Version 1.1

Standardized Inventory Methodologies For Components Of British Columbia's Biodiversity:

MACROFUNGI

(including the phyla Ascomycota and Basidiomycota)

Prepared by the Ministry of Environment, Lands and Parks
Resources Inventory Branch for the
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Resources Inventory Committee

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PREFACE

This manual presents standardized methodologies for inventory of macrofungi in British Columbia at three levels of inventory intensity: presence/not detected (possible), relative abundance, and absolute abundance. The manual was compiled by the Elements Working Group of the Terrestrial Ecosystem Task Force, under the auspices of the Resources Inventory Committee (RIC). The objectives of the working group are to develop inventory methodologies that will lead to the collection of comparable, defensible, and useful inventory and monitoring data for the species component of biodiversity.

This manual is one of the Components of British Columbia's Biodiversity (CBCB) series which present standard protocols designed specifically for group of species with similar inventory requirements. The series includes an introductory manual (Introduction to RIC Wildlife Inventory) which describes the history and objectives of RIC, and outlines the general process of conducting a wildlife inventory according to RIC standards, including selection of inventory intensity, sampling design, sampling techniques, and statistical analysis. The Introduction to RIC Wildlife Inventory manual provides important background information and should be thoroughly reviewed before commencing with a RIC wildlife inventory. RIC standards are also available for animal capture and handling, and radio-telemetry. Field personnel should be thoroughly familiar with these standards before engaging in inventories which involve either of these activities.

Standardized data forms are required for all RIC wildlife inventory. This is important to ensure compatibility with provincial data systems, as all information must eventually be included in the Species Inventory Datasystem. The manuals and data forms are available from:

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It is recognized that development of standardized methodologies is necessarily an ongoing process. The CBCB manuals are expected to evolve and improve very quickly over their initial years of use. Field testing is a vital component of this process and feedback is essential. Comments and suggestions can be forwarded to the Elements Working Group by contacting:

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The Resources Inventory Committee consists of representatives from various ministries and agencies of the Canadian and the British Columbia governments as well as from First Nations peoples. RIC objectives are to develop a common set of standards and procedures for the provincial resources inventories, as recommended by the Forest Resources Commission in its report "The Future of our Forests".

For further information about the Resources Inventory Committee and its various Task Forces, please contact:

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Terrestrial Ecosystems Task Force – Biodiversity

The background information presented in this document are based on the unpublished draft manual, *Methodologies for inventorying British Columbia's macrofungi*, prepared by Scott A. Redhead and Shannon Berch for the Resources Inventory Committee. Minor changes to the draft manual were made by Ruth van den Driessche and Tom Ethier in order to conform to the Resources Inventory Committee publication standards.

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1. INTRODUCTION

1.1 Preamble

This manual is a guide for locating and collecting macroscopic fungal fruitbodies in temperate regions of North America including British Columbia. Fungi traditionally treated as lichens or moulds are excluded. The INTRODUCTION outlines the scope and limitations on the taxonomic groups covered and may be skipped entirely if one is comfortable with the concept of macrofungi. The manual is designed to be used by individuals with biological or natural history training combined with mycological field experience, or to assist in field training of such individuals. The chapters, SAMPLING SITES BY MICROHABITATS, SAMPLING BY SEASONS, METHODOLOGY, HANDLING IN FIELD, and HOW TO PROCESS, PRESERVE, AND RECORD SPECIMENS should all be read prior to conducting actual surveys. They will assist in planning specific projects.

1.2 Definitions and delimitations of groups covered by this manual

Until recently, the kingdom Fungi included six main groups: three (chytrids, hyphochytrids, and oomycetes [water molds]) either aquatic or terrestrial but then typically borne within water films in soil or on plants via motile spores, and three (zygomycetes, ascomycetes, and basidiomycetes) primarily terrestrial. The chytrids, hyphochytrids, and oomycetes, as well as other organisms traditionally studied by mycologists (plasmodial slime molds, cellular slime molds, and slime nets) are sometimes referred to a separate kingdom from the fungi, i.e. the Protoctista (Margulis and Schwartz 1988). When treated in such a classification, the zygomycetes, ascomycetes, and basidiomycetes, are collectively considered to be the true fungi. These fungi, as defined by Margulis and Schwartz (1988), are heterotrophic eukaryotes that form spores and lack flagella (i.e. are non-motile). The Amastigomycota of Tehler (1988) is a synonymous name for the group. A more recent study (Barr 1992) indicates that "fungi" belong to at least three kingdoms, one of which, the Eumycota, encompassess the phyla Chytridiomycota, Zygomycota, Ascomycota and Basidiomycota. For convenience, he suggested that collectively the organisms traditionally considered to be fungi, but which now are known to unrelated, be considered the Union of Fungi.

This manual covers fungi in only two phyla, the Ascomycota and the Basidiomycota which together comprise the Dicaryomycotina of Tehler (1995). These fungi can be subdivided further, artificially into two morphological groupings: those whose somatic form is filamentous or hyphal, and those whose somatic form is cellular. The cellular forms characterize the yeasts, while hyphal form characterizes the vast majority of the true fungi. Most of the yeasts are identified using a combination of morphological and physiological criteria and lack fruitbodies, and therefore will not be treated further. Many fungi, especially among the species of ascomycetes, associate intimately and symbiotically with certain unicellular algae or cyanobacteria to form lichens. Lichens function like photosynthetic plants and therefore, traditionally have been collected like bryophytes, hence they are considered in a separate treatment (Goward 1994).

Nonlichenized filamentous fungi can be further divided by the mode of reproduction: sexual versus asexual, i.e., whether sporulation results from meiosis or mitosis. Moulds, which comprise the majority of microfungi, produce mitospores or conidia on relatively simple sporogenous structures. In the macrofungi, eg. mushrooms, meiospores are produced in or on specialized cells (asci or basidia) often in conspicuous fruitbodies. For this reason the hyphal fungi may also be categorized by size of the spore bearing structures (variously named as fruitbodies, fructifications, ascocarps, basidiocarps, sporocarps, sporophores, mushrooms, apothecia, morels, stromata, puffballs, conks, etc.). Macrofungi are the nonlichenized species which produce these "large" fructifications, i.e. those visible to the naked eye and generally one centimetre or more in width or height. The majority of species are from the phyla Basidiomycota, eg. mushrooms and puffballs, and Ascomycota, eg. morels and cup fungi. In fact, the term macrofungus usually refers simply to the fruitbodies and not necessarily to the mycelium. Microfungi can be defined as fungi with either microscopic spore-forming structures or minute fructifications less than few millimetres big (not more than 1 cm high). This separation is artificial because both groups include many different lineages. Microfungi and sometimes even the macrofungi are considered to be microorganisms by some definitions (Zavarzin 1995), but each of these categories involve definitions with varying degrees of precission. In nature, macrofungi are easier to identify and inventory because they are easier to find and collect than microfungi. Soil-dwelling microfungi, for instance, are usually only identifiable after they have been isolated in pure culture on nutrient media and induced to form spores. Virtually all zygomycetes are microfungi. Most ascomycetes and a few basidiomycetes (Exobasidiales, some Corticiaceae) can be considered microfungi. The following modified chart from Barr (1992) shows the relationship of the two principal fungal phyla, **Ascomycota** and **Basidiomycota**, which produce macroscopic fruitbodies.

2. INVENTORY GROUP

2.1 Union of Fungi

(Underlined phyla includes those discussed in this manual.)

Kingdom - Chromista

Phylum - Heterokonta

Subphylum - Pseudomycotina

Class - Oomycetes

Class - Hypochtridiomycetes

Subphylum - Labyrinthista

Class - Labyrinthulea

Kingdom - Protozoa

Phylum - Myxomycota

Phylum - Plasmodiophoromycota

Kingdom - Eumycota

Phylum - Zygomycota

Phylum - Chytridiomycota

Phylum - Ascomycota

Phylum - Basidiomycota

2.2 Categorizing fungi by nutritional status

While all fungi are heterotrophs, there are many ways in which they gain fixed carbon and energy and thus many ways in which they function in the ecosystem (Christensen 1989). Decomposers break down dead organic matter but often specialize in the type of organic matter attacked. Most wood decomposers may utilize either cellulose (brown rots) or both lignin and cellulose (white rots). During decomposition, nutrient elements from the substrate are either released into the environment or immobilized in fungal tissue. Fungi help develop soils by initiating primary weathering, holding together aggregates, altering ionic exchange and water holding capacity, and producing humic substances. Biotrophic fungi include those forming mutually beneficial symbioses with plants (mycorrhizae), animals (eg. bark beetles with ambrosia fungi), and cyanobacteria or algae (lichens). Parasitic fungi are also biotrophic but pathogenic fungi generally cause decline or death of their host (necrotrophic). Predatory fungi acquire carbon and nitrogen by trapping and consuming various animals (nematodes, rotifers, collembola).

2.3 Logistical limitations on inventories of terrestrial fungi

Conservative global estimates place the vascular plant:fungus ratio at 1:6, however, it may be as high as 1:9 (Hawksworth 1991). Given the fact that there are about 2,500 vascular plants in British Columbia (Jim Pojar, Ministry of Forests, Smithers, pers comm.), we can estimate that there are about 15,000 (-22,500) species of fungi in the province. In some areas of the world, such as Great Britain, the fungi are so well known that it has been possible to publish lists of extinct, endangered, vulnerable, and rare species (Ing 1992). This is not the case in British Columbia where, to date, only about 500 of an estimated 1,500-2,000 species of mushrooms (agarics, boletes, and chanterelles) have been reported (Redhead 1994). Mushrooms should be among the easiest groups of fungi to inventory because they tend to be large, so it is to be expected that other fungi, notably the microfungi, are even more poorly known in the province. Certain other groups, however, are reasonably well known. About 3,300 species of fungi are known to cause tree diseases in the province (Brenda Callan, Pacific Forestry Centre, Victoria, pers. comm.). The estimated large number of species and the small amount of information we have on their distribution are two factors complicating inventories of terrestrial fungi in British Columbia. For our purposes we will concentrate on the surveying of the macrofungi.

3. PROTOCOLS

3.1 Standard Surveys (macrofungi)

There are two basic ways to sample or survey macrofungi, along with many different techniques. One is to survey the species based on the presence of the sporophores (Vogt et al. 1992), the other is to detect the fungus based on the presence of either mycelium, spores, or other microscopic structures.

Detection of vegetative mycelium is similar to that for the detection of microfungi. It can sometimes be accomplished by direct microscopic examination, or more frequently by cultivation, isolation, and examination using a battery of different media, baits, temperature regimes, inhibitors, and dilutions or baits. More recently tests or probes utilizing antibodies or PCR produced templates, or DNA sequencing of amplified segments from minute samples have been created (Egger 1992).

Among the techniques for surveying various Kingdoms and Classes of organisms in British Columbia, the sampling of macrofungi by gathering fruitbodies is more labour intensive, and the results are more unpredictable than those employed for many other organisms. Unlike larger animals or insects the majority of fungal species cannot be trapped, nor are they attracted from long distances to baits (food or pheromones) or lights, and they cannot be forced into capture by heat, noise or light, although some may be induced to fruit by physical or chemical manipulation of the environment (Sagara 1992).

Macrofungi, like plants, must be sampled in situ, and therefore, all possible sites and microhabitats within those sites must be examined repeatedly as seasons progress. Unlike plants, nonlichenized fungi do not require direct access to light for photosynthesis, and as a consequence their fruitbodies as well as their thalli may be hidden amongst or within substrates or be subterranean. Combining the attributes of both animals and plants, the fungi may occupy a diversity of microhabitats within any one ecological zone where even an individual plant can serve as many different niches.

Phenology is also important. Sampling based upon the presence of only the fruitbody, a reproductive organ, is necessarily dependent upon the periodicity of fruiting, the length of time the species fruits, the number of fruitbodies produced, the tenacity of the fructification (ephemeral versus perennial), susceptibility to grazing by mycophagous animals, and the regularity and reliability of annual fruiting. Even the presence of a fruitbody may not ensure a positive identification beyond the generic level. The degree of maturity, the absence of different stages for some genera, and environmental damage or changes to the fruitbodies may render the fruitbodies unidentifiable using classical morphological characters.

Contrary to the situation for most plants and most animals, a fungus "individual" is very difficult to define. All hyphal fungi consist of mycelia that are perennial or long-lived relative to the life span of the fruitbodies. The analogy of apples and apple trees is often used to illustrate the situation in the fungi: fungal sporocarps are to fungal mycelium what apples are to apple trees. They are sexual reproductive structures. To understand better the challenge this poses for fungal inventory, imagine that the apple tree grows entirely below ground with only the apples forming above. One year, dozens of apples might be produced from a single tree. The next year, if conditions are bad, no apples might be produced. If we

inventoried the apples in the first year, we would count dozens of apples and the next year none, but there would still be one tree and one tree only.

An additional complication is the fact that a perennial fungal mycelium, or individual, can separate over time into more than one genetically identical, discontinuous masses. Given that with more time these separate masses might eventually fuse again, the definition of a fungal individual becomes more and more difficult. So, when the numbers or sizes of individuals is important, the tremendous difficulty of recognizing a fungus individual is another factor limiting the inventory of terrestrial fungi.

The mycelium of a fungus individual is normally much larger than its sporocarp. The mycelium of an individual of an *Armillaria* species studied in Michigan is estimated to be greater in biomass than an individual blue whale or giant sequoia (Smith *et al.* 1992) while each mushroom produced by the individual would weigh only a few grams dry. This has implications not only in terms of the "territory" or food base necessary to support a fungus individual and the appropriate area that might be needed to preserve a particular fungus and population, but also for the type of inventory that would be possible. The final factor limiting inventory is our ignorance of the space and food base required to support a fungus individual and, consequently, a viable population.

3.1.1 Minimum Personnel Requirements

To conduct a systematic survey of macrofungi requires that the surveyor combine three basic skills: (1) recognition of the variety of fungal fructifications as fruitbodies; (2) the ability to distinguish microhabitats; (3) knowledge of taxonomically important features on fresh collections. The surveyor must be trained by a field taxonomist, typically at a graduate university level, or only the more conspicuous fungi will be sampled, look-alike species will be readily confused and either mingled or be left in the field, and entire communities in neglected microhabitats will be overlooked. Collected specimens may be useless if the surveyor does not record critical data. Experience in conducting mycological surveys in Canadian national parks has demonstrated that the number of species sampled in a two week period is increased several fold when a professional mycologist takes over from an on-site general biology level student. Also the value of the specimens becomes substantially greater as more pertinent data are gathered.

3.1.2 Habitat Data Standards

A minimum amount of habitat data must be collected for each survey type. The type and amount of data collected will depend on the scale of the survey, the nature of the focal species, and the objectives of the inventory. As most, provincially-funded wildlife inventory projects deal with terrestrially-based wildlife, the terrestrial Ecosystem Field Form developed jointly by MOF and MELP (1995) will be used. However, under certain circumstances, this may be inappropriate and other RIC-approved standards for ecosystem description may be used. For a generic but useful description of approaches to habitat data collection in association with wildlife inventory, consult the manual, “Introduction to RIC Wildlife Inventory”.

3.2 Sampling Sites by Microhabitats

3.2.1 Woody plants (trees and shrubs)

Each species of tree creates an array of niches for fungi, and tree species which grow in different ecozones increase the number of niches. Additionally as a tree matures it and its mycoflora change. Seedlings, saplings, mature trees, and ancient trees all support different fungi. Hence the age, location, history, and anatomical features of a tree all combine to increase fungal diversity.

3.2.2 Wound and decay fungi

Living trees ward off fungal attack by decay fungi using a variety of defense mechanisms such as compartmentalization of infected wood by phenolic substances (see Shigo 1979), wound healing, fungistatic substances on surfaces, and sloughing of bark and branches. For these reasons only a selected group of decay species are found fruiting on living trees or recently killed trees. The macrofungi inside compartments within living hosts can usually only fruit from trunks at branch stubs, frost cracks, insect burrows, and wind damaged tops or branches. Trunks should be scanned from ground level upwards for conks, mushrooms, cankers, and other abnormal growths that may occur many meters above ground. Common examples are: *Cryptoporus, Echinodontium, Ganoderma, Hypsizygus, Phellinus, Fomitopsis, Fomes, Piptoporus, Pholiota, Pleurotus, Flammulina, Hericium.*

3.2.3 Butt and root decay fungi

Several species of decay fungi are confined to the heart wood of lower portions of mature to ancient trees, or primarily attack major roots. Two of these, *Bondarzewia mesenterica* and *Oxyporus nobilissimus*, have been flagged as old growth indicators in the Pacific Northwest of the United States (Record of Decision 1994). *Bondarzewia* occurs in B.C. around the bases of ancient conifers in coastal regions. Other butt and root rot fungi which tend to attack sap wood in younger trees in their prime, and hence are forest management pests, are *Phaeolus schweinitzii*, *Phellinus weirii*, and *Heterobasidion annosum* among the polypores and *Armillaria* sp. among the agarics. Polypores arising from the ground near trees should be carefully excavated to determine if they have subterranean stipes leading to roots or sclerotia. The crotches of roots on butts should be specifically examined for fructifications wedged in splits in the bark, eg. *H. annosum*. Similarly exposed major roots should be examined on the lower surface for appressed fruitbodies. In hardwood forests such as maple, tree bases may be covered by Xylariaceae, such as *Hypoxylon, Ustilina, Xylaria,* which form black carbonized fruitbodies having the consistency of charcoal. In the B.C. interior the agarics *Gymnopilus, Hypsizygus* and *Coprinus* may be found at the bases of poplars.

3.2.4 Bark fungi

Small agarics and many Aphyllophorales inhabit living or damaged bark around wounds on living trees. Trunks should be examined closely, particularly if dry conditions have dehydrated revivable fruitbodies. Examples are: *Mycena, Hemimycena, Laeticorticium, Peniophora*.

3.2.5 Twigs and lower senescent branches

Either through injury or canopy closure the lower branches of trees usually die while still attached and often remain shaded. For conifers, especially in moist habitats, dense concentrations of small diameter dead branches should be examined from the undersides for pendant or appressed fruitbodies such as *Aleurodiscus* and *Lachnellula*. In rain forests moss-covered lower limbs may also harbour fleshy Mycenas and bird's nest fungi (*Nidula candida*) which sit on the upper surfaces of twigs. Larger limbs have different physical properties and therefore host a different mycoflora which usually fruit along the lower surfaces, eg. *Phellinus, Datronia, Hymenochaete*. Rapidly growing species of hardwoods such as alders, willows and poplars frequently suffer branch damage and death or shading out of saplings. These standing dead small diameter corticated trunks and limbs are frequently colonized by jelly fungi such as *Exidia* and *Tremella*, revivable inoperculate cup fungi such as *Cenangium*, cyphellaceous fungi, and small pleurotoid agarics such as *Cheimonophyllum*. Because small diameter dead branches dry easily, most of the twig inhabiting fungi form revivable fruitbodies. The jelly fungi dry down to glossy or dull, dark, inconspicuous blobs and the cyphellaceous fungi or ascomycetes often curl inwards becoming less conspicuous. Searching for such fungi can be more productive in wet, drizzly weather, but is best accomplished immediately after, rather than during rains when it is difficult looking upwards.

Small branches and twigs on trees such as pines and arbutus in dry sites have yet another type of mycota because the wood tends to become dry more frequently and decays less rapidly than those in wetter habitats, thus losing bark prior to falling from the trunk. These dry, decorticated twigs and branches support fungi which are embedded in the wood or are closely appressed to the surface and have covered fruitbodies, eg. *Stictis*, Phacidiaceae. Examination with a handlens is recommended while sampling such substrates.

3.2.6 Resin

In coniferous forests old deposits of hardened resin on standing trunks harbour a unique mycota of inoperculate cup fungi, eg. *Retinocyclus*.

3.2.7 Old polypores on wood

Decaying fruitbodies of woody fungi, such as *Ganoderma* and *Fomitopsis* are themselves often colonized by fungi on their lower surfaces, eg. *Hypocrea* and *Hypomyces*. Deteriorating conks can be examined in situ or chopped from trees and examined.

3.2.8 Loose bark

Sheets of loose bark on trunks and logs in the early stages of decay create new niches consisting of humus composed of accumulated insect frass and decayed cambium tissues, and sheltered, humid surfaces. These sheets can often be pried off and both surfaces examined. As this is destructive sampling, it should be performed judiciously and randomly and ceased if not productive. Examples found in such habitats are: *Crepidotus, Claudopus, Mycena, Resupinatus,* and the rhizomorphs of *Armillaria* species.

3.2.9 Hollow trees

Older and larger trees, both conifers and hardwoods can become hollowed by heart rot and/or rot plus fire. Either standing living trees, or recently fallen hollow trees may host fungi like *Ossicaulis* which fruit almost exclusively inside trunks. When possible, and when judged to

be safe from tree collapse or resident larger animals, examine interiors from openings for visible fruitbodies.

3.2.10 Canopies

Recent research in the Carmanah Valley has revealed that forest floor fungi, *Mycena* and *Cortinarius*, can be found on accumulated moss and needle covered debris on major branches in the canopy. These representatives of both saprophytic and mycorrhizal genera suggest that a distinct community may exist well above the forest floor which should be studied where canopy research is conducted and is possible. The presence of mycorrhizal species further suggests that seedlings are taking root on the living branches.

3.2.11 Coarse woody debris

Once a tree has fallen or died standing, or when large limbs or roots are torn from trees the capacity of the wood to ward off attack fails and many species of macrofungi will immediately attack the wood. These primary or pioneer decay fungi may consist of the fungi which also attack living trees and are in place when the tree dies. Slightly more advanced decay is often caused by secondary colonizers (Boddy 1992). Fruitbodies may be conspicuous when in season, on the sides and tops of logs, stumps, trunks, and fallen branches. However, many species fruit on the under surfaces. Loose wood should be flipped, rolled, or otherwise examined on the lower, usually moister surfaces. Most appressed poroid decay fungi and other aphyllophorales are confined to these lower surfaces, with some preferring contact with soil. Common genera are *Pholiota, Kuehneromyces, Hypholoma, Ganoderma, Inonotus, Phellinus, Hyphodontia, Hyphoderma, Mollisia, Xeromphalina, Phlebia*. Following sampling, roll or flip logs back to their original positions.

3.2.12 Light woody debris (cones, twigs, sloughed bark, acorn caps)

Fallen conifer cones or piles of cone scales created by squirrels represent a specialized substrate. Most species of *Strobilurus* and *Baeospora myosura* as well as *Auriscalpium vulgare* and some cup fungi (*Ciboria rufo-fusca*) are found exclusively on partially buried or fully buried cones or cone scales. Other genera such as *Mycena* and *Hemimycena* colonize cones along with other debris. Twigs and small branches on, in and close to ground level are often attacked by *Mycena, Marasmius, Marasmiellus, Crepidotus, Xeromphalina, Psilocybe, Trametes, Nidula*.

3.2.13 Advanced wood decay

Coarse woody debris includes stumps and logs. Decay of tree trunks in temperate forests can take as long as it took the tree to grow, i.e. hundreds of years (Maser et al. 1988). After or during initial attack by primary decay fungi, secondary decay organisms invade and may replace the primary decay organisms, to be followed by tertiary colonizers. Wood in the intermediate stages before becoming humus, especially wood attacked by brown rot fungi, is often colonized by mycorrhizal plants, such as conifer saplings, which extend roots deep into the stump or log. Fungi fruiting on such wood may be either saprophytic (*Xeromphalina campanella*) or mycorrhizal (*Boletus mirabilis*), or somewhat intermediate, i.e. *Paxillus atrotomentosus*. Other genera which frequent advanced decay are: *Coprinus, Pleurocybella, Psathyrella, Pluteus, Lycoperdon, Scleroderma*, and *Galerina*.

3.2.14 Decorticated exposed wood

Fallen or standing wood with direct exposure to continual sunlight often becomes decorticated, sunbleached and cracked. Decay is inhibited by the frequent drying and raised temperatures. In such circumstances specialized genera attack wood. Examples of characteristic genera are: *Gloeophyllum, Lenzites, Schizophyllum,* and *Neolentinus*. The fruitbodies are usually coriaceous and can survive desiccation or drying conditions. Typical sites are xeric habitats (margins of range lands, pine barrens, deserts, subalpine forests), swamps (where raised water tables have led to death of many trees and subsequent loss of the entire canopy) and beached logs and branches. Additionally these species can be found on creosote treated exposed woods such as railroad ties, telephone poles and piers.

3.2.15 Intact fallen needles in needle beds

Fallen needles represent a distinct substrate which support different mycofloras depending upon the confer species. Genera such as *Marasmius, Marasmiellus, Micromphale,* and in coastal fog belts *Crinipellis*, all colonize small patches or individual needles and can be found both during wet weather or during dry weather as dehydrated but revivable fruitbodies. Other genera such as *Mycena, Hemimycena* and *Heyderia* with small fleshy sporophores occur only during wet weather. These species with small fruitbodies may occur by the hundreds (troops) on bare needle beds, or occur more selectively in sheltered hollows. Needle inhabiting species should be collected with tweezers to preserve the connection to individual needles, or enmasse along with the needle bed surface for later sorting. Fruitbodies should not be indiscriminately pulled by breaking off their bases.

3.2.16 Intact leaf litter

Freshly fallen leaves still recognizable to genus or species often posses physical or chemical barriers to colonization and therefore have selective fungal floras. Poplar leaves should be examined for *Ciborina, Hymenoscyphus, Marasmius epiphyllus, Typhula, Flammulaster, Mycena*. Coriaceous leaves of oaks, salal (*Gaultheria shallon*), and oregon grape (*Berberis nervosa*) support distinct mycotas. Petioles and veins of softer leaves such as maples and alders also serve as distinct substrates for similar genera.

3.2.17 Catkins and mummified fruits

In the spring, catkins and mummified fruits of the Ericaceae and Rosaceae, and seeds should be examined closely on the forest floor in damp locations for inconspicuous brown cup fungi (Sclerotiniaceae, eg. *Ciboria*) which parasitise these structures prior to senescence. Fruiting of these Sclerotiniaceae is timed to coincide with the flowering of their hosts at which time they infect the flowers. Hence at precisely the time alders, willows and poplars are shedding pollen the ground should be examined for apothecia arising from old catkins. Similarly when blue berries, cranberries, and other Ericaceae are flowering mummified berries on the ground bear apothecia of their Sclerotiniaceae parasites. These parasites will be mixed with saprophytes which may need to be separated by later microscopic examination.

3.2.18 Roots

In addition to wood decay fungi there are parasites of smaller roots which fruit in subterranean cavities and tunnels. *Roesleria subterranea* parasitizes grape and apple roots and *Roeslerina* spp. occur on rootlets possibly of conifers. Both genera form powdery

topped, slender fruitbodies. These fungi may only be encountered haphazardly by exposing roots through excavation or by lifting logs and other debris on the ground.

3.2.19 Bryophytes

Mosses, leafy liverworts and liverworts are colonized by several groups of macrofungi with the agaric genera *Galerina, Omphalina, Rickenella, Hypholoma*, and *Psilocybe* and the brightly coloured apothecial or clavate ascomycetes, *Leucoscypha, Scutellina, Melastiza, Octospora*, and *Bryoglossum*, being the most conspicuous. Whether in forests, open alpine areas, fields, or in bogs, each abundant genus of bryophytes is likely to host different species of mushrooms. Bryophilous mushrooms are found on mossy tree trunks, logs, moss carpets, beds, and hummocks, seepags, snow beds, and quaking bogs. *Cyphellostereum laeve* is frequently found on polytrichoid mosses in coastal regions. *Arrhenia* and *Rimbachia* species can be found in both alpine and lower elevation bogs and seepages. Seeps should be targeted during the drier summer and early fall months. For all suspected bryophilous fungi adequate samples of the surrounding hosts should be collected and connections to individual thalli preserved. Liverwort beds constitute a specialized habitat and must be examined closely for small host restricted species. Many of the bryophilous ascomycetes are small or camouflaged by being dull or darkly coloured, sessile, and nestled among the gametophytes or on adjacent wet soil. Jet black members of the Geoglossaceae (earth tongues) can also be easily overlooked, particularly on overcast days or under heavy canopy.

3.2.20 Bogs

Both living *Sphagnum* and peat moss have their own specialized bryophilous mycofloras. Species such as *Galerina paludosa* and *G. tibiicystis* will only be found in such sites. Others such as *Omphalina sphagnicola* are usually restricted to *Sphagnum* bogs but may be found in other mossy boggy sites. In addition to bryophilous species, there are mycorrhizal and saprophytic species adapted to the specialized environment in *Sphagnum* bogs. Bogs should be sampled both in early summer at about the time the ericaceae are in bloom and in late season before excessive rains have saturated the environment or heavy frosts have stopped fruiting. Even in areas of standing water mossy overhangs often harbour species of bryophilous, mycorrhizal and saprophytic species. The collector should exercise care against breaking through the bog surface when in quaking bogs or sampling floating mats overhanging pools.

3.2.21 Basidiolichens

Currently only a few species are known from British Columbia (*Omphalina, Multiclavula*) and are found in habitats along with other bryophilous or lignicolous species. If a species is suspected of being lichenized or bryophilous the hosts or thalli should be collected from around the bases of the basidiocarps with the connections left intact if possible.

3.2.22 Soil and mulch

The majority and often largest specimens of macrofungi are found on the forest floor or open fields, on or emerging from soil and well decayed litter. Without knowing more about the biology of each genus, inexperienced collectors will not be able to distinguish saprophytes from ectomycorrhizal species which together form the bulk of species from such habitats. However, collecting techniques are similar for both. Examples of common mycorrhizal genera are: *Amanita, Hygrophorus, Tricholoma, Inocybe, Cortinarius, Dermocybe,*

Hebeloma, Russula, Lactarius, Albatrellus, Ramaria, Boletus, Leccinum, Tylopilus, Suillus, Hydnum, Cantharellus, Gomphus, Scleroderma. Examples of common saprophytic genera are: *Clitocybe, Collybia, Marasmius, Pholiota, Psilocybe, Stropharia, Hypholoma, Mutinus, Agaricus, Lepiota, Psathyrella, Coprinus, Panaeolus, Panaeolina, Lacrymaria*. Several genera are of uncertain biological status. These include many of the pink-spored genera, *Leptonia, Nolanea, Claudopus*, the old growth associated genus *Phaeocollybia*, and hygrophoroid genera such as *Hygrocybe* and *Camarophyllus*. In all cases care should be taken to extract the entire fruitbody from the soil by excavating clearly to the base. Inexperienced collectors often leave volval remnants in the soil for *Amanita* species or snap off radicating bases for species arising deep in soil layers (*Phaeocollybia, Strobilurus, Hebeloma*). Care should be taken to observe surrounding vegetation, particularly suspected mycorrhizal partners for those genera of mushrooms believed to be mycorrhizal. Surveys may be directed by foreknowledge of the desired fungal species and their preferred host. Larch trees have a characteristic mycoflora which includes *Boletinus cavipes, Fuscoboletinus spectabilis, F. glandulosus, F. palustre, Hygrophorus speciosus*, and *H. laricinus*, which will not be found away from larch. Similarly many species are restricted to pine (*Suillus sibericus, S. granulosus, S. tomentosus*), Douglas fir (*Suillus caerulipes, S. lakei*), and poplars or birches (Tricholomas, Leccinums, Lactarii). Alders and willows host the genus *Naucoria* (also known as *Alnicola*) as well as Tricholomas, Cortnarii, Inocybes, and Hebelomas. Surveys to include as many species as possible from broad geographic regions need to be aimed at including all possible major ectomycorrhizal hosts in the area surveyed. This may include herbaceous or inconspicuous hosts such as dwarf willows, *Dryas, Arctostaphylos* and *Vaccinium*. Plantations or rejuvenating forests often display an abundance of species which are rare in mature forests. Young pine, Douglas-fir, or spruce groves before or just after canopy closure abound with fruitbodies of *Suillus, Inocybe, Dermocybe, Hebeloma, Laccaria, Lactarius, Cortinarius*, and *Xerocomus*.

3.2.23 Parasitized and rotting fungal fruitbodies

Macrofungal parasites of conks on trees are discussed above. Relatively few macrofungi are parasites on the fruitbodies of other fungi but because of the rarity of some they may be considered for conservation. *Hypomyces* species such as *H. lactifluorum*, the Lobster Mushroom, completely overgrow some mushrooms, transforming them into misshapen and colourful structures. Others like *Asterophora* on Russulaceae parasitize mature fruitbodies and appear to grow out of them. All species in *Collybia* sect. *Collybia* grow on decaying fungal, usually fleshy or leathery basidiomycetes. In *Collybia* the sclerotia of the fungal parasites characterize each species, being yellowish and subglobose for *C. cookei*, reddish brown and apple seed shaped in *C. tuberosa*, and absent in *C. cirrhati*. *Squamanita* has been found in adjacent Washington and has been seen in B.C. Care should be taken to gather the decaying host in all cases to determine as far as possible its identity, and to ensure that sclerotia or chlamydospores of the parasite are harvested. Agaricoid sporophore parasites are reviewed by Redhead et al. (1995).

Other conspicuous parasites are the *Cordyceps* which grow on the hypogeous truffle-like ascomycete, *Elaphomyces*. The telltale club-shaped stromata of *Cordyceps* which emerge from the soil indicate that *Elaphomyces* are also present.

3.2.24 Burns

Forest fire sites, slash burns, camp fires and even volcanic eruption will all trigger the emergence of a select group of macrofungi sometimes called fireplace fungi (Petersen 1970).

Commercial harvesters will be familiar with the phenomenon of massive fruiting of morels, particularly the black morels, *Morchella elata* and allies, which fruit prolifically in the spring on areas burned the previous year. Foresters may be familiar with the fungus *Rhizina undulata* which resembles, quite literally, a pile of dung. This ascomycete is known to attack conifer seedlings on burn sites. Less familiar are the many other species in the genera *Pholiota, Myxomphalia, Omphalina, Tephrocybe, Psathyrella, Coprinus*, and the cup fungi *Pyronema, Lamprospora, Octospora*, and *Peziza*. While some of the cup fungi and Pholiotas are brightly coloured and hence conspicuous, distracting the collector from others which are black, or dark grey to brown, and therefore are exceedingly difficult to spot amongst charred wood and soil. Burns (even single fireplaces) should be targeted and examined close to ground level by squatting down and slowly scanning small areas, particularly areas showing some regeneration of mosses. Many of the fireplace fungi are in fact bryophilous species which are associated with mosses and liverworts characterizing burns. Fireplace fungi may be induced in situ, virtually anywhere, by controlled burning, or alternatively in the laboratory by various heat shock treatments of soil samples.

3.2.25 Animal corpses

Although few in number, there are macrofungi which frequent vertebrate corpses or burial grounds. Sagara (1975) the leading researcher on this group worldwide has referred to them as either proteophilous fungi or members of the ammonia fungal group which also includes some pyrophilous species. The concentrated release of nitrogenous matter appears to be the main stimulant for growth. *Tephrocybe tylicolor* is known from B.C. and can be found around animal corpses, on human faeces, and sites with unknown, possibly urine-supplied nitrogenous sources. Hebelomas and Coprini may also be sought plus several cup fungi, eg. *Ascobolus*. Directly on horns or hoofs it should be possible to find *Onygena equina*, a stipitate ascomycete producing a powdery head of spores, while on rotting bird feathers or rotting animal fir *O. corvina* could be found. As with any study of corpses, care should be taken to avoid being infected by diseases or parasites, or being contaminated by toxins and other wastes.

Cadavers of insects or spiders under ground, moss, or under bark, serve as substrates for various *Cordyceps* species. Club-shaped stromata bearing a pimply surface revealling embedded perithecia, hence possibly species of *Cordyceps*, should be carefully excavated or extracted from their substrates. The mummified cadavers are either directly attached to the stromata or are connected by mycelial strands and must be recovered for acurate identification of the fungi.

3.2.26 Dung

Animal faeces, particularly from herbivores, are rich sources of highly specialized macrofungi. Many of the species probably require ingestion of their spores prior to germination on the expelled material. Others are carried by air currents or insect vectors. The mycoflora on dung rapidly changes as the material ages (Wicklow 1992). Fresh dung is often first covered by *Pilobolus* and various mucorales (all of which are microfungi), but after that growth dies down many species of cup fungi (eg. *Ascobolus, Saccobolus*) arise followed by basidiomycetes such as the agaric genera *Coprinus*, *Psathyrella*, *Psilocybe*, *Stropharia*, and *Anellaria*, the occasional stinkhorn (*Anthurus*) and bird's nest fungi (*Cyathus*). Some species show a preference for different dung types, i.e. horse versus cattle versus deer or moose. Even rabbit and mouse dung has its own mycoflora. If large enough samples can be obtained in the field the dung flora can

be directly sampled. However, many of the Coprini are exceedingly fragile and also deliquescent. They are best collected and then cultured from incubated dung. Because most dung inhabiting agarics survive desiccation as spores or mycelium in dung, samples of the dung may be gathered, bagged, air dried, and at a later date incubated and observed over time. Dried dung can be re-soaked by submerging it in water (distilled is best but certainly not chlorinated) and then placing it in a moist chamber. The dung should be placed on soaked material which can be paper, but soaked and squeezed *Sphagnum* peat moss works best. Adequate overhead room within the incubation chamber should be allowed for fruitbody maturation. The moist chambers should also be placed where they receive indirect sunlight since light is required for either initiation or maturation (expansion) of fruitbodies in many cases. The fruitbodies are also phototropic. It should be kept in mind than dung under different conditions may produce different mycotas. Water-soaked dung in wet depressions will produce different mycofloras from previously sunbaked excrement. Similarly, the mycota on dung in cold weather such as in early spring will differ from that in the summer.

3.2.27 Pastures and alpine meadow

Open expanses of native grasslands as well as lawns support characteristic mushroom floras. The fungi may grow in soil, on thatch or directly on culms. Nitrogen rich and nitrogen poor sites differ in their respective mycotas as do mossy versus graminoid dominated sites. Fairy rings caused by the growth of fungi, especially *Marasmius oreades*, may be more conspicuous than the fruitbodies or may be visible in the absence of fruiting. Frequently open low elevation areas are productive during summers within days after rain storms. There are also early and late season fungal floras. Pastures frequented by herbivores are usually contaminated by dung and urine. Although coprophilous fungi may be recognized when directly on dung, they grade into species groups which preferentially grow in nitrogen rich soil. Genera typical on rich sites are: *Agaricus, Psilocybe, Panaeolus, Stropharia, Bolbitius, Conocybe*. Genera typical of poorer sites are: *Marasmius, Panaeolina, Galerina, Clitocybe, Calocybe, Lycoperdon, Bovista, Entoloma, Hygrocybe, Nolanea*. In alpine meadows one finds *Calvatia, Calbovista, Entoloma, Agaricus*. A frequently overlooked niche among agaricologists but not discomycetologists is the base of hummocks. In tall grass or sedges a series of tunnels forms between tufts and under flopped over blades. These perihumid musty environments in summer and early fall support genera typical of tropical climates, eg. *Tetrapyrgos, Melanotus, Marasmius*, as well as an abundance of small inoperculate discomycetes. In heavily grazed pastures the conspicuous tufts of *Juncus* which are avoided by cattle and horses should be parted and their bases examined for small species.

3.2.28 Snow banks and snow beds

A characteristic feature of western North America is the development of snow banks from the melting snow pack in late spring or early to mid summer in higher elevations in areas which are otherwise relatively dry and exposed during the summer. A group of ecologically restricted fungi have adapted to growing just under the melting snow pack or in the wet zone adjacent to snow banks. Fruitbodies sometimes emerge from holes in the snow. Typical snow bank mushrooms occur on the east facing sides of mountains and in slightly lower elevations in the rain shadow sides. Engelmann spruce, mountain hemlock, alpine fir and larch are typical of the zone. Characteristic genera are: *Plectania, Peziza, Mycena, Neohygrophorus, Hygrophorus, Clitocybe, Heterotextus, Lentinellus, Postia, Pycnoporellus*. In wetter sites such as shaded ravines and on western facing slopes of coastal mountains where yellow cedar and alpine fir occur different species dominate. Characteristic genera are: *Pseudoplectania, Gelatinodiscus, Hygrophorus, Heterotextus*.

3.2.29 Aquatic sites

In addition to species in bogs on mosses or mycorrhizal on bog plants there exists an array of macrofungi which have evolved mechanisms for colonizing submerged or periodically submerged plant litter and wood. In moderately fast flowing streams *Vibrissea truncorum* will grow on submerged wood, while *Cudoniella* species grow on submerged leaf and woody litter in stagnant water. Both genera have apothecia on stipes which just barely raise the hymenia above water level. *Mitrula* grows in still water in bogs, forest pools, and ephemeral ponds, extending brightly coloured clavula well above the water's surface, wicking water up their porous stipes. On the sides of sedges, rushes, cattails, and grasses which may be submerged annually are many smaller mushrooms in the genera *Mycena, Resinomycena, Hemimycena, Coprinus, Marasmius. Hypholoma, Galerina, Stagnicola* and *Mythicomyces* all inhabit the bottoms of ephemeral forest pools, fruiting after they drain, while *Coprinus* species can be found on woods submerged in alkaline interior lakes.

3.2.30 Beach and shore dunes

There are several categories of dune macrofungi. A few large mushrooms such as *Psathyrella ammophila* and *Coprinus atramentarius* can arise from seemingly pure sand on the west coast, but actually are colonizing buried wood or vegetation. These fungi do not grow in salt water and therefore are above the high tide mark. Farther from the water line, beach dunes become colonized by pines, bear berry and other ectomycorrhizal plants in addition to grasses. *Leccinum, Tricholoma, Boletopsis, Cortinarius, Dermocybe, Inocybe, Hygrophorus* and *Amanita* all colonize such dunes. Also to be expected are semiburied species such as *Peziza ammophila*. Frequently moss forms extensive open carpets between trees and these expanses serve as a rich source of bryophilous fungi, eg. *Psilocybe, Omphalina, Octospora*.

3.2.31 Intertidal zones

The number of Eumycota inhabiting salt water environments, usually in the intertidal zone, is relatively few, and the "macrofungi" among them are rarer. However, careful examination of drift wood will occasionally reveal fungi with fruitbodies large enough to be seen with the naked eye, such as *Amylocarpus*, which has been found in British Columbia. For more details on collection this group of mainly microfungi on drift wood, sand grains, and marine plants see Kohlmeyer & Kohlmeyer (1979).

3.2.32 Desert fungi

This category actually covers a variety of dry land species adapted to shifting sands, silty dry soils, and dry pastures dominated by sage brush. The species in these dry lands differ from those on coastal dunes. Several genera form subterranean fruitbodies which are either pushed above the sand surface or emerge from cracks in silt following rains and then dry in place. These genera frequently have buried volva-like structures which require careful excavation. Examples are: *Tulostoma, Battarrea, Chlamydopus, Montagnea*, and *Gyrophragmium*. Others form superficial fruitbodies designed to desiccate, eg. *Disciseda, Lycoperdon, Calvatia, Agrocybe*. All have been found in B.C. or adjacent Washington and are to be expected in B.C.

3.2.33 Hypogeous fungi

These ecologically grouped species are phylogenetically quite diverse including both ascomycetes (truffles) and basidiomycetes (false truffles) as well as Glomaceae. Most form

fleshy fruitbodies which become aromatic upon maturation, attracting mycophagous animals (Castellano et al. 1989). Virtually all are mycorrhizal and have adapted to being dispersed by animals following ingestion, or in the case of *Elaphomyces* by being discarded. They constitute a significant part of the diet of squirrels, flying squirrels and voles, their vectors. The collection of hypogeous fungi is somewhat haphazard and destructive being based upon raking of the surface layers in forests using truffle rakes (three pronged garden variety). They may be found immediately below moss carpets, just within brown rotted logs, and in deeper loam or duff under needle beds, virtually always above the "mineral-organic soil interface" (Luoma 1991), hence raking down to 5 - 10 centimetres is recommended. Hypogeous species tend to be more abundant in inner mountain ranges, east sides of coastal mountains, and other well drained sites. In general they will not be found in waterlogged soils (an exception being *Alpova* under alders), and are less frequent on the wetter outer mountain ranges (an exception being *Elaphomyces*). Areas showing evidence of frequent recent shallow digs by rodents under ectomycorrhizal trees often prove to be productive sites. All small tuber or potato like fleshy structures should be gathered and sorted for later processing. Hypogeous fungi also form near the surface and can form earthen mounds which may crack the surface.

There are several other categories of hypogeous fungi. Both agarics (mycorrhizal and saprophytic) and Pezizales contain species which typically form hypogeously but which break the surface upon maturation, sometimes just barely. Examples are *Sarcosphaera crassa*, which ultimately ruptures the ground and emerges on recurved rays, and *Agaricus* and *Boletus* species in xeric environments which crack hardpacked soils, or semihypogeous *Cortinarius* or *Hygrophorus* species in xeric subalpine forests. All of these species are difficult to detect and are either found randomly, or by concentrated searches for telltale mounds. Familiarity with species likely to occur in an area, and the seasons for fruiting greatly increase the likelihood of detection.

A third category is the group which fruits in preformed cavities, tunnels created by animals, pockets on decaying litter, and under rocks or logs. Both saprophytic and mycorrhizal aphyllophorales such as *Byssocorticium* and *Piloderma* may fruit on the upper surfaces of mole and squirrel burrows, root parasites like *Roeslerina* poke out into tunnels, and *Peziza* species may fruit on the floors of dens and tunnels. Sampling of such fungi, unless excavations are systematically conducted, is haphazard. Opportunities to examine tunnels through other activities should be exploited. Raking will destroy these fragile fruitbodies, but often times will not destroy all fructifications, and therefore can be used to locate intact specimens for gentler excavation.

3.2.34 Garbage, discarded papers, fabrics, and compost piles

Saprophytic fungi thrive on uncolonized human-made relatively porous materials which are suddenly made available by being dumped in a natural setting. Soaked and decaying stuffed furniture, carpets, clothes, and shoes are rich sources of *Peziza* species, some *Coprinus* species, and along the coast a species of *Melanotus*. Garden compost, with or without manure, often supports thermophilic fungi and ones requiring high nitrogen levels. The genera *Agaricus, Lepiota, Lepista, Stropharia, Hypholoma,* and *Volvariella* may be found on such substrates. Exotic fungi can be found introduced with plant matter cultivated from foreign plants. Small wood chip and sawdust piles or spills create specialized habitats where luxuriant growth of lignicolous species in genera such as *Pholiota, Gymnopilus, Peziza*, and *Hypholoma*, can sometimes lead to the production of enormous fruitbodies.

3.3 Sampling by Seasons

3.3.1 Vernal species

Along the outer coasts in southern British Columbia, Western Vancouver Island ecoregion, Georgia Depression ecoprovince, it is often difficult to distinguish late winter fungi from early spring fungi as fungi may be present throughout the year. However, starting on the coast in February or March a flush of species may occur in virtually any habitat (lawns, forests, brushy areas) which are typically vernal. By March, April and May the remaining low elevation areas of the province except for the far north, will support the spring fungal flora. At this time morels, false morels, Sclerotiniaceae (see above), and selected agarics will fruit. Morels may be sought on burns (see above), among diseased (often insect damaged) trees, or in undisturbed forests. In unburned sites they tend to be most abundant at the altitude where the seasonal band of new growth of forest grasses has reached sufficient length to begin bowing over but is still "new growth green". Higher elevation zones where new grass and vegetation growth is just beginning is usually unproductive, and lower zones with mature blades and where the first flush of spring flowers are withering are too far advanced. This pattern repeats itself up mountain slopes as the season progresses. Mycorrhizal hypogeous fungi are said to be more frequent during the spring and summer months than in the fall months in the Pacific Northwest, U.S.A. (Luoma et al. 1991) and the same should apply to British Columbia. Snow bank fungi are discussed above.

3.3.2 Summer species

One should seek species with ephemeral fruitbodies, or those which favour either warm temperatures or require dehydration for maturation, in lowlands subject to summer rains or which are irrigated. Lawn, garden, and compost mushrooms, stinkhorns dependent on insect vectors, and species of puffballs requiring complete desiccation for spore dispersal all are found more frequently during summer months. At higher elevations snow banks provide moist habitats (see above). In deserts and arid regions stalked puffballs which emerge from sand and silt (see above) from spring rain and snow melt will be more conspicuous than at any other time of year. In the cordillera, seeps produce species which cannot fruit during times of excessive water flow, while drying bogs, swamps and ponds all yield species on mud, plant matter and other debris uncovered by retreating water.

3.3.3 Early fall species

The fall season is the primary field season in mid to lower elevations for fungi. There is a greater profusion of fruitbodies than at any other time. Many mycorrhizal species respond to the surge of nutrients to roots as trees and other host prepare for winter. Litter decaying species proliferate on the steady rain of autumn leaves and dying plant parts. Typically a new flush of different mushrooms occurs after the first frosts during days above freezing. *Russula* and *Lactarius* tend to disappear replaced by *Hygrophorus* and *Tricholoma* which often reach their peak in warm days following heavy frosts.

3.3.4 Late fall and winter species

While most of the province will be too cold for sporulation of fungi during the winter months, fungal fruiting may continue throughout the year along the coasts. Bandoni (1977)

listed *Panellus, Tremella, Flammulina, Guepiniopsis (= Heterotextus), Russula, Cortinarius, Crepidotus, Pholiota, Laccaria, Clavulina, Naematoloma (= Hypholoma), Dacrymyces, Clitocybe, Cystoderma, and Coprinus* as late fruiting genera. Some species (*Panellus serotinus* and *Flammulina velutipes*) can withstand freezing and thawing, while others appear to be capable of fruiting between frosts.

3.4 Field equipment

- **Containers:** Fleshy fungi are fragile and therefore must be individually wrapped or packaged for transportation. For bulk collecting a **reinforced backpack or carrying basket** is recommended. Traditionally individual specimens have been wrapped in waxed paper bags or sheets, but waxed paper tends to collapse in pouring rain and smaller specimens either become crushed by larger or heavier specimens, or water logged. **Aluminum foil** is now preferred in temperate climates. It forms a ridged protectant, retains moisture, protects specimens from heavy rain, and does not stick to viscid pilei. Wood decaying fungi which are not fleshy have been traditionally collected in **paper bags**. The specimens can usually withstand more mishandling because of their woody, coriaceous or corky consistency or simple structure. They may also be tucked into covered backpacks more readily that fleshy fragile mushrooms. For small fleshy fungi (mushrooms, cup fungi, coral fungi) small plastic **tackle boxes** with many compartments, or **pill vials or film canisters** may be used. **Plant presses** are used for parasites on leaves and other vegetation. Ideally a variety of containers and packing should be carried.
- **Field tools:** Handlens, sharp sturdy knife, small trowel, pruning clippers, tweezers, pencil and pad and/or minicasette recorder, and all or at least one of the following: small axe, folding saw, wood chisel and mallet (an axe or branch may suffice); and optionally a portable field microscope (binocular sized).
- **Survival gear** should also be considered for any collecting trip in rugged terrain. Consider taking some or all of the following depending upon conditions: insect repellent, layers of clothing, gloves, boots, emergency food and water, waterproof matches, compass, whistle, jangling noise maker and bear repellent, space blanket, first aid kit, and, under some conditions, fire arms.
- For **photography** consider the following: tripod, flash, shutter release, reflectors, umbrella (to protect the camera), water spray bottle (to freshen or clean specimens in situ), scales (mm and cm).

3.5 Field Procedures

3.5.1 Terrestrial epigeous fleshy fungi

In general for moderately sized fruitbodies, attempt to remove the entire, intact fruitbody from the base upwards. Keep all fertile surfaces as clean from dirt as possible. In soil and litter most can be picked by hand by simply digging slightly below the fruitbody. Avoid pulling with only the stem without first digging the base loose or determining if it is attached to something. When in doubt, use a trowel or knife to investigate the depth of origin. Extraction can be complicated by a radicating base which may have started well below the surface. It may be attached to dead or living roots or rhizomes, a sclerotium, a specific substrate such as a cone, another fungus (eg. *Cordyceps* on *Elaphomyces*), an insect cadaver (*Cordyceps*), bark, buried twigs, mummified fruits (Sclerotiniaceae), a rhizomorph (*Armillaria*), or the base may have arisen from a volva or similar structure (eg. *Amanita, Volvariella, Chlamydopus, Battarrea, Phallus, Mutinus*) which is easily torn from the stem. Additionally some mushrooms have exceedingly slimy stems which make them difficult to pull.

In the genera *Hygrophorus, Hygrocybe, Cortinarius,* and *Mycena*, differentiation of species is frequently based on the presence or absence of viscosity on the stipe. Extremely watery mucilage cannot be readily detected microscopically and therefore viscosity should be investigated in the field using dry hands. If there is any doubt about the viscosity of the stem, care should be taken not to inadvertently transfer mucilage to the stipe from the pileus (or other mushrooms) when harvesting or transporting specimens.

Test suspect species of *Mycena, Hydropus, Lactarius, Peziza*, and other lactescent species for latex production while still in the field if adequate material exists. Note whether a conspicuous and extensive mycelium is present around the bases of the fruitbodies. Attempt to gather different stages of growth such as buttons along with maturing and mature specimens. The colour of the immature lamellae is critical in the genera *Dermocybe, Cortinarius* and *Phaeocollybia*. Note odours in the field (and again when processing). Gentle excavation, or removal of some moss may reveal buttons in the area surrounding larger fleshy species in soil or coming through moss carpets.

Wrapped or packed fragile ephemeral specimens (i.e. deliquescent Coprini or stinkhorns), or specimens in need of immediate spore printing, should be conspicuously flagged and be the first to be processed. If possibe, avoid placing fruitbodies of genera known to expand rapidly on their sides, for within hours genera like *Amanita, Pluteus, Coprinus*, and *Mycena* will tilt their pilei in response to geotropic stimulus. *Amanita* and *Pluteus* are notorious for quickly reorienting their pilei; not only will these specimens make poor photographic subjects, but the reorientation of their lamellae, like shut venetian blinds, will block spore print formation.

Zip lock bags, double wrappings, or air tight containers can be used for collecting dried or nearly dried specimens of most puffballs, stalked puffballs, false puffballs, earth stars and their allies. Each produces millions of dry hydrophobic spores which rapidly cover and contaminate every other collection.

3.5.2 Lignicolous fungi

To remove fruitbodies from their substrates, cut, chop, saw, or chip the wood preserving the connection of the fungi to the wood or bark. If possible, also salvage decayed wood from

deeper areas below the fruitbodies as an indication of the decay type. Two broad categories of wood decay are the white rots and the brown rots. These and other rot types have ecological, biological and taxonomic significance.

Fungi on small branches and on twigs may be collected by clipping and trimming the branches in segments short enough to fit into herbarium boxes. This is best done while the materials are fresh (in the field or before drying). After drying specimens may be fragile or may break off while trimming branches.

Care should be taken to collect intact growing or delimiting margins and edges of the aphyllophorales which are appressed to wood and bark, and to include any rhizomorphs and mycelium associated with them. The features of the margins are sometimes used taxonomically.

For extra large polypores, particularly perennial fruitbodies, and especially for either common species, or rare and endangered species, it should be possible to take a small sample from the fruitbody rather than the entire structure. It is not necessary to remove and carry huge *Ganoderma applanatum* fruitbodies when a small pie-shaped segment will suffice. Learning the common species is helpful. Similarly, if a rare species such as *Oxyporus nobillisimus* were to be found in B.C., a small sample of the fruitbody (5 - 10 cm wide) along with notes on size and attachment would be sufficient to record and confirm the identity, but not to obliterate it from the site.

Lignicolous fungi are often hosts to fungal parasites, eg. *Tremellales*, *Hypocreales*. While surveying lignicolous fungi examine the fertile surfaces with a handlense for blemishes, flaws, and gelatinous drops, or examine rotting portions closely. Flag suspected specimens for examination at a later date. Parasites often dry down to inconspicuous smears or dots which are not easily relocated.

3.5.3 Foliar pathogens

Most foliar pathogens are microfungi but they may become conspicuous. Foliar pathogens are traditionally collected and preserved using plant presses, just as their hosts are collected. Plant presses consist of a stack of corrugated cardboard between which are sandwiched sheets of absorbent paper such as newsprint. Specimens are flattened between the layers of absorbent paper. The entire bundle of cardboards, paper, and specimens is compressed and bound together by a frame and straps (see drying below). Do not press fleshy fungi such as mushrooms or polypores, even ones on plant materials. Compression of macrofungal tissues decreases their value as taxonomic features.

3.5.4 Small litter and moss colonizing species

Small agarics (*Mycena, Marasmius, Hemimycena, Psilocybe, Hypholoma, Tetrapyrgos, Coprinus*), inoperculate cup fungi (*Hyaloscyphus, Beledonium*), cyphelloid fungi (*Lachnella, Cellypha, Calyptella*), and clavaroid fungi (*Typhula, Clavulinopsis*), along with microfungi frequently colonize senescent, slightly lignified, herbaceous tissues. These substrates include grass culms, stems of larger annuals or biennials, (eg. thistles, burdocks, False Hellebore, cattails), leaf petioles, cones, fruits, fallen twigs, shrub and vine litter (eg. sage brush, clematis). The collection of these fungi is relatively simple once they have been located. The substrates should be clipped to convenient size for packing and storage. Durable to pliable specimens may be carried in paper bags, paper folders, or other small containers. Fragile specimens should be tucked into vials, tackle boxes, or canisters. Leaves or moss may be

used as additional packing to prevent jostling and abrading of specimens or to maintain 100% humidity. Delicate specimens collected from amongst wet mosses or at the bases of decaying vegetation live in saturated atmospheres and begin to shrivel immediately upon removal, sometimes collapsing in 30 seconds). Specimens may also be collected and packed in moss or paper towels dipped in water and squeezed till not dripping. Packing materials can be selected from the site to remind the collector of the environment for each specimen.

Bryophilous species should be handled similarly, always ensuring that adequate quantities of the substrates are included to allow for identification and documentation of the hosts.

3.6 Processing Spores

3.6.1 Note on spores as taxonomic features:

Fungi, whether microfungi or macrofungi, are basically microorganisms at the reproductive and assimilative level. Although mushroom fruitbodies are large organs, many critical features distinguishing families and genera are microscopic. Basidiospore morphology has proven to be one of the most reliable features for identification and classification. Over 150 years ago it was learned that living agarics and other hymenomycetes will discharge a constant rain of spores yielding a powder, which mirrors the shape of the hymenophore when it is created on a flat surface. These deposits are called spore prints. Mushrooms previously not distinguishable were shown to have spore prints of different colours, and hence were identified as distinct. Modern classification of agarics is based on these pigment differences, and in many cases spore print coloration has proven to be correlated with spore wall ultrastructure and relatively stable pigments. These differences support the recognition of well delimited genera or families, eg. *Entoloma, Clitopilus, Agaricus*. Using standard dichotomous taxonomic keys to families, genera, and species, it is virtually impossible to identify an unknown agaric without first obtaining a spore print. Asking a novice or introductory level student to key mushrooms in the absence of a spore print will lead to frustration. However, most agaricologists bypass this process by the simple facts that other features, both microscopic and macroscopic, characterize genera, and that unless colours are subtle, they can be seen microscopically. Fortunately, in the absence of spore prints there are some common mushrooms which can be identified by their overall appearance. The Shaggy Mane, *Coprinus comatus*, can be recognized from a car moving along a highway because of its combined distinctive shape, colour, size, and habitat.

Depending upon the relative importance placed by monographers on spore print colour in a genus, the absence of a print may prevent the identification of a specimen. For example, precise spore print colour is rarely used to distinguish species in *Inocybe* or *Cortinarius*, whereas spore print color can be critical in *Clitocybe*. Weighting of the importance of spore print colour reaches its zenith in the genus *Russula* (Russulaceae), one of the largest, showiest, and most common forest mushroom genera. With few exceptions, if a spore print is not obtained, a collection cannot be keyed out or identified. Spores coloration in *Russula* ranges from white to yellow or orangish brown, with every possible shade in between. The fact that such importance has been placed on spore coloration (a feature which is not absolutely stable and which in this case varies with quantitative) has helped to creat a taxonomic nightmare in this genus. It is notable that given either the presence of any other easily used feature, or the inability to obtain a spore print in closely related taxa, spore mass colour is "taxonomically" less significant. For example, in the genus *Lactarius* (Russulaceae), which produces differently coloured latexes, spore print colour is less often used to distinguish species. Spore colour is not required to identify species in the gasteroid genera, such as *Macowanites* (Russulaceae), which have lost the ability to discharge spores.

3.6.2 Making Spore Prints (an exercise in common sense)

To obtain spore prints from most mushrooms (agarics and boletes) it is recommended that the cap (pileus) be cut off and lay it gill-side (lamellae-side) or tube-side down on a piece of white paper and cover or store away from air currents. Under the right conditions most

usable spore prints should be produced anywhere from 1 hour to 24 hours later. For medium to large sized specimens this process will suffice in many circumstances. Another technique which is sometimes useful is to place under the cap a perforated or slit sheet of paper through which the uncut stem in inserted. This latter technique works well for large genera such as *Russula* and *Amanita*, etc. and can be started in the field in the collecting basket itself.

Copiously sporulating specimens may produce spore deposits when not desired, in collecting baskets on one another if mixed, and on table tops or books where they are placed. However, it is often difficult to obtain useable prints. The following paragraphs address these problems.

3.6.3 Spore print trouble shooting

Maturity

When possible select fully opened (but not overly expanded or senescent caps) for printing. Young sporophores may not have started sporulation while older sporophores may either have ceased sporulation, or give only weak prints. Where possible use more than one specimen to increase the likelihood of success. Basidiomes are more likely to sporulate in the hours immediately after harvesting than after storage, therefore it is best to try more than one the first day. Occasionally completely sterile specimens will be collected (members of the Strophariaceae seem particularly prone to this condition) which will look normal but never form spores.

Loss of humidity

Small or delicate mushrooms will either dry completely or lose cell turgor pressure in minutes. Spore release by basidiomycetes requires living cells. For small fungi place the caps on small pieces of paper and enclose in small containers. Consider placing soaked paper, moss, or another source of moisture on, over or next to the cap to prevent rapid dehydration. For minute specimens and exceedingly delicate structures, the caps can be stuck by their sterile surfaces to agar (or gelatin) in Petri plates using simple capillary attraction (use razor blades and tweezers for handling) and suspended over a small slip of paper (or microscope slide coverslip). Surprisingly heavy (thick) prints can be obtained from small pilei this way.

Excessive humidity

Usually this is not a problem and temperature is the main problem in suspect cases. Excessive humidity can lead to condensation of water and exudates on the specimen and on the paper. As the spore printing process is dynamic, a slow loss of humidity is beneficial and may lead to heavier and heavier spore production. In saturated environments, genera like *Russula* do not print well. Consider the natural environment. If a species grows in a perihumid habitat it will sporulate better in a saturated atmosphere, if it grows in arid regions, it will likely do better in humid but not saturated air. Excessive moisture will also lead to rapid contaminating (parasitic) mold growth, especially on boletes. Do not completely enclose printing caps if excessive moisture is anticipated.

Temperature

Consider the natural environment before storing caps for printing. Except in the summer, most fungi are sporulating at outdoor temperatures well below room temperature, sometimes near freezing. Alternatively, specimens collected during the summer may not do well in air

conditioned rooms where the temperature is too cold. For these reasons, many fungi do not sporulate well at room temperature. The ideal situation is to store them outside in the shade where they are undisturbed. On field trips they can be stored in their containers on trays in a parked car provided the daytime interior heating of the car has dissipated and the temperature does not drop significantly below freezing at night. As most species do not do well near zero degrees centigrade, storing them in a fridge while obtaining prints is not recommended. Other solutions are to place the containers on window sills (with or without cracking the window open to moderate temperatures), or under shelter outdoors (protected from foraging animals - mice, raccoons, insects), or in an unheated room. Avoid placing the containers in direct sunlight which may overheating, condensation, or drying.

Orientation

Basidiomycetes actively discharge their basidiospores from living basidia by a mechanism which is not fully understood. However, the net effect is that the spores fly out possibly less than 20 to 100 μm in many cases, aftr which they free fall until caught in air currents. Hence, in nature one will always find the lamellae of agarics, the tubes of boletes and polypores, and the spines of tooth fungi, completely perpendicular to the earth's surface (i.e. exhibiting geotropism). This permits the spores to fall straight down after their forward motion is slowed. For specimens with widely spaced gills or large pores, a slight tilt to the fertile surface will not significantly affect the build up of a spore deposit on paper. However, for specimens with exceedingly narrow pores (polypores and boletes) or crowded gills, a slight tilt will cause all the spores on one side to fall on the other, and for spores to fall back on the surface facing upwards. Few or no spores will be deposited on the paper. Ensure that specimens with crowded gills or tubes have the spaces between the hymenophore perpendicular to the earth. Normally this can be accommodated by placing a single cap upside down so it rests symmetrically on its gills or tubes. In cases where only half a cap is used, it must often be propped up to its normal orientation in nature (use another half cap, the edge of the container, or a block of agar). For large fleshy species it may not be desirable to print the entire pileus.

Boletes, especially if only a single specimen is found, can decay or be totally eaten by maggots in one night, and therefore it is best to dry a portion immediately. Pie shaped segments may be used for printing but these are likely to topple away from the perpendicular position. Trim inrolled margins away or with a razor blade cut a clean flat surface across the gills or tubes (perpendicular to the spaces) and lay the piece down on paper. Segments may also be suspended slightly (1-2 mm) above the surface of paper (a few millimetres) using embedded tooth picks or pins.

Problems arise in the genera with free lamellae, eg. *Amanita* and *Pluteus*, because the lamellae may have closed like venetian blinds during transport or storage due to tilting and a rapid geotropic response. These genera should be printed as soon as possible before the lamellae reorient themselves.

False and misleading spore prints

More than one novice mycologist has vehemently claimed to have a mushroom with a spore colour completely at odds with the true colour. More often than not this has resulted from a combination of two events: 1) a failure to obtain a true deposit for reasons outlined above, and 2) pigments, decay by-products, or condensed water on the edges or the lamellae or tubes have stained or watermarked the paper. Watermark false spore prints are usually interpreted

as white spores, when in fact it could be almost any spore colour. Stains are usually brownish or grey, and the false print is interpreted as being dark. Endless and futile efforts to key these fungi will result. Even worse, they could be "identified" to the wrong genus and species. A rule of thumb, in the absence of a microscope, is when in doubt scrape the suspected print with the back of a thumb nail (or gently with a knife) to see if a small accumulation of spores results. If this cannot be done, there probably are so few spores present that the true colour will not be revealed.

Spore prints should be made on pure white paper without sizing or glossy surfaces to obtain a true colour. Spore prints may be white, but come in various shades of creamy, greyish or bluish "white" and these can only be seen on absolutely white paper. Deposits on black paper, as has sometimes been recommended will certainly leave no doubt about the presence of a print if the spores are pale, but it will also obscure subtle differences. Additionally, colours of darkly pigmented spores are best observed on white.

A common macroscopic and microscopic reaction used in fungal taxonomy is the "amyloid" reaction to iodine, usually in Melzer's reagent. It may be tested on spore prints but not reliable on white paper because paper itself will sometimes give positive blackish reactions. In such cases use glass slides for printing.

3.6.4 Spore Prints from other fungi

Polypores and other aphyllophorales

Generally it is less critical to obtain spore prints for the identification of these two broad groups of fungi. Traditionally the taxonomy has not been built up around this feature because there is less variation, most are identifiable in its absence, and spore prints are less often attainable. Nonetheless, spore prints can be obtained using the same techniques as used for agarics and boletes, and prove useful. For example, some *Inonotus* species have vivid yellow spore prints. In the Coniophoraceae spore prints range from yellow to yellow brown. *Ramaria* and some other coral fungi have brown prints.

Ascomycetes

Again traditionally it has not been critical to obtain spore deposits from most ascomycetes. Distinguishing species and genera has never been based upon spore print colour, although spore pigmentation has been utilized at the microscopic level. In spite of the lack of tradition, it is still advisable to obtain spore prints whenever convenient because greater accuracy in measuring mature spores can be ensured. For large ascomycetes such as the large cup fungi (*Peziza, Plectania, Morchella*) laying the specimens on paper or a slide hymenium side down and then air drying them will usually suffice. Even specimens placed on a drier will usually discharge spores onto paper or a slide placed under it. Possibly the shrinkage of the asci as they dry promotes the discharge.

3.7 Recording Data on Fresh Specimens

The classification of fleshy fungi is heavily dependent upon taxonomic features which are lost or modified upon drying or preservation in liquids. For most species, the microscopic features are best preserved and most conveniently preserved by dehydration. Therefore, taxonomic features, which are required for identification or for confirmation by others, must be recorded for each specimen. One method of recording some of these features is by photography. Good quality colour slides, prints, or more recently by compact discs, can eliminate much tedious effort in recording data. However, features such as taste, odour, texture, colour changes through time, macrochemical reactions and latex changes cannot always be recorded this way. Reliance on photography sometimes fails to capture features, colour may not be exact, and not all features can be shown in one photograph. Written record is recommended in most cases. These may be minimal, varying by genus and by the experience of the taxonomist. Appendix A consists of standard sheets which may be used as guides to prompt the collector to record the most important features.

3.8 Preserving Herbarium Specimens

With the exception of foliar pathogens which are pressed and dried similar to plant collections, most macrofungi should be dried and stored in boxes or packets. Commercially available small personal food dehydrators are now recommended, but custom made driers work just as well. Basically mushroom driers consist of stacked trays or shelves with moderately finely meshed screen bottoms (gaps 1-2 mm diam), over a source of low heat, which can be a heating coil on a rheostat, incandescent light bulbs, or a space heater with a fan. In the absence of electricity catalytic space heaters can be used but the bottom screens must not be placed too close to the heating surface. Whether commercial or custom made the driers should form a chimney for conducting warm dry air up through the screens, past the specimens, and out the top. Convection currents usually suffice if the drier is vented at the base and open at the top. Forced air will dry specimens faster but specimens lose weight and may be blown about when dried if the fan speed is too great. The heat on the bottom shelf should feel hot to the palm of the hand but not hot enough to burn skin. At the top of the drier the air must still feel warm and circulating. Excessive heat cooks the specimens which destroys much of their microscopic structure. Similarly poor air circulation can have the same effect. For both reasons "ovens" are not suitable. Specimens may become blackish or otherwise discoloured, and the tissues become agglutinated and difficult to separate when re-soaked.

Specimens should be dried until brittle. All traces of moisture should be gone. Large fleshy specimens may be sliced to accelerate drying but care should be given to preserve features of the intact specimen which might be of use taxonomically. Reshuffling the stacking order of the drier helps promote even drying when it is fully loaded. Small specimens may be placed on small squares of tissue paper on the screens (provided forced air will not blow it away), or small open boxes may be used.

It is important to number all parts of a collection as they are processed. One collection may be represented by specimens on a drier, spore prints, cultures, photographs, notes, and in a collecting book. Numbering specimens is the only way of tracking all these parts.

Silica gel may be used for small specimens, or in areas lacking electrical power. The use of zip lock plastic bags with or without the addition of a pinch of slica gel helps preserve specimens taken off driers and temporarily stored while still in field conditions. Puffed up zip locked bags also provide a protective cushion for packing delicate specimens. Exceedingly small specimens may additionally be wrapped gently in soft tissue paper before bagging them.

A few microscopic fungal tissues do not survive dehydration and rehydration. Examples are the sphaerocysts in *Peziza* apothecia and in some *Coprinus* cuticles and veils. These are large, thin-walled, inflated cells which collapse and will not re-inflate. Samples of these species may be preserved in liquid such as FAA (see glossary). *Coprinus* species also under go autolysis as part of their maturation process. Fixing young specimens in vials with FAA allows for their preservation to supplement dried mature materials. Small slips of paper with pencilled numbers dropped in with the specimens can be used to label the collection in the field.

3.9 **Sampling Design**

Site selection is dependent upon the objectives of any given inventory. Objectives of terrestrial fungal inventories could include assessment of: 1) general fungal biodiversity of sensitive or protected areas, 2) distribution of target species such as commercial edible wild mushrooms or forest pathogens, 3) impact of forest practices and other land management on ecosystem function, 4) long-term changes in species composition due to succession, pollution or climate change.

There are important issues of scale to be considered in the setting of objectives, choice of inventory type, and selection of sites. To determine distribution of a species, a presence/not detected (possible) inventory would be appropriate and the area of focus would be the possible range of the species. The very broad nature of an inventory used to determine the distribution of a fungus necessitates that it not be very intensive. Determining possible range assumes some foreknowledge of the species in question. The amount of information available on any given species would depend on the amount of attention it has attracted. Mushrooms draw attention if they have an economic impact - either negative (eg. tree pathogens), or positive (eg. commercial marketted edibles). Regional information depends on the activities of local amateur or professional observers and tends to reflect human population densities.

Vogt et al. (1992) provide an overview of sampling techniques for epigeous and hypogeous fungi based upon sporophore production. Their Table I lists sampling parameters used in different studies which range from transects once-visited, to permanent plots in multiples of 4 m^2 to 1,000 m^2, and either repeatedly visited in a one to 5 year period, or simply checked once. In each case the experiments were designed to answer specific questions regarding overall species numbers, biodiversity relative to compared sites, biomass, species abundance, numbers of sporocarps, and the effects of disturbances. At this time there is no established standard methodology. Virtually no research laboratory employs experimental designs identical to those in another laboratory and the relative accuracy of each has not been tested.

In virtually all cases where species richness is to be determined or compared, the minimum area to be surveyed in each comparative "site" should be 1,000 m^2, either as contiguous units or scattered smaller units. Additionally, the period of time should not be less than 3 years and ideally would be longer. Even under these conditions, for studies being conducted in western forests in the United States, either increasing the sampling period or increasing the size of the plots or transects will lead to increased numbers of species. In old-growth sites even after 10,000 m^2 were sampled the number of species new to the survey continued to increase steadily without statistically significant levelling off of the species/area growth rate (Glenn Walker pers. comm.).

Based on several years of experimental surveys in complex ecosystems, J. Ammirati and colleagues recommend that several types of survey techniques be employed simultaneously. Mycorrhizal fungi appear to be best sampled in large plots (or many randomly or patterned smaller plots yielding a large area in total) over a minimum 3 year period. Litter inhabiting and bryophilous fungi can be sampled in a series of smaller plots or fewer plots over the same period of time. For lignicolous species on coarse woody debris (and other specialists such as dung fungi) the substrates themselves can be targeted and specifically visited, each serving as a unit. Dung should be identified by animal species while logs and stumps should be classed by species, age, and diameters.

Sampling of hypogeous fungi is the most destructive. In one successful study, Luoma et al. (1991) used 25 circular, 4-m^2 plots (=100 m^2) placed every 25 m along 3 transects running parallel to the contour slope and spaced 75 m apart. These were raked to 5 - 20 cm depth and the fruitbodies harvested. New transects were placed in each stand over the 4-year study period. The transects were sampled in spring, summer, and fall ensuring there is no re-sampling in the same patch.

Surveys of tree diseases and the fungi that cause them have been carried out in the forests of B.C. by staff of the Forest Insect and Disease Survey (Canadian Forest Service), and a manual exists describing the methods (Shore 1984). General methodology for determining incidence or percentage of the stand affected by foliage diseases, cankers and other diseases includes the examination of 25 trees selected randomly at 10 m intervals along a transect. Among the specific methodologies, symptoms for the detection of six species causing root rot are described and a method for determining the percentage of stand affected which also involves the determination of symptoms on trees encountered along a transect is presented.

Sampling by targeted species may be appropriate in some cases. For example, the pine mushroom, *Tricholoma magnivelare*, is one of British Columbia's commercially harvested forest mushrooms. It begins fruiting in August in Alaska and northern B.C., continues through November in southern parts of the province, and into January in northern California. Historically the industry was unregulated so no production or economic data has been collected. Because of this, it's value is unknown but is assumed to be high (estimates of value of exported wild mushrooms for 1990 from Oregon of $35 million and from BC of $9 million). The industry in British Columbia now relatively undeveloped compared to that in the United States, might be worth considerably more if fully developed. One study might involve monitoring the effects of intensive mushroom harvesting. Here two levels of resolution and inventory would be appropriate. At the higher (provincial) level, field work would be required to test the accuracy of anecdotal reports and to test the predictive value of the distributional model. At the lower (landscape) level, intensive field inventory could provide production, relative abundance, or even density information.

4. SUMMARY

The number of parameters which need to be considered when conducting surveys of fungi are so great that each survey must be tailor made. No simple set of guidelines suffices to ensure consistency over a broad spectrum of habitats, microhabitats, seasons, and taxonomic groups. Each question, be it quantitative, qualitative, or dynamic adds further dimensions. In this manual methodologies are given for locating and processing all types of macrofungal fruitbodies within the study area.

GLOSSARY

AGARIC - a gilled mushroom

AMMONIA FUNGI - ecologically grouped fungi characteristic of terrestrial sites rich in ammonia, sometimes associated with corpses, urine, faeces

AMYLOID - for fungal structures, a positive reaction (bluish to black) when treated with iodine such as in Melzer's reagent or Lugol's solution

ANNULUS - an attached to loose ring of cottony or membranous veil tissue left on the stem of a mushroom after expansion of the cap

APHYLLOPHORALES - an order of basidiomycetes generally characterized by a hymenium which does not form lamellae like an agaric

APOTHECIA - a cup-shaped fruitbody of an Ascomycetes

ASCUS (PL. ASCI) - reproductive cell characterizing the ascomycetes where meiosis takes place followed by the internal formation of ascospores

ASCOCARPS - an Ascomycete fruitbody

ASCOSPORES - spores formed in asci

ASCOMYCETES - see Ascomycotina

ASCOMYCOTA - fungi characterized by the formation of asci and ascospores

BASIDIOCARPS - a Basidiomycete fruitbody

BASIDIOLICHENS - a lichen where the fungal partner is a Basidiomycete

BASIDIOMYCETES - see Basidiomycotina

BASIDIOMYCOTA - fungi characterized by the formation of basidia and basidiospores

BASIDIOSPORES - spores formed on basidia usually following meiosis

BIRD'S NEST FUNGI - Nidulariales, an order of Basidiomycetes characterized by fruitbodies resembling miniature bird's nests with "eggs"

BOLETES - Boletales, an order of Basidiomycetes characterized by fleshy, putrescent, pileate fruitbodies having fertile masses tubes under their caps

BROWN ROTS - for wood decaying fungi, species which preferentially digest cellulose in wood leaving a brownish residue primarily consisting of lignin

BRYOPHILOUS - living on or with bryophytes

BRYOPHYTES - mosses, liverworts, leafy liverworts

BUTT - off a tree, the flared out base of the trunk

CANKERS - a distorted, infected woody growth on trees induced by parasites

CARTILAGINOUS - for fungi, tissue, often in a stem, having the consistency of cartilage

CHLAMYDOSPORES - a thick-walled resting asexual fungal spore formed terminally or in hyphal segments

CLAVAROID - club or coral shaped Basidiomycete fruitbodies

CONKS - woody, perennial basidiomycete fruitbodies on trees and often hoof shaped

COPROPHILOUS - living on dung

CORIACEOUS - for fungi, having a consistency of leather

CORTEX - sterile tissue forming the outer layer of a fruitbody

CORTICATED - referring to woody plant substrates having bark in place

CUP FUNGI - Ascomycetes with cup-shaped fruitbodies (apothecia)

CYPHELLACEOUS - Basidiomycetes with cup-shaped fruitbodies

CYPHELLOID - see cyphellaceous

DECORTICATED - referring to woody plant substrates which have had bark removed

DIKARYON - a nuclear state found only in basidiomycetes following fusion of compatible mating strains wherein the compatible nuclei are paired throughout the vegetative mycelium.

DIKARYOTIZE - to form a dikaryon

DISCOMYCETES - cup fungi

DISCOMYCETOLOGISTS - one who studies discomycetes

EARTH STARS - puffballs, mostly in Geastrum, in which the outer peridium splits into radiating triangles

ECTOMYCORRHIZAE -a symbiotic condition between the roots of a vascular plant and a fungus which forms an external sheath around the root tips in addition to a net work a round its cortical cells

FAA SOLUTION - Formalin (13 ml), glacial acetic acid (5 ml), 50% ethyl alcohol (200 ml)

FALSE PUFFBALLS - Scleroderma a genus in the Sclerodermataceae rather than the Lycoperdales, true puffballs

FALSE TRUFFLES - basidiomycetes such as the genus Rhizopogon which resemble truffles

FALSE MORELS - morel like ascomycetes in genera like Gyromitra and not a species in the genus Morchella

FIREPLACE FUNGI - fungi commonly and sometimes exclusively found in and around burned ground and vegetation (see pyrophilous fungi)

FLUSH - for fungi, a nearly synchronous crop of many fruitbodies followed by a period of fewer or no fruitbodies

FRUCTIFICATIONS - fruitbodies

FRUITBODIES - macroscopic fleshy or woody reproductive organs formed by fungi in or on which spore are formed by either asci or basidia, rarely by other means

FUNGISTATIC - a chemical which inhibits fungal growth

GASTEROMYCETE - a group of basidiomycetes which form spores internally, usually in membrane bound sacs, and which do not actively discharge basidiospores from the basidia

GILLS - radiating plate-like spore bearing organs on the underside of mushroom caps (lamellae)

GLEBA - fertile, sometimes powdery, sometimes gelatinous spore laden contents of gasteromycete or truffle fruitbodies

HEART ROT - with regard to wood decay, a condition where the heart wood decays ahead of the sapwood

HYMENIUM - an often membrane like layer of either basidia or asci which forms the fertile surfaces on fungal fruitbodies

HYMENOPHORE - either the entire fruitbody or the portion of the fruitbody which bears the hymenium

HYPHAE (SING. HYPHA) - filamentous cells of a fungus thallus

HYPOGEOUS FUNGI - fungi which fruit below ground

IN SITU - in place where naturally occurring

INNER VEIL - fungal tissue which does not cover the entire fruitbody but which does cover the developing hymenium (often the gills), and which tears open leaving remnants on the stem and/or the cap margin as a cortina, annulus, bands, patches, or hanging marginal scales

INOPERCULATE - with regard to ascomycetes, those with thin-walled asci lacking an operculum and which are not double walled, i.e. bitunicate

JELLY FUNGI - basidiomycetes such as Tremellales, Dacrymycetales, and Auriculariales, with gelatinous fruitbodies

LACTESCENT - exuding latex when cut

LAMELLAE - see gills

LAMELLULAE - short gills near the cap margin which do not reach all the way to the stem and which are inserted between full sized gills (lamellae)

LATEX - with regard to fungi, a milky liquid which is exuded and coagulates into rubber when the fruitbody is cut

LIGNICOLOUS - growing on or in wood

MACROFUNGI - fungi which form macroscopically visible fruitbodies usually over one centimetre in height or width

MELZER'S REAGENT - a stain containing iodine which causes amyloid or dextrinoid reactions (chloral hydrate 100 gm, potassium iodide 5 gm, iodine 1.5 gm, distilled water 100 ml)

MICROFUNGI - fungi which do not form fruitbodies or form minute fruitbodies

MONOKARYON - the haploid unpaired nuclear state of fungi which form dikaryons

MORELS - fleshy fruitbodies of the genus Morchella which resemble coarsely pitted cones or sponges on a stalk

MUMMIFIED FRUITS - with regard to fungal parasitism, fruits from a host plant which are infested by a fungus and which dry like a mummy to form a type of false sclerotium

MUSHROOM - a general loosely defined term for fleshy fungal fruitbodies, usually bearing gills

MYCELIUM - the cottony or net-like vegetative thallus of a fungus which consists of many filamentous hyphae

MYCOFLORA - fungal species in a region or habitat (mycota)

MYCOPHAGOUS - that which eats fungi

MYCORRHIZAL - that which forms mycorrhizae

PCR - polymerase chain reaction, a methodology used to produce multiple copies of selected segments of DNA molecules

PERENNIAL FRUITBODIES - fruitbodies which last more than one year and usually increase in size by the addition of layers

PERIDIUM - with regard to fungi, the outer layer of a fruitbody

PETRI PLATES - a glass or plastic sterilized shallow cylindrical dish with an overhanging matching lid used for holding sterilized media for growing microorganisms

PILEATE - having a pileus or cap

PILEUS (PL. PILEI) - a cap as in a mushroom cap

PINK- SPORED - with regard to basidiomycetes, species having a pink spore deposit

PLEUROTOID - with regard to mushrooms, species lacking or nearly lacking a` stipe, eccentrically attached, often fan to spathulate shaped, often on wood

POLYPORES - basidiomycetes in the Aphyllophorales, but not the fleshy boletes, which form fruitbodies having many pores on the underside, which are the mouths of fertile tubes

POLYTRICHOID - mosses in the Poltrichaceae, eg. Polytrichum, Atrichum

PRIMARY DECAY FUNGI - the first decay fungi to attack sound wood

PROTEOPHILOUS FUNGI - fungi which colonize protein rich substrates such as animal matter

PSEUDOCOLUMELLA - the extension of a stipe into the gleba of some fungi, especially gasteromycetes, which is still recognizable as stipe tissue

PSEUDORHIZA - the radicating base of certain mushrooms which resembles a root but is actually an ascending organ which gave rise to the fruitbody

PUFFBALLS - a group of gasteromycetes, mainly the genera Lycoperdon, Calvatia, and Bovista, which when dried have thin flexible perdidial walls, and dry powdery spores which are discharged in clouds often by a bellows effect

PYROPHILOUS FUNGI - fungi associated with burned sites (see fireplace fungi)

RADICATING - a mushroom with a pseudorhiza

REVIVABLE - with regard to fungi, fruitbodies which shrink when dried but remain viable and revive when rewetted

RHIZOMORPHS - root-like fungal organs with differentiated cortices and which grow from one food source to another, eg. the "shoe strings" of Armillaria species

SAPROPHYTIC - living on dead matter

SCLEROTIA - a multicellular resting or storage fungal organ, usually with a cortex and not incorporating host tissue

SECONDARY COLONIZERS - fungi which replace primary colonizers

SNOW BANKS - snow drifts at high elevation which melt after most of the ground cover has run off

SPORE PRINT - a powdery deposit of fungal spores which forms an image of the hymenophore, eg. gills or pores, when the living fungus is placed over another surface

SPORES - microscopic reproductive structures, either sexually or asexually produced, and consisting of one to several cells, but not hundreds of cells

SPOROCARPS - fungal fruitbodies

SPOROPHORES - fungal fruitbodies

STALKED PUFFBALLS - a group of puffballs with long stems, eg. Tulostoma, Chlamydopus

STINKHORNS - a group of gasteromycetes, the Phallales, which produce fragile rapidly expanding erect tissues partially coated in slimy, foul smelling, sweet tasting mucilage laden with spores which are dispersed by insects such as flies and beetles which attracted to the fruitbodies

STIPE - the stem on fungal fruitbodies

STIPITATE - have a stipe or stem

STROMATA - a mass, mound or growth of sterile fleshy tissue on or in which are produced many small ascocarps

SUBICULUM - a cottony to membranous sheet of tissue on or in which many small fruitbodies are formed

TRANSLUCENT STRIATE -the radially striate pattern formed by the lamellae on a mushroom cap as viewed through the translucent surface

TROOPS - with regard to fungal fruitings, occurring in masses as many distinct individuals in a compact area, literally looking like "troops" with helmets

TRUFFLES - ,hypogeous ascomycete fruitbodies resembling plant tubers, usually highly aromatic when mature and prized as food by man and animals

TUBES - with regard to fungal fruitbodies, tubular structures lined on the inner surface with basidia

UNIVERSAL VEIL - sterile fungal tissue, often membranous, cottony or granular, which envelops the young fruitbody and is torn open or apart during expansion of the fruitbody. Remnants may form scales on the cap, scales`on the stem or lower annulus surface or a volva.

VOLVA - remnants of a universal veil at the base of a stem which when most highly developed forms a membranous sac, but which may also be fragile, cottony, or granular in nature

VOLVATE - having a volva

WHITE ROT - for wood decaying fungi, species which at first digests lignin in wood fibre creating a paler, whiter patch of decayed wood prior to complete digestion of the cellulose component

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APPENDIX A SAMPLE DATA RECORDS: AGARICS, BOLETES, AND OTHER PILEATE FUNGI

Group:
Date:
Collector:
Number:
General Impression:
Habit:
Habitat:

- Associated plant cover:
- Substrate or host:
- Age, state of decay, or condition of substrate:
- Site orientation, cover, immersion or topography:

Most striking feature:

Pileus:

- Shape (profile__, from above__, or):
- Width ___cm to ___cm (or height___cm to ___cm):
- Colour variation:
- Transparency
  (Translucent striate __, opaque__, or):
- Margins (straight__, incurved__, inrolled__, or):
  (entire__, or):
- Cuticle texture (moist__, viscid__, dry__, or):
  Scales (absent__, or):
- Veil tissues (location, colour durability):
- Consistency (fleshy or):
- Thickness of flesh:
- Colour and consistency of flesh:
- Bruising or spotting:

Hymenophore: lamellae__, tubes__, teeth__, ridges__, smooth__, or:

- Attachment of hymenophore to stipe (adnate or):
- Degree of crowding (subcrowded__, __ per cm/mm, or):
- Lamellulae (__ tiers):
- Pattern (radial__, or):
  Forking (absent or):
- Colour (general impression enmasse):

- Margins, mouths, or tips:
  Coloration (concolorous with hymenium__, or):
  Outline (entire or):
- Colours of hymenophore face views:
- Shape of hymenophore in face view (tapered or):
- Width of lamellae or lengths of tubes or spines:
- (note latex & bruising at breaks)

Spore print colour:

Stipe:

- Attachment (central__, eccentric__, lateral__, or):
- Attachment (firm__, detachable__, or):
- Generalized shape (cylindrical__, or):
- Length of aerial portion: ___cm to ___cm
- Width: ___mm to ___mm plus variation:
- Shape of base (slightly tapered__, rounded__, or:
- Pseudorhiza (absent__, present__):
- Cuticle texture (see veils also):
- Colour patterns (see veils also):
- Veil tissues:
  Inner veil (a cortina__, membranous__, or):
  Universal veil (dry__, viscid__, and):
  Annulate no__, yes__ (see below).
  Volvate no__, yes__ (see below).
- Consistency of flesh (fleshy__, cartilaginous__, or):
- Core (solid__, stuffed__, hollow__, or):

Annulus: Movable or affixed, orientation (flared, pendant, sheathing), single or double, upper versus lower surface texture & colour, edge(s), location (superior, central, inferior), other:

Volva: Membranous__, crumbly__, banding__, other__; outer colour, inner colour, gelatinous layers, free margins__, sheathing__, other__, bruising__.

Latex: Exuded colour

- Colour change
- Staining ability
- Beading__, dripping__, scant__
- Where?

Odour (nondescript__ or "fungal"__, versus):
(fresh__, as unwrapped__, when cut or crushed__, dried or drying__)

Taste (nondescript__ or "fungal"__, versus):

Rhizomorphs(?):

Sclerotium (?):

Macrochemical reactions:

APPENDIX B SAMPLE DATA RECORDS: TRUFFLES AND FALSE TRUFFLES

Group:
Date:
Collector:
Number:
General Impression:
Habit:
Habitat:

- Associated plant cover:
- Substrate or host:
- Age, state of decay, or condition of substrate:
- Site orientation, cover, immersion or topography:

General Impression:
Most striking feature:

Peridium:

- Colours:
- Bruising reactions:
- Surface texture:
- Openings to gleba:
  Rhizomorphs:
  Rhizomorphs colours:

Cortex:

- Consistency
- Thickness
- Colour
- Bruising after cuts:

Peridium adherence to gleba:

Gleba:

- Colour:
  Immature:
- Mature:
- Consistency:
- Chambering, cavities, mucilage, wall colours:
- Latex:
  absent___
  initial colour:

final colour:
staining:

Pseudocolumella:

- absent___
- colour:
- consistency:
- length:
- width:
- type of attachment:
- degree of penetration or projection:

Odour (nondescript___, or “fungal”___, versus):
(fresh___, as unwrapped___, when cut or crushed___, dried or drying___)

Taste (nondescript___, or “fungal”, versus):

Macrochemical reactions:

APPENDIX C SAMPLE DATA RECORDS: APOTHECIAL ASCOMYCETES

Group:
Date:
Collector:
Number:
General Impression:
Habit:
Habitat:

- See Habitat Attribute Table
- Associated plant cover:
- Substrate or host:
- Age, state of decay, or condition of substrate:
- Site orientation, cover, immersion or topography:

General Impression:
Most striking feature:

Apothecia:

- Stipitate (no__, yes__ - see below)
- Shape of hymenium (eg. shallowly__ or deeply__ concave;
  plane__, convex__, saddle-shaped__, wrinkled__, pitted__, other):
- Width of apothecia or head:
- Depth or height of apothecia or head:
- Colour of hymenium:
  Immature:
  Mature:
- Colour of apothecium or pit rims:
  Marginal features (entire__, hairy__, membranous__, split__, or):
- Exterior of apothecium:
  Texture:
  Distribution of hairs (entire surface__, rim only__, or):
  Colour of surface (glabrous__, or):
  Colour of hairs:
- Flesh:
  Colour:
  Latex:
  Consistency:
- Stipe:
  Length:
  Width:
  Texture (glabrous__, or):
  Grouping (solitary__, or):
- Subiculum (absent__, or):

- Stromata (absent__, or):
- Sclerotium (absent__, or):

Odour (nondescript__ or "fungal"__, versus):
(fresh__, as unwrapped__, when cut or crushed__, dried or drying__)

Taste (nondescript__ or "fungal"__, versus):

Macrochemical reactions:

Appendix A. Aphyllophorales (including polypores)

Group:
Date:
Collector:
Number:
General Impression:
Habit:
Habitat:

- Substrate or host:
- Age, state of decay, or condition of substrate:

General Impression:

Type of fructification:

Dimensions:

Consistency:

Colour of fresh hymenium:

Stains (not notable__, or):

Latex (absent__, or):

Odour (nondescript__, or):

Taste (only if fleshy - nondescript__, or):

Spore print colour:

Macrochemical reactions: