WHAT IS AN ADEQUATE COLLECTION OF FUNGI?

J.A. Simpson [1], J. Walker [2], C A. Grgurinovic [3] & P. Buchanan [4]

[1]Research Division, State Forests of NSW, P.O. Box 100, Beecroft, N.S.W. 2119, Australia
[2]5 Cook St., Baulkham Hills, N.S.W. 2153, Australia
[3]Australian Biological Resources Study, P.O. Box 787, Canberra, A.C.T. 2601, Australia
[4]Landcare Research, Private Bag 92170, Auckland, New Zealand

Abstract

The fungi of Australasia are still inadequately known as compared with phanerogams. Guidelines for collection in the broad sense and for scientific study are presented. If followed carefully these guidelines should ensure that collections are adequate in amount and quality for future scientific study. A *Code of Conduct for Collecting* is presented for both general mushrooming and for scientific collecting. It is hoped the guidelines will be of value to managers of conservation areas when deciding upon the requirements to issue permits to collect fungi for scientific studies. The importance of voucher specimens of high quality is emphasised.

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A. Introduction

The collection of fungi in Australasia for scientific study is covered under various land management and conservation laws. In Australia fungal collecting is now regulated by diverse land management agencies and private land holders (Environment Australia 2001). Often, those issuing permits have little knowledge of fungi and what constitutes an adequate collection. Furthermore there are proposals to regulate access to biological resources by permit under Section 301 of the *Environment Protection and Biodiversity Conservation Act 1999* that came into effect in Australia in July 2000. These regulations would make a benefit-sharing contract between the collecting body and the owner/custodian of the biological resources a mandatory pre-requisite to issuing a permit (Voumard 2000). As part of this process it is proposed to extend export controls on micro-organisms. This will require the parties to the contract to lodge voucher specimens and information about the collection with a CITES-approved authority in Australia that has facilities for preservation and further dissemination, when appropriate, of this material. Queensland for example has passed the *Nature Conservation (Protected Plants) Conservation Plan 2000* (Queensland Government 2000) under which (Schedule 1) all fungi, algae, lichens, mosses and liverworts are declared '*Protected plants for which additional limitations apply*'. Collecting protected plants requires compliance with the '*Code of Practice for the Taking and Use of Protected Plants*' (Queensland Department of Environment 2000). However, the Queensland Code does not provide specific guidance for collection of fungi.

Permission to collect fungi in New Zealand is controlled by the Department of Conservation for all lands managed by the Department (including National Parks and Nature Reserves). Regulations governing such 'concessions' can be found in the *Conservation Amendment (No. 2) Act 1996*, the *Conservation Act 1987*, the *National Parks Act 1980*, and the *Reserves Act 1977* (Department of Conservation 2001). Fungi are not differentiated from 'flora and fauna', and in fact are often grouped under 'plants'. Permission to collect on land managed by local authorities is governed by each authority.

It is now recognised that the fungi, as generally accepted until about 1980, are a polyphyletic assemblage of heterotrophic eukaryotic organisms that in modern classifications are included in at least three Kingdoms (Walker 1996). However, since for example *Myxomycota* and *Oomycota* are usually studied by mycologists and phytopathologists rather than phycologists or protoctistologists, they are included here with the true fungi. Botanists continue to have difficulty accepting that lichens are fungi in symbiotic relations with cyanobacteria or green algae and not true plants.

B. A Code of Conduct for Collecting

The Australasian Mycological Society (AMS) has prepared a draft Code of Conduct for mycologists in Australia and New Zealand. It is rather different to that of the British Mycological Society (2000) reflecting the relative paucity of knowledge of the Australasian mycobiota. The AMS Code is voluntary and subject in Australia to Federal, State and Territory laws and regulations and in New Zealand to national legislation and/or local bylaws concerning the collection of fungi and plants. It is intended that this document will be available on the AMS website and will be revised and amended as necessary.

a. General guidelines

1. The Australasian Mycological Society is committed to the conservation of communities and populations of fungi and to maintenance of biodiversity. The Australasian Mycological Society recognises the importance of wild fungi as a resource to be utilised, but accepts harvesting of fungi for food or medicinal values only where it does not endanger the viability of fungal populations, and their associated organisms and habitats.
2. Do not collect on publicly owned lands (*e.g.* National Parks, State Forests, Nature Reserves) unless you have the appropriate permit(s). Details of permit requirements can be obtained for Australia from Environment Australia (2001) and for New Zealand from Department of Conservation (www.doc.govt.nz) or from the relevant local authority. For private land, it goes without saying that permission from the landowner is obligatory.
3. Always seek the landowner's or site manager's permission before you enter a site. Explain the purpose of the collecting and what will be done with the collections.
4. Try to minimise damage to vegetation, litter, logs, rock, soil and other features. Return disturbed sites as close as possible to the conditions before collecting commenced. Rake disturbed litter back over the plots, roll logs back to their original position and orientation, and do not trample vegetation unnecessarily. If chiselling lichens from rocks, do not deface prominent features or rock faces. The aim is to not impair the natural beauty of the site. Where possible, avoid collecting within sight of the public so as not to encourage non-scientific and non-permitted collecting.
5. Collect what you need and can adequately describe and curate. Sort collections before leaving a site and return any excess material to the native habitat.
6. If collecting is permitted for food, only collect from localities where the fungus is plentiful. In any case do not harvest more than half the fruiting bodies of any single species present. A maximum of 1.5 kg per person per visit will usually be adequate. Avoid collecting immature fruiting bodies; harvested mature fruiting bodies will have had the opportunity to discharge spores in the field. It is improbable that managers of National Parks, Flora Reserves, or Nature Parks will grant permits for collection of fungi for culinary purposes.

b. Scientific collecting

1. **Collect** only the amount needed for reliable identification, description, isolation and voucher specimens, including possible duplicates for examination by specialists in the group. It is essential to ensure that all duplicates of the one collection contain the same taxon.
2. **Keep** accurate field records of locality, habitat and host. Geographic coordinates are becoming highly relevant for inclusion in collection data so that distribution maps can be easily generated. GPS devices have made accurate determination of position fast and very accurate. In the future it is likely that GPS data will be used in conjunction with Geographic Information Systems to model site factors associated with occurrence of particular fungi. A specimen without field data (at least locality, host, date and collector) is of little scientific value. Details of how to collect botanical specimens can be obtained from the Centre for Plant Biodiversity Research (2001) website.
3. **Give** each collection an individual field collection number that will stay with the collection for the rest of its life. This must correspond to the number given in the field notebook, in which relevant notes about each collection are made. The field collection number should be written on a piece of paper and wrapped with the collection.
4. **Treat and conserve** all specimens in ways that ensure the preservation of important characteristics and the stability of the genome. Molecular characters are being used increasingly in taxonomic studies and specimens need to be preserved in such a way as to cause minimal disruption of the nucleic acids. For molecular work, drying at high temperatures and the use of nuclear radiation for preservation are unacceptable. Forced air at 45°C are recommended drying conditions to preserve DNA. If material is to be held for some months before deposition in a recognised herbarium it should be disinfested of arthropods by freezing at -15°C for 72 hours. Always avoid using liquid preservatives, except for small fragments that may be placed in various fixatives for later embedding and sectioning or DNA extraction.

5. **Retain voucher specimens.** They provide a permanent, checkable record of the fungus and host studied. It is important that voucher specimens be prepared so as to maximise their usefulness in the future. Accompanying microscope slides need to be prepared carefully and sealed (Volkmann-Kohlmeyer & Kohlmeyer 1996). Full descriptions, collection details and field collection numbers should accompany all specimens. They should be lodged in a registered herbarium and often the appropriate herbarium will be specified in the collector's permit; in Australia it is usually the herbarium of the State or Territory where the collections were made, at least for holotype collections, while in New Zealand there are only two recognised fungal herbaria. It is highly desirable, some would say essential, that the dried holotype collection of a holomorph, teleomorph or anamorph, and where possible a living culture from the same collection, are housed in the one herbarium. All holotypes should be lodged in an Australasian herbarium. The major registered public collections for fungi in Australia are the Herbaria at Adelaide (**AD**), Brisbane (**BRIP**), Canberra (**CANB**), Orange (**DAR**), Hobart (**HO**), Melbourne (**MEL**), Perth (**PERTH**), Sydney (**NSW** for lichens only), Knoxfield (**VPRI**). In New Zealand, the New Zealand Fungal Herbarium is at Auckland (**PDD**), with a collection focused on fungi of importance to forestry at Rotorua (**NZFRI-M**). 'Voucher collections are invariably necessary, not only when new fungi are described, but also in connection with any scientific study, whether by taxonomists, systematists, physiologists, chemists, molecular biologists, pathologists, ecologists, clinicians *etc.* dealing with organisms. It is essential to preserve voucher specimens as dried material and, where possible as permanently preserved living cultures' (Agerer *et al.* 2000). This applies to fungi from mammals and other vertebrates, invertebrates, and plants (Agerer *et al.* 2000, De Hoog & Guého 1985). Records 'that cannot be verified are mere waste of paper' (Dennis 1960). Many scientific journals now require that specimens referred to in publications be appropriately accessioned in a recognised institution. The addresses of Herbaria can be obtained from the *Index Herbariorum* or on the internet at http://www.nybg.org/bsci/ih/ih.html or from Puttock & Cowley (1997).
6. **Offer** the results of your collecting and copies of any papers published to landowners, land managers and issuers of permits. Indicate the significance of the results obtained.

C. Importance of cultures

Wherever feasible it is desirable that the fungus collected be isolated into axenic culture, preferably from single spores in addition to isolation directly from the fruiting body or substratum. Sexual compatibility or incompatibility, cultural characteristics, and the presence of an anamorph are of increasing importance in determining the identity of many fungi. Many ascomycota and some basidiomycota have characteristic anamorphs and culturing is an excellent way of confirming anamorph-teleomorph connections. Methods for isolating fungi are outlined in Choi *et al.* (1999) and Goh *et al.* (1999). Methods of storing and maintaining fungal cultures are discussed in Smith & Onions (1994) and Ryan *et al.* (2000). Cultures should be stored under suitable conditions for long-term storage, such as lyophilization or freezing in liquid nitrogen, and deposited in a recognised national or regional culture collection. In Australia, the National Fungus Collection (Orange (DAR), Indooroopilly (BRIP), and Knoxfield (VPRI)) has the facilities to maintain culture collections; in New Zealand culture collections are located at Auckland (ICMP) and Rotorua (NZFS).

D. DNA for molecular studies

Live cultures are the preferred source of DNA for PCR, sequencing or other molecular studies. For taxa that cannot be cultured, hymenial tissue from fresh field-collected ascocarps, basidiocarps or sporocarps are placed in 2× CTAB (hexadecyl trimetyl-ammonium bromide) buffer (Doyle & Doyle 1987) and stored at room temperature until the DNA is extracted and purified (Hibbett & Vilgalys 1993). Provided dried exsiccata have not been subject to high temperatures during drying, or to a δ-ray or X-ray radiation sterilisation treatment DNA can also be extracted from dried herbarium specimens (Humpert *et al.* 2001). Weir & Blackwell (2001) recommended storage in 95% ethanol of insects and their ectomycoparasitic fungi for DNA studies of the fungi. It is essential that specimens of collections to be used in DNA studies are retained in an herbarium for future reference.

E. Size of collections

One of the purposes of this document is to provide a concise guide as to what constitutes a good collection of a fungus. Given the diverse size, hosts, habitats, mechanical strength, and resistance to desiccation of different fungi, there is considerable variation in what represents a sufficient collection. The Mycological Society of America produced a 'comprehensive' guide, a book of 703 pages (Stevens 1974). The following comments are a brief guide of what and how much to collect and how to treat collections.

With **all** fungi it is important to:

1. collect/provide sufficient material for detailed examination and/or culturing;
2. collect a full range of developmental stages but not overmature, decomposing or invertebrate-infested material unless you are specifically interested in organisms associated with senescing fruiting bodies or with hyperparasitic taxa;
3. pack or store material bearing fruiting bodies in such a way as to protect those structures from physical damage, desiccation and mould and insect damage;
4. press and dry host specimens before dispatch;
5. not send fresh collections in plastic bags unless they will get to the recipient in less than 24 hours (during cool weather) or are hand-delivered;
6. avoid liquid preservatives. They wash off spores from their parent organs, damage specimens, distort colours and are completely unsuitable for long-term storage. Moreover, specimens in liquid cannot be sent through the post or by airfreight. For preparation of thin sections, appropriate small pieces can be placed in a suitable fixative in small tubes for later treatment; and,
7. segregate field collections from laboratory cultures to minimise the risk of mite infestations.

F. General points on collecting

There are several points to note when collecting fungi:

1. **Fresh characters.** Record in the field in a notebook (complemented if possible by photographs or drawings), those characters that can only be recognised on fresh material. This is especially important with colours.
2. **Host records.** With all fungi on plant parts, and for mycorrhizal fungi, a separate botanical collection of the host should always be made to ensure that its accurate identity can be determined and, if there is any later change in concepts of host taxonomy, that a host specimen is available for examination. Such host collections should be filed with the fungal collection. Make sure collection of a botanical sample for identification is specified in your permit application. With fallen plant organs, it is sometimes difficult to determine which of the surrounding plants is the parent and great care needs to be taken; in some cases, 'undetermined fallen leaf' is the only satisfactory solution.
3. **Plastic bags.** These should be used with the greatest care and usually for the shortest possible length of time (Millar & Richards 1974). Many taxa of microfungi would never have been discovered if not induced to sporulate in a moist bag. However, hyphomycetes and some other fungi can quite quickly produce abnormally long conidia and conidiophores in the humid atmosphere inside a plastic bag, thus distorting observations on the true morphology of the species. Others ooze or discharge spores from fruiting bodies. Moreover, secondary mould development can occur rapidly inside a plastic bag, with the production of mould spores and sporing structures. Thus material collected into plastic bags should be examined soon after collection or airdried as soon as practicable.
4. **Handling.** Considerable care is needed in handling all specimens bearing microfungi. Many microfungi produce delicate fruiting structures that can be easily damaged or removed from plant surfaces if the specimens are not handled properly during collection. Avoid rubbing or handling leaf surfaces and other plant organs bearing rust sori, mildew colonies, leaf spots, or superficial growths on twigs. Do not hold them between fingers and thumb. All that can be seen on some leaves with the unaided eye is a spot or yellow blotch and the fine superficial fruiting structures will only be detected under the microscope. Practices such as cutting off leaves with a small pair of secateurs directly into a bag, or breaking leaves off by holding the petiole, and similar care when transferring the material to the plant press will pay dividends in the quality of the specimen preserved.
5. **Repeated sampling.** Collecting in the same place at several different times of the year is particularly useful in locating different stages of the life cycle of both parasites and saprobes. For example, teleomorphs may be found on dead fallen leaves that, when living, bore the anamorph earlier in the season.
6. **Different states.** With rusts, which may have several spore stages, plants should be examined carefully to see if more than one spore stage is present. In the case of heteroecious species, where two botanically

unrelated hosts are involved, surrounding plants should be carefully examined to see if possible alternate stages of the life cycle are present. If suspected, these would then need to be the basis of cross-inoculation work to see if the rusts on the two hosts were part of the same life cycle. With downy mildews and *Albugo* spp., sporangia and oospores may occur on different plant organs; similarly with powdery mildews, cleistothecia may occur on different organs from the conidial structures. The whole plant should be thoroughly searched.

7. **Fungi of quarantine significance.** Fungi known or suspected to be exotic and of quarantine significance should be sent very securely packaged, double wrapped, and either by express post or by courier. Ensure you do not breach State, Commonwealth, or national quarantine regulations.

G. Larger fungi

This category includes all the larger Ascomycota and Basidiomycota. A range of specimens at different stages of development should be collected. Terrestrial fruiting bodies should be dug up, not pulled, so that the base is retained. Those on wood should be carefully cut or chiselled off or, for those growing on bark, taken off with small pieces of bark. Resupinate species can be removed with a portion of the substratum, ensuring that each specimen contains both the central and marginal portions of the fruiting body. For large species, a minimum of three fruiting bodies should be collected; for small species, for example tiny marasmioid taxa and most microscopic fungi, collection of 100 fruiting bodies may not be excessive as each examination could use up at least one complete fruiting body. For resupinate species, hand-sized portions are usually sufficient.

A comprehensive description of the fungus should be made before it is dried. All collections should be kept separate from one another in the collecting basket or container by wrapping them separately in pieces of dry newspaper or greaseproof wrapping paper. A sample of the wood on which a lignicolous species is growing should be removed with a knife or hatchet and kept with the fungus specimen as the kind of rot is an important species characteristic.

H. Microscopic fungi

a. On living plants

Plant parasitic species such as rusts, smuts, mildews, leaf-spotting species, diffuse hypogenous fungi and others occurring on plant hosts can be pressed and dried between sheets of newspaper in a plant press. A collection of 15–25 average sized leaves or equivalent is usually sufficient for one collection in most cases; if duplicates are required, a larger collection may be needed. For small leaves (*e.g.* clovers, wattle pinnules), a larger number of units should be collected. Leaves of grasses, rushes and sedges that tend to inroll lengthwise after collection should be pressed as collected. For large structures such as rust galls and witches' brooms, three or four pieces should be sufficient. Twigs and similar structures bearing fungal structures can be pruned out; five to ten pieces each 10–15 cm long is an appropriate collection, as long as several fruiting structures are present on each piece. Small pyrenomycetes, pycnidial fungi and similar structures are often embedded in the woody plant tissue and may be at various stages of maturity or even old and empty. A thin slice taken across the top of a few fruiting bodies, which are then viewed with a hand lens, will show if they are filled with mucilaginous contents or have a shiny interior, indicating that contents are still present. If they appear dull and empty, the fungus is probably old and dead.

b. On dead parts of plants

Saprobes and saprobic stages of plant parasites may be found on dead plant parts, either still attached to the parent plant or as fallen dead organs. These are often difficult to observe in the field. For dead attached parts specimens of a similar size to those collected for fungi on living plants will usually be adequate. Several bags (paper or plastic) of fallen litter can be returned to the laboratory for examination or incubation in moist chambers.

I. Specialised groups of fungi

a. Aquatic fungi

If discovered in the field, they are collected into containers with some water from the site. More commonly, baiting techniques are used to discover what species are present. Freshwater spora can be sampled by membrane filtration of 250 mL samples of water (Iqbal & Webster 1973). The entire filters are scanned under the

microscope at 100–400× magnification and spores can be removed for culturing. Jones (1971) and Hyde & Pointing (2000) give a full selection of techniques for handling freshwater and marine fungi.

b. Chytridiomycota
Sparrow (1960), Karling (1977) and Barr (1987) give details of how to collect and isolate chytrids.

c. Dung fungi
Fungi may be found fruiting on dried dung in the field and pieces of the dung bearing the structures can be collected into paper bags. Often, fresh dung is collected and incubated in moist chambers to see the succession of species that develop. Portions of such material can be dried to preserve the fungi and the specimens stored in the herbarium. Care should be taken when handling dung of carnivores or omnivores; plastic gloves are advised. Avoid breathing in any dung dust and fumigate specimens before storing. Bell (1983) provides a comprehensive guide to collecting and isolating dung fungi.

d. Fungi on arthropods
Diseased insects, spiders, *etc.* may be found in the field bearing fruiting structures of fungi parasitising them. Larger fruiting bodies such as those of *Cordyceps* and some of the more prominent conidial anamorphs may be collected and dried as explained for the larger fungi above. The search for microscopic species often involves collection of the animals and use of specialised techniques for detecting the presence of the fungi. Details for the Laboulbeniales may be found in Tavares (1985) and in Thaxter (1896–1931) and for Trichomycetes in Lichtwardt (1986) and Misra & Lichtwardt (2000).

e. Lichenised fungi
Details of the collection, preservation and examination of lichens are summarised by Hawksworth (1974), Filson (1992) and Malcolm & Galloway (1997).

f. Mycorrhizal fungi
Brundrett *et al.* (1996) provide detailed and practical advice on collecting and processing ectomycorrhizal and vesicular-arbuscular fungi, the isolation of ectomycorrhizal fungi into axenic culture, and the establishment of pot cultures of Glomalean fungi.

g. Myxomycota
Specimens should be collected with a portion of the substratum and placed immediately into individual small containers and preferably secured so that they do not shake about and risk damage. Some collectors pin them to small pieces of cork in the bottom of the container to minimise damage to the delicate fruiting structures. Myxomycota generally keep well when dried out, if handled and curated carefully. Details about collecting and handling myxomycota are given in Martin & Alexopoulos (1969 pp. 12–15), Martin, Alexopoulos & Farr (1983 pp. 19–24), Stephenson & Stempen (1994) and Ing (1999).

h. Oomycota
Downy mildews and the white blister fungi (*Albugo* spp.) have been mentioned above under the microfungi. For the remaining Oomycota, cultural studies are usually necessary and details are given in Fuller & Jaworski (1986), Sparrow (1960) and van der Plaats-Niterink (1981).

i. Thermophilic and thermotolerant fungi
Much remains to be learnt about these fungi in Australasia where they are not uncommon in soils and decomposing organic matter. Cooney & Emerson (1964) is an excellent starting point.

j. Yeasts
Yeasts are ascomycetous and basidiomycetous fungi whose vegetative growth predominantly results from budding or fission and which have sexual states that are not enclosed within or upon an ascocarp or basidiocarp. The presently accepted methods for the isolation, maintenance and identification of yeasts have been reviewed by Yarrow (1998). Collections of yeasts from the field will involve collection of substratum from which the yeasts can be isolated. Living cultures are essential for yeast taxonomy.

k. Zygomycota
The methods for collecting and isolating the sporangiospore-producing species of Zygomycotina are detailed in Benjamin (1959) and O'Donnell (1979). Methods of studying the mycorrhizal Glomales and Endogonales are given in Brundrett *et al.* (1996).

References

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