sensitive skin of the upper lip surface and then equally gently and slowly pull it away—if there is any sticking sensation it will be very easily detected and a viscid pileipellis can be recorded. If the cutis is dry, a small drop of distilled water (never use saliva as this has enzymes in it that may alter the pileal surface) will revive the viscidity sufficiently. In all the cases so far encountered by the author, the 'lip test' has never failed to confirm a suspected ixocutis or ixotrichoderm.

(My apologies to the person who told me of this technique—I cannot remember who you are but thanks very much for the information.)

Getting that Fungus out in the Field

There are various suggestions that are made as regards how you should equip yourself in the field. I have tried numerous methods, but I would like to share the tried and true ones that I now seem to use very consistently. As regards collecting the fungus, most authorities recommend carefully digging it up with a tiny bit of soil if it is a ground species. This is so you can see if there is a volva that is critically important for the genus *Amanita* or any other species with below ground structures. You can carry a small garden trowel or a specially made implement, but my preference is now an old and battered 'Swiss army penknife', carried in a belt pouch. Before anyone asks, it has only a single, rather battered blade

and nothing else—I've yet to see the fungus that needs to be extracted with a corkscrew. This knife blade allows all the digging normally required. It can remove species from wood (except of course, those like *Phellinus* which need a small axe) and it has the added advantage that when you want to have lunch, it can be cleaned and used to butter your sandwich and peel the apple or orange. Even very deep fungi can be removed using this small blade. The most spectacular case was a *Cordyceps gunnii* which needed about 30 cm of painstaking and very careful excavation through a tree fern trunk and then into the soil.

A small, ×10–×20 hand lens is essential. It allows examination of quite small fungi in order to see details that may sometimes be absolutely critical in the field before the fungus wilts in any way.

I have also found that a set of forceps is absolutely essential. These are used to collect very tiny and delicate species. Forceps are also one of the easiest things to lose and I have not lost my pair of forceps in 10 years. (With one exception that I am sure Alan Mills is delightedly waiting to mention.) What I have done is drilled a hole at the top of the forceps and threaded through this a very brightly coloured cord—orange or yellow for preference as it stands out like a beacon on the forest litter. You just can't lose them or put them down because a final 'look around' always shows the bright cord and the attached forceps. The only time I have ever 'lost' my forceps was when I was in Tasmania in April/May 1998: before my final packing, I emptied a box with some forest litter outside on the ground—the forceps were of course, underneath the litter. Alan found my forceps about two weeks later in the back yard, solely because the bright cord was attached—I have been suitably grateful as I value those particular forceps rather highly. Incidentally, I also use a small piece of plastic tubing placed over the sharp ends of the forceps so that when they are placed back in my top pocket I don't get impaled on the sharp ends.

Storing the fungus to bring it back as carefully as possible is very dependent on how much you want to carry and exactly what it is that you are looking for. If 'anything is grist to your mill', the best things are probably small plastic containers (margarine containers with lids are excellent) and some grease proof paper to wrap up the larger items. If, as in my case, the target fungi are all smallish and delicate, then a very useful container is the 'bait box' with its compartments which can be stacked on the floor of a basket and carried without banging or damaging the specimens.

In the Laboratory

Detecting the Presence of an Ixocutis or an Ixotrichoderm on Herbarium Material

One of the most aggravating things is trying to decide if the 'withered piece of agaric fungus' being studied, originally had a viscid cuticle on the pileus. A very good starting procedure is always, and I **do** mean ***always***, examine the surface of the pileus using a stereo dissecting microscope under about ×20–×40 magnification. From considerable experience, an originally viscid pileipellis will almost always make its presence known by a 'varnished wrinkled' appearance under this magnification. The surface looks 'polished' as a result of the dried 'gluten' (or whatever caused the viscidity) and as the surface dries, it produces a very distinctive 'skin' over the surface that wrinkles. Over perhaps 300–400 collections examined this way, I am unable to re-call any collections that demonstrated this character which did **not** eventually prove to have had a viscid pileus when first forming in the field.

Cutting a Section

I suspect that most agaricologists find that hand sections are quite good enough for what has to be examined as regards sections of pilei or lamellae. It takes practice and a stereo dissecting microscope, but once mastered the technique is simple and extremely effective. I never bother with scalpel blades—unless there is *absolutely* nothing else—because I have found they are too thick. Better blades can always be obtained from the old, 'double edged' razor blades such as Gillette or Wilkinsons. The original Gillette blades were made of high carbon steel and snapped beautifully, however, the modern stainless steel blades will not do this very successfully. I find the best way is to have a very small pair of scissors devoted to 'tin-snip' work and cut very small, scalpel shaped sections of a blade as I require them. These tiny pieces of blade can then be mounted in the handles of dissecting needle holders. Use the screw-ended ones and then you can replace the used blade sections as often as you need to.

Recently I also found another source of even better blades. The double bladed 'plastic combinations' that are used in modern pivoting razors have an even finer metal foil than the old Gillette type twin blades. I very carefully pulled apart a blade after considerable bathroom use and even then found that the edges on these used blades were superior for cutting very fine sections when compared with new twin blades. My thanks to Katrina Syme who first informed me of this method in July 1998.

Tweezers and Dissecting Needles
No matter how you try, needle-pointed tweezers still end up looking like crowbars under a stereo microscope. This is all the more irritating if you have a very fine section just cut and the result is rather like 'trying to delicately manipulate a postage stamp-sized piece of tissue paper with a pair of fencing pliers'. One way out of this is to use the razor blades that have been cut with your dedicated 'tin-snip scissors'. You can cut very tiny pieces of blade and due to the action of the scissors, these tiny pieces are also a little curled. Mount one of these in a dissecting needle holder. The result is a very fine, almost hook like manipulator which can be used to tease out the hand cut section and very delicately place it on the microscope slide.

Rehydrating a Section While Keeping its Side View Position
When you are just rehydrating a 'lump of tissue' and only intend to splay it out, then there is no need to worry about placement of the section on the slide. However, if the material to be examined is a carefully cut hand section and it is critical that the details of the section be seen from the side, then just placing it on the slide is absolutely out of the question. From very, very bitter experience, the traditional method of 'place the section on the slide, add a drop of reconstituting mountant—such as ammoniated congo red—and then place a cover slip delicately on top' is just not good enough. If you do use this method, you will find it will work successfully in about 30–40 per cent of the attempts; the remainder of the time, the material twists as soon as the mountant strikes it and when the cover slip is put on, the twisted material has taken up a position which usually negates the effort put into getting as thin a section as possible and you have to start all over again—often thinking some very interesting words about mycology in general.

A large number of very frustrating moments can be avoided by the most simple and obvious procedure—but again, it's so obvious that no one ever tells you. Once the section is cut, place it on the microscope slide. Now, put the cover slip on the dry material and check to see that it is still flat—use the stereo microscope. Next, place a drop of the reconstituting mountant on the slide at the edge of the cover slip. It will immediately be drawn under the cover slip and rehydrate the section. Because the cover slip is present to hold the section firmly, it cannot twist or distort and virtually a 100 per cent success rate is guaranteed using this method of looking at sections.

Keys, Keys and 'Schtinken' Keys
I remember once about 15–20 years ago, when I was ever so naive, that I thought a single key was all I needed to completely discover the world of fungi. Better yet, I thought that a key—any key—was absolutely correct in all details. Ummmmmm...how things change. About 10 years ago, I recall spending an afternoon in my study with Alec Wood while we pored for about two hours over a collection obtained that morning from the Bunya Mountains. We were using the agaric keys published by a very well known and world respected agaricologist—who shall remain nameless. We believed that what we had was a species of the genus *Marasmiellus* because all the characters, apart from one, were present. But after examining the pileus' cuticle something like five or six times we could find nothing of the rameales hyphal structure that was supposed to be present. The problem was that our main key said that the hyphae had to be there—or it wasn't in the genus. After two hours of brick walls, we mentally 'tore our hair' and decided that it was a *Marasmiellus* whether the key to the genera agreed or not. So we finally went to the sub-section that contained the genus *Marasmiellus* and there, part way down the key to the species was a couplet which essentially said: 'Rameales hyphae absent from the pileus in this small group'. I forget exactly what we said following this discovery but it meant that although the group was known to the mycologist who constructed the keys, there was no way of getting to this group validly from the main part of the keys.

The lesson was re-learnt fairly abruptly. Don't just use one key on a new species, try several by different authors and if about four out of the six give the same answer, you are probably on to the correct result. If you come to a dead end, try some different paths and assumptions because quite often these will eventually lead to the correct answer.

'Photographing the Brute'
Most of my photographs are taken in the laboratory under contrived conditions. This is because mostly I only require photographs that allow diagnostic processes, not 'prettiness' of the subject. Nevertheless, there is a place for these pretty photos and in any event, without them, the fragile beauty of these natural gems would not be appreciated by the larger public—something to be greatly desired and encouraged. However, a couple of tips in the photographic area are worth knowing, especially if they can save money.

I mostly use colour slide film because I can show the pictures on screens for audiences, I can always get colour photographs made, and I can scan the slides easily into a computer. If possible, the film I use is Kodachrome 25ASA. This is extremely slow but very, very fine-grained and therefore allows a scanner to produce excellent images from the slide. Of course, this means that most field photographs must be taken by flash. If 25ASA is unobtainable (it is still made and can be got in specially by any good camera store if they normally don't stock it) then 64ASA is almost as good.

The ultimate way of photographing fungi in the laboratory is to use a copying stand. This allows the camera to be positioned directly above the specimen(s) and held absolutely still. The specimens can be arranged nicely and the picture taken with a flash attachment. A copying stand is quite an expensive item, between $300 and $800, but a simple multi-purpose copying stand can be had for about $50. What you do is purchase a tripod but make sure that it is one of the type that will allow you to take the central adjusting column out and invert it *i.e.* suspend the camera from underneath and inside the tripod legs. The specimen is now set up inside the tripod legs and the camera attached. There will be a few 'bugs' that have to be worked out of the system—including how to see down through the view-finder, and of course making sure that the camera does not fall downwards under any circumstances—but they are not insurmountable. Always use a shutter release cable to take the photograph and as a general rule, use a flash diffuser over the flash bulbs to soften the light and reduce 'blooming' where large glows of light appear on the photographic slide. This method was the one I used extremely successfully throughout my Tasmanian trip during 1998 and the results were all I could have wished for.

Some extra tips on the photography include the fact that the fungi should be placed on a ground glass sheet about 20 cm off the ground so that shadows are not photographed. The background should be a neutral grey colour. If you use a photographer's standard grey, the slides you take can produce images that can always be brought back to the original colour.

Drying the material for preservation

I have found two methods which are equally suitable depending upon where you are situated. My home is solar powered and normally I don't like using electrical heating elements to dry fungal specimens—a heating element is one of the most energy consuming methods of employing electricity. Instead I use a gas-powered dryer made of freshwater tank strainers fitted with a pilot light from a gas fired hot water system. The pilot light is also fitted with a small hot plate that acts as a heat diffuser. My specimens are usually dry overnight and the amount of gas used is extremely small: a one and a half kilogram gas bottle is good for about a week's continuous drying use.

The other method was introduced to me when I was at the Wilson's Promontory meeting of the Victorian Field Naturalist's group in May. I had never thought of this one but seeing the results has resulted in my now having my own drier which is taken with me if I am ever collecting in a situation where I have access to mains electricity. The drier is simply a proprietary brand food drier. These have a series of temperature settings from about 35–50 degrees and blow the warmed air over the fungal material that is placed on a series of racks. These driers are very effective and contrary to my misgivings, the constant air flow doesn't seem to mix the spores from any of the fungi to any extent. I have to thank Tom May for introducing me to that obvious use of a commercially available unit. The cost is (was) under $150 but I have seen dedicated mycological driers at costs of over $250.

And finally, again my plea—if you have any good ideas for any area of fungal research—tips that allow better applications of well known techniques, let me know for the future. My email address is generally examined daily if you wish to contact me rapidly.