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Microfungi on Wood and Plant Debris

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Microfungi are defined as fungi with microscopic spore-producing structures (Hawksworth et al. 1995). They comprise an unnatural group of organisms that cuts across the classification schemes for Fungi and for the fungal-like organisms in the Protista, Chromista, and Myxomycota, some members of which previously were placed in the Fungi. The single criterion for inclusion in the group is the small size of the spore-producing structures, in contrast to the large macroscopic fruiting bodies of the macrofungi. Despite their lack of a common phylogenetic history, the microfungi are grouped together because they, or their asexual states, are collected using the same techniques. In this chapter, we focus on procedures and protocols used to collect microfungi that live on or in wood and plant debris. Microfungi also can be found in other habitats, but techniques for collecting them from those environments are covered in other chapters (see "Culture Techniques," later in this chapter).

Although the criterion for defining the microfungi is arbitrary, at least some species from most groups of fungi qualify for membership. We include the following groups:

Ascomycota. The Ascomycota comprise the largest division of fungi including approximately 3250 genera containing 32,250 recognized species (Hawksworth et al. 1995). Most ascomycetes are regarded as microfungi, and so members of this phylum account for the bulk of the group. Nevertheless, the Ascomycota includes some large-size species with macroscopic fruiting bodies, especially among the Pezizales and the Xylariales, that are collected and observed with techniques used for macrofungi.

Anamorphic Fungi (deuteromycetes, asexual fungi, anamorphs, conidial fungi). This unnatural group comprises around 2600 genera and 15,000 species, characterized by mitotic rather than meiotic production of spores (conidia). Clear cytological evidence of the mode of spore production is available for only a small proportion of those species. Likewise, the number of meiosporic states that have been correlated with teleomorphs is limited. Most of those meiospores have teleomorphs in the Ascomycota; a lesser number have teleomorphs in the Basidiomycota. The majority of species in the group have no known link; those forms are presumed to be ascomycete-related.

Microscopic Basidiomycota. The basidiomycetes comprises about 1400 genera and 22,250 species. Although most macrofungi are basidiomycetes (a few are ascomycetes), not all basidiomycetes are macrofungi. Some species—for example, the Tulasnellales (tiny resupinate saprotrophs)—are only microscopically visible and thus are sufficiently small to be grouped, for convenience, with the microfungi. Basidiomycetous anamorphs frequently have minute fruit bodies or lack them completely, and many are observed directly and cultured using microfungus protocols. Microscopic basidiomycetes are found especially among microfungi colonizing woody, rather than soft, plant debris.

Zygomycota. The zygomycetes that coexist with other plant-inhabiting microfungi are species in the order *Mucorales*. Although we include them in our treatment, almost all species in the order can be sampled readily using techniques described in the chapters on fungi in soil (see Chapter 13) or on animal excrement (see Chapter 21). Fifty-six genera and around 300 species currently are known from the order.

Other groups of microfungi associated with nonplant substrata are considered elsewhere. They include the following:

1. Lichenized ascomycetes encountered on plant surfaces (see Chapter 9).
2. Lichenicolous and fungicolous fungi treated together as fungicolous fungi (see Chapter 17). Many fungi gaining nutrition from other microfungi will be sampled using the protocols described later in this chapter.
3. Chytridiomycota. Some are encountered on aquatic plants and plant detritus (see Chapter 23); others are found in soil (see Chapter 13) or associated with living terrestrial plants (see Chapter 7).
4. Oomycota. Almost all are found either associated with living plant tissue (see Chapter 7) or in soil or sediment samples (see Chapters 13 and 23).
5. Myxomycota (treated in Chapter 25).

EXTENT OF HABITATS

The most important factor influencing the distribution and lifestyles of fungi is their heterotrophic nutrition (Cooke and Rayner 1984). Fungal growth therefore is confined to environments in which suitable supplies of organic compounds (primarily organic polymers) are more or less immediately available for use, although dispersal propagules and resting spores independent of their mycelium may be sampled from a very wide range of habitats. Fungi vary widely in their capacity to gain access to and exploit carbon compounds, which differ in availability, location, and configuration. As a result, microfungi grow on plant debris in every conceivable habitat, including those subjected to the extremes of climate that characterize the Arctic and Antarctic and deserts and rain

forests, as well as the milder subtropics and temperate environments. The nature and quantities of nutrients together with physical features of the substratum, water availability, humidity, and temperature determine the success of colonizations, subsequent survival of individuals, and species composition of a community.

Large numbers of fungal species colonize plant debris, a rather general term, which at its simplest can be defined as nonliving plant fragments. More precise characterizations may be required, depending on the objectives of the study, to ensure use of an appropriate sampling strategy. Such characterizations involve consideration of additional factors such as the following:

1. Origin of the material: roots, pneumatophores, stems, trunks, bark, wood, branches, twigs, petioles, leaves, phyllodes, lamina, veins, hairs, bracts, flowers, fruits, seeds
2. Condition of the material: age and degree of senescence (recently fallen or otherwise), fragmented mechanically or by a biological agent, such as insects or mammals; digested by insects; rotted by other fungi; tunneled; secondarily utilized; degree of decomposition; chemical composition
3. Location of material in the habitat: superficial, buried, immersed, litter layer, canopy, on or in other organisms

Microfungi, because of their small size, can occupy minute sites. The importance of precise definitions of such microhabitats cannot be overemphasized. Attempts to quantify and define microhabitats within particular environments are few, although this should be an important first step in designing an efficient sampling program. What information is available generally has been gathered as part of studies on successional patterns (e.g., for colonization of woody tissue; Dix and Webster 1995). Hawksworth and colleagues (1998) noted the importance of this issue for the design of sampling programs in tropical ecosystems, although the list of niches included was not exhaustive and the authors did not define them specifically.

TROPHIC RELATIONSHIPS

Three nutritional strategies, which are not necessarily mutually exclusive, have been recognized for fungi (Cooke and Rayner 1984). Individual species can, in different circumstances, manifest all three, and fungi frequently shift their nutritional strategies in the course of their life cycles. Saprotrophy, which is the most frequent nutritional habit of microfungi colonizing plant debris, occurs when a fungus uses nonliving organic material from a plant that it did not kill. In necrotrophy, which frequently involves processes similar to saprotrophy, the fungus kills the living tissues of a plant and then uses them for nourishment. For some necrotrophs, the completion of a full or abridged life cycle depends on a switch of modes from necrotrophy to saprotrophy; often the transition is gradual. Biotrophy, in which only living host cells are exploited as nutrient sources, is much less common among microfungi colonizing plant debris, and biotrophic species largely are excluded by definition. Some biotrophs later become necrotrophs, however, and a small proportion subsequently can function saprotrophically. The timing and rate at which the transition occurs clearly is important in the context of plant debris colonization.

Because the processes by which dead plant material is altered and returned to the environment are many and varied, it is inevitable that niches and trophic strategies of microfungi overlap. For example, we include leaf lesions in this chapter because the dead or dying host tissues resulting from action by a necrotroph or biotroph almost always are colonized by secondary saprotrophs. Bark of living trees, which is dead, often supports growth of microfungi (bark fungi are treated with the endophytic fungi; see Chapter 12). Consequently, temporal, spatial, and physical transitions from species of microfungi that colonize living tree tissue and to those that colonize dead tissues occur at the interface between the bark and the living tree cambium.

A large proportion of endophytic fungal species become saprobic on the death of their host, an advantageous strategy because their intimate association with the plant allows immediate utilization of the dead tissue. Furthermore, many endophytic species enter an intermediate necrotrophic stage as the host tissue becomes moribund, allowing even earlier colonization and utilization of energy resources. Nutritional metamorphoses have not been studied adequately, although observations such as that of Bills and Peláez (1996) integrating information from endophytes and saprobes are beginning to reveal what are frequently very complex ecological systems. Culturing techniques for examining the diversity of microfungi associated with dead plant tissue are largely the same as those for endophytes, although initial surface sterilization is not necessary. Endophytic fungi are treated in detail in Chapter 12.

It may be years before plant debris decays fully and its residues are incorporated into the soil (Dix and Webster 1995), although in humid tropical ecosystems the process may be much more rapid (Anderson and Swift 1983). Therefore, a temporal, as well as a physical, interface between plant debris and soil needs to be taken into account in microfungal community analyses. Microfungi

that sporulate can be readily observed directly even on heavily decayed plant debris (often after incubation in moist chambers), which means that fruit bodies, spores, and mycelium may be removed and isolated if required. Although the latter technique may substantially undersample species that are present in plant debris compared to culture methods such as particle washing (Polishook et al. 1996), identification problems caused by nonsporulating cultures are largely avoided.

LIFE STRATEGIES

Concepts of life strategies in fungi have been derived largely from the work of Grime (1977, 1979) on higher plants. First brought to the attention of mycologists by Pugh (1980), they subsequently were accepted, modified, and extended by Cooke and Rayner (1984) and Andrews (1992) and summarized by Dix and Webster (1995). Pugh and Boddy (1988) emphasized that trophic nutritional types, which may change at different phases of growth, should not be used to classify fungi. Rather, they should be used to define behaviors shown at particular stages of fungal life cycles because their behaviors may change according to different phases of growth.

The three primary life strategies are explained in the following paragraphs.

RUDERAL

Short life spans and high reproductive potential characterize fungi found in disturbed but productive environments. Species are ephemeral, but growth can be rapid and devoted almost entirely to reproductive structures. Many *Mucorales* are ruderals, and they are abundant in soil. Ruderal fungi appear to be particularly sensitive to transient factors such as water stress (Pasricha et al. 1994) and play an insignificant role in litter decomposition (Webster 1957). A modified ruderal strategy is shown by coprophilous fungi, which include zygomycetes, basidiomycetes, and ascomycetes.

COMPETITIVE

High competitive ability is dependent on characteristics that maximize growth in productive, relatively undisturbed conditions, by active, antagonistic means, rather than through invasion of a niche vacated by a previous occupant. They are persistent and long-living, are capable of defending captured resources, have rapid or slow growth and spore germination, exhibit slow or intermittent reproduction, and have good enzymatic competence.

STRESS-TOLERANT

Endurance of conditions of environmental stress or resource depletion select for other communities of microfungi. Most known stress-tolerant fungi, which are not symbiotic, can be grouped in well-defined ecological niches, each of which is adapted to a particular type of stress. They are persistent as long as stress conditions prevail and are replaced if stress is alleviated. They lack noticeably rapid growth, spore germination, or reproduction, but are enzymatically competent.

LIMITATIONS TO THE STUDY OF MICROFUNGI

SIZE

Large numbers of fungal species are so small as to defy sampling except by the most meticulous and time-consuming means. Their lack of conspicuousness (often even when fruiting) is a serious barrier to understanding their diversity and the roles they play in an ecosystem. Fruiting bodies are frequently as small as 50 µm in diameter, and some hyphomycetes have conidiophores only about 10 µm long. Spores may be as small as 2–3 µm long and are often less than 1 µm wide, although a few species have spores greater than 100 µm long.

The minute nature of microfungi makes their direct observation in the field difficult; consequently, collecting focuses on adequate sampling of entire substrata and niches rather than on the collection of individual species. The success of this approach varies according to collector experience, but even an expert, who may be able to achieve targeted collecting for some groups, often has little idea of what actually has been collected until the material is examined microscopically unless the habitat is already well known. Almost all plant debris supports more than a single species. Painstaking mounting of vegetative and spore-forming structures and their examination using a compound microscope is the only way to find out what is actually present in mixed populations of microfungi without culturing from the material using techniques borrowed from soil analysis, such as particle washing or particle filtration (see "Principal Isolation Methods" in text and "Appendix" of Chapter 13). Some systems are overwhelmingly complex. Individual

leaves in tropical litterfall can harbor up to 30 different species of hyphomycetes (Sutton 1986), only some of which grow in colonies well defined by zone lines. In temperate regions it is common to find similar substrata—for example, cupules of *Fagus* and *Quercus* (the seeds are colonized by another group of microfungi) or leaves of *Laurus* and *Ilex*, colonized by similar numbers of species. The spines of fallen cupules of *Castanea sativa* in the United Kingdom are found regularly with 5–10 species of hyphomycetes and ascomycetes (Sutton 1973b, 1975); other fungi colonize the fleshier tissues.

Accessory Vegetative Structures

Most microfungi are relatively long-lived, but sporulate (and thus are directly identifiable) only for short periods. They may produce melanized fruiting structures, which serve as protection for the enclosed spores during adverse conditions of drought, low temperature, high ultraviolet rays, and so forth. Melanized hyphae also can withstand adverse conditions. Other vegetative structures such as chlamydospores, sclerotia, bulbils, appressoria, and setae also may be produced partially or entirely for dispersal or survival of cold or dry periods. Unless the morphology of such structures is very distinctive, species are difficult to identify in this condition and must be cultured to induce formation of their sporulating states.

Teleomorphs and Anamorphs

Many microfungi have more than one morphologically distinct spore-bearing stage in their life cycles and can produce not only a teleomorph but also one or more anamorphs (Kendrick and Di Cosmo 1979). Anamorphs may be formed without teleomorphs and vice versa, and additional conidial states (synanamorphs) may be formed. Large numbers of species appear only to produce mitotic spores, but because the production of the meiotic morph is frequently cryptic in comparison with the formation of conidium-bearing structures, the proportion of species that actually reproduce sexually is unknown. Even if both (or all) morphs are produced, the timing of events is often unknown even in relative terms. The anamorph may or may not precede the teleomorph, which may or may not be produced at the same location on the mycelium. Two or three synanamorphs may be formed (sometimes even in the same conidioma), and one may be formed earlier in the season than the other. This is the situation in some species of *Phomopsis* (B. C. Sutton, personal observation). For ascomycetes such as *Diplocarpon rosae* or *Rhytisma acerinum*, the anamorph (*Marssonina* or *Melasmia*, respectively) is formed as a necrotroph on leaves. After leaves fall, the anamorph ceases to sporulate, and a teleomorph stroma is formed. The fungus overwinters in this state, and at leaf emergence, the ascospores reinfect new leaves to produce the anamorph again (Knight and Wheeler 1977). The need to recognize and link these different reproductive and vegetative states is a significant restraint on biodiversity sampling strategies.

Ecology

Detailed ecological information is lacking for most microfungi, except the more well-known and widespread plant pathogens, which have been studied mainly in agricultural systems. The only systems that have been explored systematically are some temperate successional systems in litter and wood (Hudson 1968; Cooke and Rayner 1984; Dix and Webster 1995). Microfungi overcome the constraints on colonization, especially in extreme environments of high or low temperatures and limited water, by exploiting narrowly defined microhabitats. Hudson and Webster (1958) discovered that fungal succession varies even between upper and lower internodes of grass stems, and Webster and Dix (1960) found differences between standing and uprooted culms. A. Rambelli (in litt., March 1996) observed that primary colonizers of newly fallen *Pistacia lentiscus* leaves in Sardinia are different on the upper and lower surfaces, even of individual leaves.

Knowledge of microfungal ecology, and of the range of species colonizing particular niches and microhabitats, is restricted, especially in regions that are subject to climatic extremes. To take two opposite examples, in *Agave* and similar desert plants healthy leaves and stems seemingly are devoid of epiphytic fungi (although they may be colonized by endophytes). However, microfungi do develop in sites where moisture is available (i.e., in the rosettes and leaf axils). Thus, the bases of older leaves are often colonized. The novelty and composition of the mycota of this niche do not appear to depend on the plant species (B. C. Sutton, personal observation). Further evidence for this apparent lack of host specialization comes from examination of the restricted diversity of microfungi associated with cacti (Cannon and Hawksworth 1995). At the other end of the water-availability gradient, similarly distinctive guilds of species occur in association with the water traps within bromeliad rosettes in wet tropical forests (M. M. Dreyfuss, personal communication) and among the wide range of aeroaquatic fungi that grow in conditions of periodic inundation (Park 1974; Ando 1992).

Emerging evidence suggests that many saprobic fungi are specialized to colonize substrata with particular

physical characteristics rather than to particular host taxa (Polishook et al. 1996). Features such as substratum longevity, topography (e.g., spininess, venation patterns), rigidity, and water retention capability must have encouraged the evolution of species guilds, and climatic features such as extent and pattern of rainfall also play a part in promoting specialization.

Currently no easily available source of information on distributions and ecological preferences of fungal species exists. This is yet another major barrier to inventory work, to identification and characterization of taxa, and to knowledge of conservation status. Such a publication would allow surveyors of ecosystems to reduce dramatically the numbers of species with which their taxa should be compared when attempting identifications. Such data would have to be amassed on a systematic basis with well-developed habitat definitions such as those used by phytosociologists (Barkman et al. 1986) and supplemented by experimental work detailing temperature ranges, nutrient levels, water levels, and other environmental parameters necessary for growth of individual species in culture. Such an undertaking would be a mammoth exercise, but agreement on a logical framework for the data would allow a gradual buildup of information.

Species Concepts

Most species of microfungi associated with dead plant tissue continue to be identified based on morphological characters observed from collections from host material rather than cultures or molecular characters. Identifications are sometimes unreliable because of difficulties of definition and delimitation of genera and species. Furthermore, key morphological features are often subject to environmental modification over time, often to unquantifiable degrees. Therefore, many species are probably distinguished unsatisfactorily from their relatives on morphology alone. Lack of information on morphology of fungal structures in culture for many species makes identification of these species particularly difficult, not only because sexual structures are produced more rarely in culture but also because other structures frequently used for taxonomic analysis in field specimens, such as fruit bodies and conidiophores, may be absent or poorly developed in culture.

Nutritional status clearly plays a large role in the evolution of fungal species, a fact that has not been sufficiently taken into account for the vast majority of taxa (Brasier 1987; Cannon and Hawksworth 1995). For many groups of biotrophic fungi, current opinion is that species are restricted to relatively small taxonomic units of their associates (often genera, sections or groups of related genera), although most of the evidence presented is based on observations of morphological similarity rather than by experimental techniques (e.g., Cannon 1991). Many necrotrophic fungi have traditionally been regarded as strongly host-specific, resulting in a plethora of species names, in which the species are defined more in terms of their host identity than by features of the fungi themselves. The accepted species concepts for some necrotrophic genera with traditional taxa largely based on host identity, such as *Colletotrichum* and *Phomopsis,* are in the process of revision (Sutton 1992; Rehner and Uecker 1994). DNA analysis (particularly ribosomal DNA) is showing that such genera are systematically diverse, but the species recognized by this new evidence do not always correlate significantly with host-based classifications. Aproot arrived at similar conclusions while studying distribution of the common species of *Didymosphaeria* on decaying dicotyledonous plants (Aproot 1995). Such work increases the need to develop appropriate and, as far as possible, comprehensive species concepts that conform to modern evolutionary theory.

Saprobic fungi do not suffer the same nutritional constraints as necrotrophs because there is no need to evolve ways to penetrate living plant defenses. That does not mean that saprobes never prefer particular substrata because many are adapted to specific microhabitats or hosts (e.g., Læssøe and Lodge 1994). Such adaptations may involve the ability to metabolize, or at least to tolerate, noxious plant compounds, especially the wide range of defensive metabolic products produced as protection against pathogenic heterotrophs of all kinds (Bharat et al. 1988). Species that are apparently saprobic and that show clear host/substratum preferences may be endophytes during their initial growth stages; many species of Xylariaceae fall into this category (Petrini and Petrini 1985). Fungal taxonomic groups often include species varied in their nutritional mode; Laessøe and Lodge (1994) found that the strictly saprobic *Xylaria axifera* was strongly host-specific to dead petioles of species of Araliaceae and is not found as an endophyte. Similarly, Whalley (1987) found that *X. carpophila* does not develop stromata from freshly fallen fruits but only from partially degraded fruits in the litter layer. Some substratum-specific saprobes, particularly species of the Xylariaceae, may be present as endophytes in unrelated hosts but do not develop after the host material dies. *Verticicladium trifidum*, typically observed on conifer debris, is widespread and a non–host-specific endophyte (G. F. Bills, personal communication). Variations in nutritional mode, type of host specificity, and transient states of the life cycles present particular problems in esti-

mating diversity of endophytic fungi and in understanding their interaction with the host plant.

Species definitions for many microfungal groups are problematic at present as a result of the apparently frequent lack of sexual recombination, absence of information about gene exchange, and arguments as to the relative merits of biological and phylogenetic species concepts (Cannon and Hawksworth 1995; Colwell et al. 1995). Lack of information on anamorph phylogeny and anamorph/teleomorph connections (Kendrick and Di Cosmo 1979; Sutton and Hennebert 1994; Sutton 1996) are further barriers to proper characterization of microfungal species. Molecular data already are improving our knowledge in these areas, but current techniques of nucleic acid analysis must be adapted for groups that cannot be cultured. Unfortunately, DNA extracted from herbarium specimens is often too degraded for full analysis (Haines and Cooper 1993).

Numbers of Species

Described species of fungi of all groups number about 72,000 (Hawksworth et al. 1995), which represents less than 5% of the conservatively estimated total number of fungi (Hawksworth 1991). The probability of encountering a large proportion of unknowns in niches, habitats, and geographic areas previously unexplored for fungal diversity is high. This issue is considered in detail by Cannon and Hawksworth (1995).

Availability of Data

Diversity studies of fungi, especially of microfungi, are currently seriously hindered by the lack of information in easily accessible and usable formats. Comprehensive databases of fungal species names are available only for limited geographic areas and for limited taxonomic or ecological fungus groups (e.g., Cannon et al. 1985; Farr et al. 1989). Few data on names accepted by modern experts or placed into synonymy are easily available. The lack of modern monographs and other accounts of fungi that can be used by nonspecialists is even greater. The work by Ellis and Ellis (1997) on microfungi in northern Europe is a shining example of what can be achieved. The United Kingdom's Natural Environment Research Council has provided funding for important descriptive manuals of the ascomycetes of Great Britain and Ireland that are currently in preparation, but, for tropical regions little is available that does not require extensive training to use. Problems caused by the high proportion of undescribed taxa are even more acute.

TAXONOMIC AND BIOTIC STATUS

Patterns of Distribution

With a few notable exceptions, knowledge of fungal biogeography is very limited, in large part because of lack of data. Except for some economically important groups (mainly plant pathogens) and a few relatively conspicuous species, the number of collections of microfungus species is inadequate for portraying the extent of their distributions and likely biogeographic origins. Difficulties in accessing available data (most collection records are not computerized) compound the problems faced even in assessing current knowledge. General treatments of this subject are provided by Pirozynski and Weresub (1979) and Galloway (1994).

Distribution patterns of fungi are linked intimately to their modes of nutrition. Clearly, fungi that are confined to particular host groups (i.e., social groups; not necessarily implying parasitism or pathogenicity) have distributions that are, at most, coextensive with those of their plant associates, although other factors may limit their range further. Some fungi, especially ruderal species, are distributed widely, and many of these are observed abundantly in ecosystem analyses. This has led to a traditional view that fungi tend to be universal in their occurrence. Some taxa, such as members of the Caliciales (Tibell 1984), for example, are presumed to be cosmopolitan as a result of their ancient evolutionary history. Many dung fungi and perhaps soil and litter fungi, in contrast, are distributed widely (e.g., Gams 1992) at least partly because of extensive human introduction. The most prominent (or, at least, most commonly encountered) members of the microfungal component of ecosystems will be those that sporulate profusely, are easy to isolate into pure culture, and have short generation times. Such species are inherently more likely to spread widely than those with restricted growth and sporulation and are recorded much more reliably in samples. Research on fungal diversity in genuinely undisturbed habitats is badly needed as a basis for proper assessment of natural distribution patterns of saprobic microfungi. Recently, many fungal species that previously were considered to be cosmopolitan have been subdivided into geographically well-defined segregates (e.g., Brasier 1987; Otrosina et al. 1993; Mueller 1992). This trend may prove widespread as additional taxa are analyzed.

It is difficult to prove that individual species are rare or have limited ranges, given that evidence supporting such hypotheses is always negative. Nevertheless, it is likely that distributions of a significant proportion of saprobic microfungi are restricted. Questions of distribution and rarity of many microfungi will be answered

authoritatively only after extensive inventory of a series of sites.

Current Knowledge of Species Diversity

The number of species of microfungi currently known that are associated with dead plants is difficult to estimate, even roughly, for two reasons. First, although a few reasonably complete databases (although much editing is required) for fungal names now exist, there are no comprehensive registers of accepted species in which synonyms are indicated and linked with correct names. Such information is available only for restricted groups (rarely higher than family in the taxonomic hierarchy) and is confounded by disagreements over species concepts and the status of poorly known taxa. Second, taxonomic and ecological divisions rarely coincide, and data on substratum preferences of fungi are not available in an easily accessible form. The value of organizing distribution data both taxonomically and ecologically is frequently significant for distributional, as well as systematic and nomenclatural, reasons. Unless species designations have changed significantly over time, historical occurrence records should be valid and can provide valuable information on changes in distribution patterns, conservation status, and similar responses.

Some basic estimates of species diversity are possible. Hawksworth and colleagues (Hawksworth et al. 1995) gave a conservative estimate of 72,000 described species, although other experts (e.g., Rossman 1994) have suggested that the number may be much more than 100,000. The large discrepancy is probably at least partly a result of differences in the ways poorly known taxa were included in the estimates. Hawksworth's group included only species that were established as "good" by modern experts. It is not unreasonable, perhaps, to speculate that we have at least basic knowledge of about 100,000 fungal species, although large numbers of those have not been characterized adequately.

A crude estimate of the proportion of fungal species that are microfungi on dead plant tissue can be made by examining the numbers of fungi known that belong to the major taxonomic group that inhabits such substrata (see also Dick and Hawksworth 1985). According to the *Dictionary of the Fungi* (Hawksworth et al. 1995), about 13,000 of a total of 32,250 species of Ascomycota, or about 40%, are likely to be microfungi found on dead plant parts. The remaining species are lichenized, confined to living plant tissue, or primarily associated with soil. About 45% of the Ascomycota are lichenized, so about 73% of nonlichenized Ascomycota are likely to be sampled using the techniques described in this chapter.

About 15,000 species of mitosporic fungi are accepted in the *Dictionary of the Fungi*. Some of those have established teleomorph connections, although the methodology for establishing connections needs further development (Samuels and Seifert 1995) and inevitably some species have been counted twice. A large proportion of mitosporic fungi on dead plant tissue are categorized as microfungi because few lichenized mitosporic fungi have been described so far, although the number is increasing rapidly. Counterbalancing this to some extent are the many necrotrophic fungi that produce conidia from living plant tissue and form teleomorphic structures once the plant has died. Given our poor knowledge of mitosporic fungi, it is likely that a larger proportion of fungi currently classified as mitosporic than of fungi of Ascomycota will be sampled using living plant protocols. Because information is not available to assess these data accurately, the ratio of 73% (for nonlichenized taxa) therefore is reduced to a somewhat arbitrary 65% of mitosporic fungi likely to be sampled using dead plant techniques. A known species estimate of 10,000 is established, therefore, for mitosporic fungi on dead plant parts.

The numbers of species of microfungi in the Basidiomycota and Zygomycota are relatively small compared with those of the Ascomycota, and most species of the former two groups will be sampled from dung and soils. We include a token 500 species to represent the Basidiomycota and Zygomycota on dead plant tissue that would not be discovered using the techniques described in other chapters.

We estimate, therefore, that the total known diversity of fungi associated with dead plant tissues is 23,500 species, or 32% of the 72,000 described species estimated in the *Dictionary of the Fungi* (Hawksworth et al. 1995). Estimations of total fungal diversity are currently of considerable interest (Rossman 1994; Cannon and Hawksworth 1995). The figure of 1.5 million species proposed by Hawksworth (1991) remains the best-researched estimate to date, although that number includes organisms from other kingdoms traditionally treated as Fungi. About 1500 known species fall into that category, with perhaps a proportion of species detectably associated with dead plant tissues similar to the proportion estimated for the true fungi. If we use the proportion of known fungal species associated with dead plant tissues with the 72,000 estimated total species of microfungi, then the total species associated with dead plant material can be assessed. Those calculations suggests that about 480,000 fungal species could be collected using the protocols described in this chapter. Some investigators have suggested even higher estimates for total fungal diversity, whereas others believe that the very high local diversity of fungi will not translate into

such large numbers on a global scale (Hawksworth and Kalin-Arroyo 1995).

Assumptions that known microfungi on dead plant material represent the same proportion of the total of known fungi as the actual figures may not be justified. Hammond (1995) demonstrated that the proportion of organisms in a given group that is known to science varies according to size of the organisms concerned. Hawksworth (1993) provided some limited evidence of such a relationship within the fungi. Those arguments suggest that the figure of 480,000 may underestimate the number of fungi associated with dead plant materials. The sheer number of species involved (more than 95% of which have not even been described) presents special problems for designing and carrying out fungal inventories.

STUDIES OF LITTER DIVERSITY

A number of studies of microfungi associated with dead plant parts have been carried out in recent years, although formal diversity assessment has been the primary aim of only a few. The studies may be grouped roughly into those that focus on fungi associated with particular species of substrata and those that concentrate on habitats.

HOST-SPECIFIC DIVERSITY ESTIMATION

The large number of fungi that are host- or substratum-specific, combined with the fact that plants frequently occupy well-defined ecological niches, suggests that estimates based on the ratios of plant to fungal species may provide the best working hypotheses of total diversity for planning inventories. Even so, such estimates must be used cautiously, especially when incomplete surveys are being considered. The diversities associated with selected plant species are listed in Table 11.1. Many of the earlier studies were not intended to be comprehensive, focusing primarily on species succession rather than diversity. The number of fungi associated with an individual plant species may be quite high, even with incomplete sampling.

TABLE 11.1
Fungal Diversity Associated with Dead Tissues of Selected Species of Plants

Plant species	Plant part	No. fungal species	Reference
Abies alba	Leaf	95	Aoki et al. (1992)
Abies firma	Leaf	104	Aoki et al. (1990)
Acanthus ilicifolius	Decayed tissue	130	Vrijmoed et al. (1995)
Agropyron pungens	Debris	98	Apinis and Chesters (1964)
Alchornea triplinervia	Submerged leaves	81	Schoenlein-Crusius and Milanez (1995)
Atlantia monophylla	Leaf	73	Subramanian and Vittal (1979a, 1979b)
Carpinus caroliniana	Living and dead bark	155	Bills and Polishook (1991)
Carex paniculata	Leaf litter	60	Pugh (1958)
Castanea sativa	Cupule	>27*	Sutton (1975)
Eucalyptus regnans	Leaf litter	>24	Macauley and Thrower (1966)
Fagus sylvatica	Leaf	29	Hogg and Hudson (1966)
Gymnosporia emarginata	Leaf	52	Subramanian and Vittal (1980)
Helianthus annuus	Achene	98	Roberts et al. (1986)
Heliconia mariae	Decaying leaves	56–98	Bills and Polishook (1994)†
Heracleum sphondylium	Stem	51	Yadav (1966)
Juncus roemerianus	Dead leaves	86	J. Kohlmeyer (personal communication)
Laurus nobilis	Leaf	137	Kirk (1983, personal communication)
Nypa fruticans	Leaf	63	Hyde and Alias (2000)
Pinus sylvestris	Leaf	120	Hayes (1965)
Pinus sylvestris	Leaf	70	Kendrick and Burges (1962)
Pinus sp.	Leaf	73	Tokumasu et al. (1994)
Pinus sylvestris	Decaying leaves	127	Tokumasu et al. (1997)
Pteridium aquilinum	Petiole	114	Frankland (1966)
Shorea robusta	Leaf litter	38	Bettucci and Roquebert (1995)
Quercus germana, Q. sartorrii, Liquidambar styraciflua	Leaf	46	Heredia (1993)

* Incomplete survey.
† Four individual plants sampled.

Frankland's (1966) study of the succession of fungi colonizing dead *Pteridium aquilinum* rachides remains one of the most influential. She found a total of 114 fungal species. Some recent studies have charted similar or greater levels of diversity. Cornejo and colleagues (1994) reported the recovery of about 500 species of fungi from leaf litter of only six tree species in Panama, although their methodology might not have accurately discriminated litter fungi from soil fungi. Bills and Polishook (1994), using a particle filtration method, recorded between 56 and 98 species of microfungi for samples derived from individual leaves of *Heliconia mariae* from Costa Rica. Roberts and associates (1986) isolated 98 fungal species from achenes ("seeds") of cultivated sunflowers (*Helianthus annuus*). Aoki and co-workers associated more than 90 fungal species with litter from each of two species of *Abies* in Germany and Japan (Aoki et al. 1990, 1992). Other recent studies have reported relatively low levels of diversity. Bettucci and Roquebert (1995), for example, identified only 38 taxa from leaf litter of *Shorea robusta* in Malaysia. That number may in part reflect deficiencies in experimental technique; their incubation of dilution plates at 25°C or 35°C and use of high-nutrient media probably resulted in overgrowth by ruderal saprobes. The high proportion of species detected from genera such as *Trichoderma, Gliocladium,* and *Penicillium* suggests that this was the case.

There is little evidence that even the more detailed studies of fungal diversity associated with litter of particular plant species have produced a near-complete inventory. Perhaps the most comprehensive survey available is one made by P. M. Kirk who carried out a series of direct observations of fungi on leaf litter of *Laurus nobilis* (Lauraceae; Kirk 1981, 1982, 1984). He identified 137 species of microfungi from 44 collections of leaf litter made over a period of 12 years in the southern United Kingdom. Because data were obtained by one person, inconsistencies of observations and their interpretations associated with multiple collectors and identifiers are reduced. We produced a rarefaction curve (Fig. 11.1A) for his data by plotting cumulative species number against cumulative number of collections (P. F. Cannon and B. C. Sutton, unpublished data). It suggests that, at least for this host in the southern United Kingdom, the total number of species of associated microfungi is likely to be about 150, although use of a different sampling technique might reveal another suite of fungi. The data also show (Fig. 11.1B) that no species was found in more than 75% of the 44 samples, that 35% of the species were found only once, and that a majority of species (64%) was found in less than 9% of the samples. Those data strongly suggest that many species of microfungi are rare, although lack of knowledge of host specificity and the fact that *Laurus nobilis* is not native to Britain make such interpretations less reliable.

The estimates provided earlier in this section and those in Table 11.1 suggest that the number of microfungi associated with the litter for a plant species is likely to range well over 100. In Kirk's study, and in those listed in Table 11.1, a large proportion of the species recovered were wide-spectrum saprobes. Some crude estimates of the diversity of host-specific species might be attained by combining those disparate character sets, but variations in species concepts and sampling techniques and the large number of incompletely identified taxa included in each study would render any estimates obtained of very uncertain value. Studies by Polishook and associates (1996) on the complementarity of fungal species isolated from litter of two plant species growing together in a forest in Puerto Rico addressed the question of specificity and ubiquity of microfungi on different host plants. Additional studies of the variation in complementarity between different host species and the effects that are genuinely host-related versus those that primarily are the result of physical differences in the host tissue are essential. Such an experimental program would be of considerable value in planning future sampling protocols, especially if it were carried out in a speciose site such as a tropical rain forest.

Non–Host-Directed Diversity Estimation

A small number of surveys have focused on the diversity of microfungi associated with litter in general (Table 11.2). Such an approach has advantages and disadvantages. Identification of species recovered can be more difficult because host identity cannot be used as a limiting character. Sampling is faster, however, and can be carried out by someone untrained in plant fragment identification.

Two of the most extensive general litter studies are those of Rambelli and colleagues (1983) and Bills and Polishook (1994). Rambelli and his co-workers studied leaf litter samples from four tropical forest plots in the Ivory Coast, comparing undisturbed vegetation with sites that had been cleared using traditional agricultural practices. They periodically examined litter samples incubated in moist chambers. They identified between 129 and 165 species from undisturbed and disturbed plots, respectively. The disturbed sites showed greater diversity than the undisturbed ones, presumably because native species persisted in competition with taxa introduced or encouraged by the agricultural process. The similarity of the fungal species lists from the sites was high, suggest-

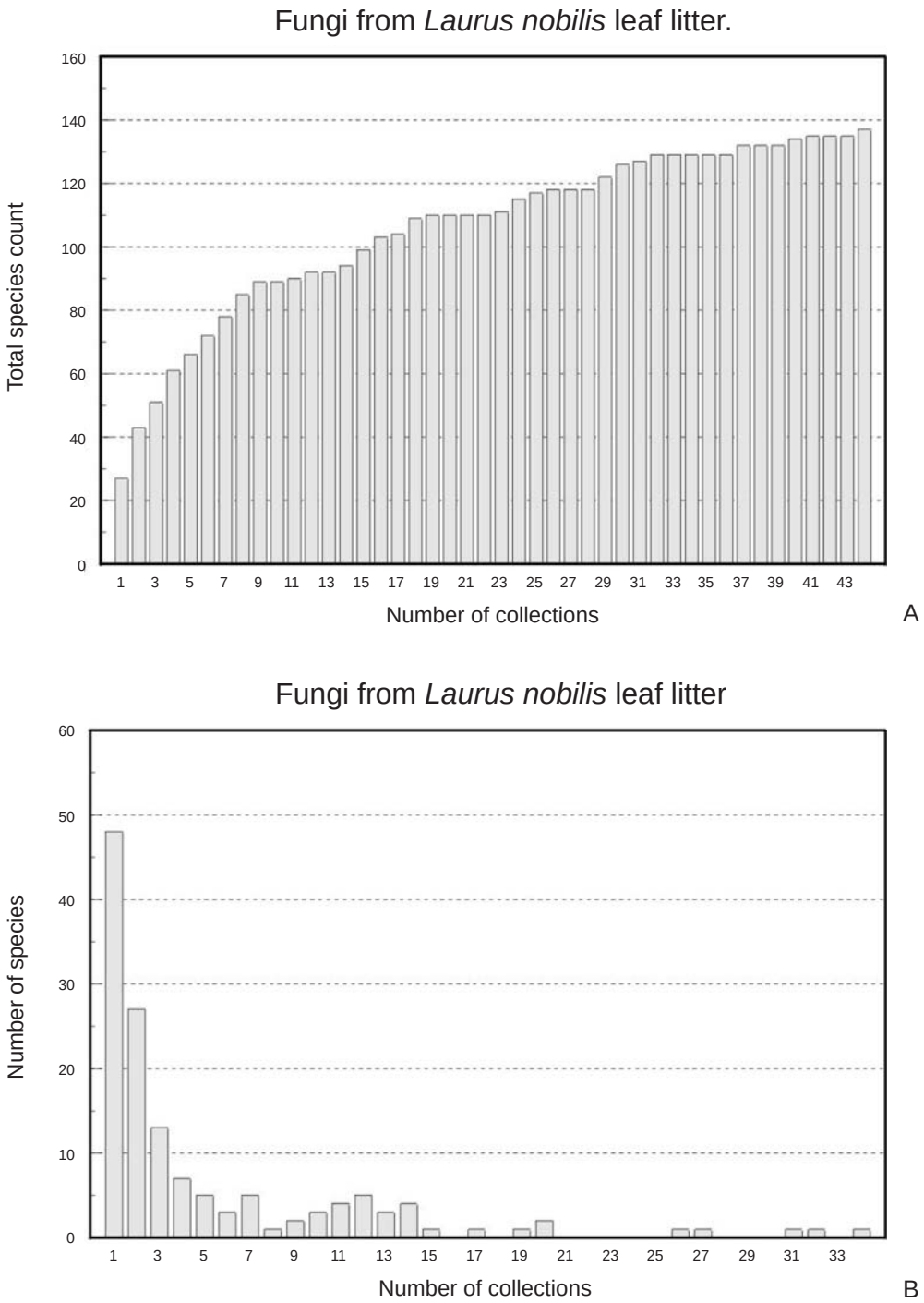

Figure 11.1 Fungi from *Laurus* leaf litter in the United Kingdom. **A.** Rarefaction curve showing cumulative species of fungi against cumulative number of collections. **B.** Frequency diagram of incidence of fungal species.

ing that many species were distributed widely and evenly. The plots constituted two pairs on somewhat different soil types, which appeared to have very little effect on the microfungal litter diversity, although how the mycota varied between plot pairs was not stated. In continuing studies, Rambelli and colleagues (1984) found that the fungal biota in the cultivated plots appeared to be gradually returning to the natural, precultivated state, although in a later paper (Rambelli et al. 1991) they noted that continuing cultivation had the potential of

TABLE 11.2
Microfungal Diversity Associated with Mixed Leaf Litter

Locality	Habitat	No. species	Methods	Reference
Great Britain	Salt marsh	141	Unspecified	Apinis and Chesters (1964)*
Austria	Alpine sedge	128	Direct observation	Nograsek (1990)†
Ivory Coast	Forest	129–165	Moist chamber	Rambelli et al. (1983)
Costa Rica	Wet forest	78–134‡	Particle washing	Bills and Polishook (1994)
Puerto Rico	Wet forest	338	Particle washing	Polishook et al. (1996)
Puerto Rico	Wet forest	24	Direct observation	Polishook et al. (1996)
Panama	6 tree species	500	Dilution plating	Cornejo et al. (1994)

*Ascomycota only.
†Ascomycota only, excluding inoperculate discomycetes.
‡Number of species per sample.

TABLE 11.3
Proportions of Major Taxa of Fungi Recorded at Different Sites in England

Taxon	Mickleham Down (4 ha)	Slapton Ley (211 ha)	Esher Common (400 ha)	Warwickshire County (250,000 ha)	Southeastern England (1,050,000 ha)
Mycetozoa	5%	5%	3.5%	6%	No data
Oomycota, Zygomycota	1%	3%	1.5%	6%	No data
Ascomycota	31%	43%	33.5%	40%	28%
Basidiomycota	46%	32%	40.5%	44%	42%
Mitosporic fungi	16%	17%	21%	8%	24%
Total species recorded	1380	2500	2700	2700	No data

Data sources: Mickleham: A. Henrici (in litt. 2000); Slapton: D. L. Hawksworth (in litt. 1997); Esher: B. Spooner and P. Kirk (in litt. 2000); Warwickshire: Clark (1980); Southeastern England: (Dennis 1995).

reducing diversity as a result of the climatic changes induced by cultivation.

Bills and Polishook (1994) carried out an intensive study of litter microfungi from four sites in lowland Costa Rica by culturing fungi from washed litter particles, in part with the aim of developing methodologies for diversity assessment. They detected from 78 to 134 species in each collection and found that rarefaction curves comparing total species number with total culture number showed little sign of leveling off despite identification of an average of 424 isolates from each sample. That finding suggests that they did not achieve anywhere near a complete sampling of the fungal diversity. The undersampling of the species diversity was underscored by Lodge and Cantrell (1995b), who reanalyzed Bills and Polishook's data and found only a 15–28% overlap of named species between samples.

The diversity of fungi in leaf litter is significantly greater than that in soil (Bills and Polishook 1994; Bettucci and Roquebert 1995). In a study in São Paulo State, Brazil, only six of 87 taxa identified from soil and litter samples were found only in the soil (Attili and Grandi 1995; Schoenlein-Crusius and Milanez 1995).

SPECIES DIVERSITY OF MICROFUNGI OVER LARGE AREAS

Study Sites

The data available on which to base estimates of fungal species diversity in large study areas, wherever they are located, are minimal. The most complete (or, perhaps more accurately, the least incomplete) information on fungal species numbers for specific areas is from three sites in southern England (Table 11.3): Slapton Ley National Nature Reserve (211 ha, of which nearly half is open water), Esher Common (ca. 400 ha), and Mickleham Down (4 ha). Each has been surveyed over a long period by a succession of expert collectors. Slapton Ley has been studied for more than 25 years by a large group of mycologists led by D. L. Hawksworth (International Mycological Institute [IMI]); Esher Common is largely a joint effort between B. M. Spooner (Royal Botanic Gardens, Kew) and P. M. Kirk (IMI); and Mickleham Down is primarily by an expert amateur mycologist, A. Henrici. Esher and Mickleham are rather

drier than Slapton, and the diversity of habitats is somewhat greater at Slapton. Plant species overlap considerably among the sites, however, which makes limited comparisons valid. More than 2500 fungal species are known from both Slapton Ley and Esher Common, but only about 30% of those are common to both areas. That observation and the fact that large numbers of microhabitats at both places have not yet been examined in detail suggest that the inventories are nowhere near complete for either site. An estimate of 3000 species for the total fungal diversity for either site does not seem unreasonable. Because Slapton Ley contains around 500 plant species, the figure corresponds well with Hawksworth's (1991) working hypothesis that fungal diversity outstrips plant diversity by a factor of around six. Of the species currently known from each site, the proportion associated with dead plant parts is somewhat higher than the overall estimates calculated earlier, but the research interests of the collectors may explain the high numbers. More than 1300 species of fungi currently are known from Mickleham Down, despite its small area, uniform vegetation, and lack of open water.

Extrapolation of these incomplete data to a large (50,000-ha) sample area, even in southern England, is more or less impossible because information on fungal distribution patterns is so inadequate and comparable data for species numbers over larger areas do not exist. The best data for a large study area in Britain are for Warwickshire County (Clark 1980), which covers approximately 250,000 hectares. Around 2700 fungal species have been recorded, although some speciose groups (especially the mitosporic fungi) are substantially underrecorded (Table 11.3). The relatively small increase in number of species encountered in this large area in comparison with the figures for Slapton, Esher, and Mickleham suggests that extrapolating from data associated with such a large area may not be a particularly good way of estimating fungal diversity. The large variation among the sites in proportions of species from major taxonomic assemblages (Table 11.3) provides further evidence that historical sampling procedures have been biased and that the proportion of species remaining to be sampled is significant.

Tropical versus Temperate Areas

Many circumstantial data, but no direct evidence, support the commonly held view that species diversity of the fungi as a whole is greater in tropical regions than it is in temperate regions (Lodge et al. 1995). Dennis (1970) compared the mycotas of Venezuela and the British Isles in fair detail, noting particularly that leaf epiphytes and inhabitants of living leaves in general are significantly more diverse in tropical, than in temperate, zones. Tubaki and Yokoyama (1973) observed that the diversity of fungi associated with litter of *Castanopsis cuspidata* and *Quercus phillyraeoides* was greater in southern Japan than in central Japan. Batista and co-workers frequently observed 10 or more microfungus species during direct examinations of single leaves in Brazil (Cannon and Hawksworth 1995; da Silva and Minter 1995), emphasizing the enormous diversity associated with neotropical plants. The same workers recorded as many as 26 fungal species from a foliicolous lichen community (da Silva and Minter 1995). However, large numbers of microfungi also can be found on plant parts in temperate (e.g., Sutton 1975) and Arctic regions. H. Knudsen (in litt.) estimated that the number of fungal species in Greenland was about 3000, conforming closely to the 6:1 ratio of fungal-to-plant species suggested by Hawksworth (1991).

Direct evidence that species diversity is greater in tropical than temperate regions does exist for individual fungal groups. For example, Cannon (1997a) found that the number of species of the Phyllachoraceae (Ascomycota) known between latitudes 40 and 60 N and 40 and 60 S is only 38% of that between latitudes 20 N and 20 S, and the corresponding figure for latitudes above 60 N (there is no vegetation below 60 S) is only 6% of the figure for the tropics. The true proportions are likely to be even more extreme given the under-recording of tropical species compared with temperate zones. Similar data might be gathered for a range of other fungal groups, providing a more balanced estimate of relative diversity in the two regions. Inventories are the only way to obtain such data.

FACTORS INFLUENCING SITE DIVERSITY

Many of the factors influencing site diversity affect all fungal groups similarly, but in some circumstances effects on diversity of microfungi associated with dead plant material are specific to that group. Factors affecting plant distribution and diversity, for example, will have proportionately greater influence on such microfungi than on fungi from some other groups (e.g., lichenized fungi or animal-associated taxa). The greater within-site habitat variation in plants and microhabitats, the larger is the expected microfungal diversity.

Temperature, Water Relations, Seasonality, and Physical Factors

There is little reliable information charting the effects of temperature on fungal diversity (in contrast to a plethora

of information for individual species), but physiologists appear to agree that the majority of fungi grow and sporulate best at temperatures greater than 15–20°C (e.g., Cooke and Whipps 1993). Although supporting data are based on a species assemblage that is not representative of the world mycota, Cooke and Whipps (1993) suggested that most fungi are adapted to grow in warmer climates. That then might lead to the conclusion that diversity is greatest in warm regions. A small proportion of fungi is known to be psychrophilic or thermophilic (e.g., Petrini et al. 1992b; Mouchacca 1995; see Chapter 14). It is likely that microfungi on dead plant parts conform to this rule. It is also likely that humidity plays a much more prominent role in influencing fungal diversity than does temperature alone. Elevation effects also may be relevant.

Again, data on the requirements of individual species for particular levels of moisture in the environment are abundant, but statistical analyses of overall species diversity under different water regimens are rare. Field mycologists know, at least, that spore production in a wide range of fungi depends on adequate rainfall. The considerable diversity of fungi in aquatic environments (e.g., Kohlmeyer and Kohlmeyer 1991; Bärlocher 1992; Shearer 1993) is indicative of the importance of water as a factor limiting fungal existence. Conversely, some species are adapted to xeric environments, both natural and manmade (e.g., Hocking 1991; Zak 1993; Cannon and Hawksworth 1995; see "Xerotolerant and Xerophilic," in Chapter 14). The diversity of microfungi closely associated with plants is small in arid areas compared with that in mesic areas, reflecting reduced host-plant diversity and restriction of fungi to very specific microniches where water is more available (Zak 1993; Fisher et al. 1994). In contrast, soil fungus diversity seems similar regardless of moisture level, if sufficient nutrients are available (see Chapter 13), although individual species may have specific water requirements.

Water availability directly affects rates of decomposition (Dix and Webster 1995). Abundance of water promotes fungal growth in general. It also may affect diversity, because fungi vary in their tolerances to water stress, and interspecific competition (Pasricha et al. 1994). Relatively low water availability may result in (at least in the sampling of) greater diversity in culture because slower-growing taxa are less likely to be crowded out by weedy species. For microfungi on dead plant tissue, water relations are important, although the substratum itself may act as a reservoir, allowing continued growth in dry conditions. Variation (seasonal fluctuation) in water supply can have dramatic effects on the microbial biomass of soil and litter (Lodge 1993; Lodge et al. 1994, 1996a), although not necessarily on microbial diversity.

The effects of seasonality on microfungal diversity are connected intimately with the effects of temperature and water. Significant seasonal variation in either temperature or water availability will result in a fungal biota different from that developing when conditions are constant (Lodge and Cantrell 1995b). Factors such as the aspect of the site, topographic variation, and soil type will affect microfungal diversity but primarily through their influences on the flora of the study area.

Plant Diversity and Distribution

The taxonomic diversity and structural variety of plant taxa exert the greatest influence on microfungal diversity at a site because such a large proportion of the microfungi has at least some degree of host and substratum preferences. Fungal diversity also is affected by variations in host life form; lignified tissues, such as wood, bark, and bamboo stems, decay much more slowly than non-lignified remains and therefore exhibit more extensive succession of fungal species. Distinct guilds of fungi are associated with dead bryophytes, algae, grasses, and other such groups, and although direct evidence is not available, it seems likely that the diversity of the fungi will be strongly positively correlated with the numbers of these plant groups.

Diversity of Other Organisms

The diversity of organisms other than plants should have little effect on the diversity of plant-associated microfungi but will be relevant in multitrophic relationships involving animal dispersal of fungal propagules. In addition, the rate of decomposition of litter material may be affected substantially by animal species, especially termites and bark-boring beetles (Anderson and Swift 1983), and competition for nutrients among fungi and other organisms may influence fungal diversity considerably. Again, the data available are too limited for proper analysis.

Human Influence

The effects of disturbance on microfungus diversity rarely have been quantified, but any physical disturbance, such as logging, trampling, and chemical pollution, should have a dramatic effect on microfungus diversity. Fungi may be affected indirectly through the influence of the disturbance on plant diversity, but increasing evidence (primarily from studies of lichenized and endo-

phyte taxa) indicates that fungi also may be affected directly.

A comprehensive study of the effects of human disturbance on microfungus diversity in litter was carried out in seasonally dry forests of the Ivory Coast by Rambelli and associates (1983). They compared two sample plots from each of two locations that differed in soil type and plant-species composition. One plot was left undisturbed. The other was cleared, planted with rice using the traditional agricultural techniques of the region, and then left to revert to natural forest after a single planting cycle. Sampling was initiated weeks after forest clearing and continued for a 3-year period. Fungi were sampled using moist chamber studies and direct observation of developing sporulating structures. Species identified from the litter of each sample plot ranged from 129 to 165, with species diversities at the disturbed sites exceeding those in the undisturbed ones. That increased diversity presumably reflects the persistence during cultivation of most fungi associated with the undisturbed habitat and the influx ruderal species and species associated with the introduced crop. However, the widespread practice of clearing forest and growing crops continually until the soil fertility deteriorates has a marked deleterious effect on diversity of litter microfungi (Rambelli et al. 1991).

A major survey of soil fungi is also of interest because fungal diversity in soil and litter may be closely correlated. Using isolation techniques, Miller and colleagues (1957) also showed higher diversity of fungi in cultivated soil in Georgia than in undisturbed forest soil. They found that of 165 species isolated, 115 were only found in forest soil, 141 only in cultivated soil, and 91 in both habitats. Although the differences in species profiles may not be numerically significant, it is likely that disturbance favored increased diversity of ruderal and rapidly colonizing species, which are sampled more efficiently using standard isolation techniques such as suspension plating. Other smaller-scale soil studies, however, have shown that persistently disturbed ecosystems exhibit lower microfungus diversity than natural, undisturbed sites (e.g., Joshi et al. 1994).

UNDERTAKING A MICROFUNGAL INVENTORY

Sampling Strategies

The resources necessary for a complete microfungal survey of even a small area are enormous, and most species are so small that it is impossible to be certain of their absence even after extensive sampling. Although the case for conducting a limited number of comprehensive inventories in distinct types of ecosystems throughout the world is scientifically compelling, realities of current funding levels argue for surveys that emphasize reproducibility rather than completeness (Cannon 1997b). Partly because of the lack of widely promulgated protocols, fungal surveys historically have been poorly defined, making it difficult to compare them.

Sampling strategies will differ somewhat depending on whether a total inventory or an assessment of diversity is required. For either goal, standardization of sampling strategies is crucial, both for comparative purposes and for the establishment of stop rules. Methods can involve direct observation (either at the time of collection or after incubation in moist chambers) and protocols for cultures.

Direct Observation

Moist chamber (Appendix I) and isolation techniques have been compared by several research groups. Watson and colleagues (1974) and Visser and Parkinson (1975) concluded that each method sampled distinct species assemblages, with a significant level of complementarity (Colwell and Coddington 1994). Polishook and associates (1996) found that at least 79% of the microfungal species identified by direct observation of decaying leaves also were obtained by particle filtration and subsequent culturing. Complete surveys require that both direct observation and culture techniques be used. Fungal cultures provide major benefits; they offer additional systematic characters for use in identification and determining relationships, living cells for genetic analyses, and the opportunity to screen for technological applications.

Advantages of direct observation methods over cultural techniques include the following: (1) lower cost because agar media and incubators are not required; (2) less airborne contamination; and (3) more complete identification because a higher proportion of observed species produce spores or other distinctive structures. Disadvantages of direct observation include the following: (1) greater difficulty in observing many taxa simultaneously; (2) vegetative structures immersed in host tissues; (3) greater difficulties in recognizing and separating species in mixed communities; (4) suppression of growth of some taxa as a result of competition among fungal and bacterial species in host tissue; (5) conditions in moist chambers suboptimal for sporulation of some species; and (6) absence of cultures for subsequent studies.

To ensure comparable results among samples, incubation conditions of natural substrata must be monitored closely, and the host tissue used must be clearly defined. Similar samples usually are achieved by cutting areas (circles or squares) of designated size from broad-leaf or bark tissue or using given lengths of petiole, needle, or stem. Samples should be as uniform as possible, for example, including a given proportion of midrib for leaf tissue. Samples should be placed in closed containers of appropriate size on damp filter paper, paper towel, moistened sterile vermiculite, or moistened sterile sand. Heredia (1993) attached microfungi from leaf surfaces to microscope slides with double-sided adhesive tape to facilitate repeated observation and to make voucher slides. Insecticides and/or molluscicides can be used to prevent degradation of specimens by small invertebrates, but some fungi are probably sensitive to these chemicals. Emerging pests also can be removed by hand. Samples must be inspected frequently for newly emerging fungal species and to ensure that moisture levels are correct. Variation in moisture content through the experiment may alter the diversity being recorded (J. Zak, personal communication). For additional information, see Appendix I on preparation of moist chambers.

Culture Techniques

The methods of analysis of litter microfungi in culture are similar to those described for soil fungi (see Chapter 13), and thus we only briefly summarize them here. The most effective method described to date is that of Bills and Polishook (1994), in which plant material is mixed with sterile water and pulverized in a sterilized blender. The resulting slurry is washed through a series of mesh filters using distilled water. Particles from 100 to 200 μm in diameter are suspended in a small volume of distilled water and washed again. The resulting suspension is pipetted onto agar plates of two selective media, a malt agar medium amended with cyclosporin A and dichloran-rose bengal medium (Appendix II). New emerging colonies are subcultured onto a range of standard media, and sporulation is encouraged, where necessary, by additional culturing on cornmeal agar media containing pieces of sterilized banana leaves. The method was developed for tropical rain forest leaf litter but is equally appropriate for temperate litter and for analysis of dead twigs, wood fragments, and other substrata.

Various factors influence the diversity of fungi isolated from litter samples. Those include the following:

Particle size. If litter fragments are too large, multiple colonies may arise from the same fragment and overgrow and obscure one another. If the particles are too small, then the fungal cells may be disrupted. Bååth (1988) recommended fragmentation resulting in a colony to a particle ratio of 1:1 or less. A technique in which plant tissues are cut with a sterile scalpel into small, equal-size pieces before culture has been used for endophytes (see Chapter 12). The spatial order of the plated fragments is recorded, and the number and kinds of fungi are referenced to each fragment. That procedure allows for the investigation of the spatial relationships of the various fungal mycelia or propagules (Wilson and Carroll 1994; Lodge et al. 1996a). The technique is labor-intensive compared with fragmentation in a blender and cannot be used effectively with well-degraded samples.

Growth medium. Nutritionally selective media can also play a major role in maximizing the diversity of taxa recovered, although their effects are not always obvious and the extent of their selectivity has rarely been tested adequately (Cannon 1996; G. F. Bills, personal communication). Many of the most effective media are nutrient-poor (e.g., soil extract agar and carboxymethylcellulose medium, CMC; Appendix II). These media restrict hyphal growth for most species and are inexpensive to prepare. Many of these media are transparent, which facilitates observation of minute colonies.

Growth inhibitors. If growth, especially of the common ruderal species, is inhibited, slower-growing fungi are more likely to be recognized and subcultured before they are overwhelmed by invasive molds. Growth can be inhibited either by incubation of cultures at relatively low temperatures or by the inclusion in the medium of chemical inhibitors such as cyclosporin (Dreyfuss 1986). Cyclosporin-containing media permit germination of a wide range of fungal propagules, but subsequent hyphal extension is strongly restricted. The media, therefore, are not strictly selective but allow easy separation of colonies on agar plates from materials with high fungal densities. See Appendix II for media containing growth inhibitors.

Inventories that rely heavily on culture protocols can produce large numbers of nonsporulating cultures, which are difficult to identify. Whereas specific identification is not essential for diversity studies, separation of taxa is necessary. Bills and Polishook (1994) adopted a wide taxon concept and aggregated individual nonsporulating cultures into a general category. At least 25% of species detected belonged to the category of once-observed unidentifiable species. The separation of nonsporulating cultures into taxa is inevitably more difficult than if spores are produced, but careful study of characters such as growth rates, colony morphology, pigmen-

tation, and hyphal structure usually allows for an acceptable estimate of species diversity.

Molecular techniques such as random amplified polymorphic DNA analysis (see Chapter 6) can be used to confirm the taxonomic integrity of groups of nonsporulating cultures, although their cost may preclude widespread use. Basic biochemical tests and molecular fingerprinting are also of value for characterization, although these methods are expensive compared with traditional classification techniques. The lack of reference data for other than a very small proportion of fungal taxa means that direct identification of isolates from diversity investigations using sequencing techniques is impractical for the foreseeable future.

For large surveys, the division of taxa into both nonsporulating cultures and sporulating taxa of uncertain identity is problematic because of the large numbers both of individuals and species. We recommend, therefore, that investigators set up a detailed database of character expressions to facilitate comparison among taxa.

Observation and Isolation from Washed Litters

Fungi whose dormant propagules land on or become mixed with a substratum can be excluded from a sample of fungi nutritionally associated with the dead plant material by litter washing (Harley and Waid 1955; Parkinson and Williams 1961). Tokumasu and associates (1997) successfully adopted such protocols in studies of fungi associated with pine needles in Thailand. They washed the needles in detergent and rinsed them in distilled water using a shaker. After washing, material either can be placed in moist chambers for observation, or it can be cultured.

COMPLETE INVENTORIES

There are two primary ways to carry out a total inventory of microfungi on dead plant tissue. The first uses a sampling strategy based entirely on host-plant identity and will cover most microfungi, excluding only those that occur on unidentifiable, rotted wood. The second approach involves sampling substrata, especially those in a highly decayed and unrecognizable state, uniformly across a defined geographic area. With a double-pronged protocol, both host-specific and plurivorous taxa are sampled adequately.

The two approaches must be balanced to prevent unnecessary duplication of sampling and to avoid overlap with other sampling strategies (primarily for living plants/endophytes and soil). Preliminary research is needed to identify that balance because few data on this subject are available and site characteristics (especially patterns of host diversity) may affect the outcome significantly. Minimum resources necessary to carry out the inventory will depend on characteristics of the sampling site (e.g., ease of access, climate, seasonal variation) and familiarity of the investigators with the mycota.

Sampling on a Host-Species Basis

Sampling using host-plant identity as an organizational tool depends first on an adequate knowledge of the plant species at the site, although common, familiar host species can be surveyed while rarer and difficult taxa are located and identified. If possible, an investigator should sample several widely separated populations of each species at a large site, taking into account differences in local community structure caused by varying physical features, soil types, aspect, and so forth. Fungi associated with living plant tissue (biotrophs and endophytes) should be sampled at the same time. It will be necessary to have detailed knowledge of the locations of rare and targeted host species (preferably using geographic information system, or GIS, technology) or to sample alongside plant collectors. Ideally, suitably trained plant experts should be consulted during the planning stages. In some cases, it may be feasible for plant collectors to collect dead plant materials for examination by fungal experts.

Sampling on a Geographic Basis

A small proportion of the fungal species associated with plant parts in late decay stages will probably be missed with host-species–directed sampling because plant parts will no longer be identifiable to species. The soil specialists will sample some of this material among the litter and humus, but microfungi on rotten wood and bark will need special attention. Such material is best sampled on a geographic basis, using a series of transects designed to sample the widest possible environmental diversity at the site by crossing valleys, streams, rocky outcrops, and other physiographic features. Similar transects may be used by macrofungus specialists, and sampling can be carried out in conjunction with that group. Samples with recognizable fungal presence should be taken from rotten wood and bark at specified points along each transect. A small number of apparently asymptomatic specimens should be taken in addition for close examination in the laboratory and for study with moist chambers. Samples should be taken at intervals during seasons of

active growth and at least once during dormant spells. Care should be taken to minimize the effects of site disturbance and repeated and destructive removal of samples on the study sites. A formal monitoring process to ensure that species are not being lost from a habitat as a result of the sampling may be appropriate.

General litter study (i.e., well-decayed, fragmentary plant parts) is carried out more effectively with culture techniques than with direct observation because of the difficulties in manipulating small pieces of substratum for microscopic observation.

Rapid Assessment Techniques

A primary aim of rapid estimation techniques is to minimize reliance on experienced fungal systematists, who are too few in number to provide extensive widespread diversity assessment (Cannon 1997b, 1999). Assessment of the diversity of poorly known and speciose groups will require radical methods if meaningful results are to be obtained in an acceptable timeframe. There is considerable interest in developing methods for biodiversity estimation that do not rely on identification of all the species sampled. The use of parataxonomists (highly trained collectors without formal academic credentials; Janzen et al. 1993) has been discussed widely, and similar workers are potentially able to process collections in laboratories. Untrained staff have assessed the diversity of speciose groups of spiders, ants, polychaetes, and bryophytes successfully (Oliver and Beattie 1993). Some of the most extensive fungal surveys have been performed almost exclusively by dedicated nonprofessionals (e.g., Clark 1980).

Industrial screening programs represent a practical extension of those concepts; such programs use methods developed to maximize diversity of target organisms extracted from natural samples so that their metabolic, biochemical, or genetic attributes can be assessed. In some cases, the work may be carried out by technicians without taxonomic training; formal identifications are required only if a strain producing potentially valuable metabolites is discovered (Bills 1995).

Methods of Estimation

Inventories can be carried out using direct or indirect methods and with or without reference to plant associates. Because of the cryptic nature of microfungi, direct observation methods require extensive field trips by highly experienced collectors. Even then, identifications will rely heavily on laboratory studies of materials collected for confirmations. Direct observation protocols may be valid for well-known ecosystems where suitably qualified staff members are available.

One of the simplest methods to reduce the sampling required for a diversity assessment is to restrict collection of material to substrata derived from a small range of plant species. Ideally, a range of types of substrata from a number of plant species that represent the range of botanical diversity of the sample site should be chosen, but even this can result in an unacceptable level of work. Of the various substrata available, leaves are probably the most satisfactory to work with because they are abundant; have a range of structural features such as laminae, midribs, and petioles; and decay relatively quickly so that successional studies in moist chambers can be completed rapidly. They can be identified relatively easily, even when detached, compared with bark or wood and are present in the vast majority of vascular plants.

PROTOCOLS FOR SAMPLING PROGRAMS

We suggest protocols here for analysis of microfungi associated with dead tissues of a single (woody) plant and for a complete survey of a small wooded area. We stress that even restricted surveys involve considerable work and that their value for comparative purposes can be increased immeasurably if protocols are rigidly defined and followed. Nevertheless, environmental variation both within and among sites is complex and difficult to define, and local conditions sometimes may preclude adhering to the quoted methods precisely. In those circumstances, investigators should record and explain departures from the planned protocols.

Site Selection

Sites may be chosen for practical reasons, such as ease of access, to complement surveys of other organisms that have been carried out or are planned or because of environmental threats such as proposed development or encroaching offsite pollution. Because of the lack of baseline data on fungal diversity and distribution patterns, it is often useful to select sites that are typical of well-defined vegetational or ecological categories such as old-growth northern coniferous forest, tundra or tropical deciduous forest. Ideally, development of international consortia of biologists will promote integrated surveys of particularly valued environments with benefits for all.

Site Analysis

Once a study site is chosen, a comprehensive analysis of within-site environmental variation to identify the full range of microclimates and niches is critical both for single-species surveys and complete inventories. Such information allows for an efficient and complete survey of the area and can ensure the comparability of results between sites. The site should be surveyed fully, with variation in physical features, such as elevation and aspect, accurately mapped. Temperature, precipitation, and insolation should be measured regularly, ideally over a number of years. Geologic features such as underlying rock type, soil acidity, composition, and water content must be recorded for the entire site. Special features such as rivers, caves, and rock outcrops should be noted as providing distinct microclimates that potentially may harbor different fungi, even if the host plants present are the same.

Even if a single-species survey is envisaged, information on the plant species present and their distributions will provide accurate characterization of the vegetation at the site. For a complete inventory, investigators must sample fungi associated with each plant species at the center of its within-site distribution as well as in isolated spots.

Sampling Frequency

To determine sampling frequency, the investigator must know the climatic regime, including the timing of dormant periods during hot, cold, or dry spells, as well as which perennial host species are deciduous. Sampling can be less frequent (but not suspended) during dormant periods. Many fungal species in both temperate and tropical ecosystems sporulate seasonally. In temperate regions, that seasonality often is related to nutritional demands and requirements for spore dissemination. Most larger basidiomycetes fruit in the autumn, when the nutrient supply available from leaf fall is at its maximum. Many pathogenic microfungi, in contrast, produce spores in the spring when host tissues emerge from dormancy and have not yet developed extensive physical and chemical defenses against infection. In the tropics, sporulation can be associated with dry or wet seasons (Cornejo et al. 1994; Lodge and Cantrell 1995b).

Frequency of sampling may be reduced by extensive use of moist chambers, which allow samples collected at perhaps 2-month intervals to be observed continuously. Such an approach requires adequate laboratory space but is advantageous in reducing collection time and site disturbance and in allowing for more extensive and objective successional studies. Succession is extended in many systems, so moist chamber observations must be continued over a considerable period. Sealing the ends of cut branches slows desiccation so that a wider variety of fungi, particularly endophytes adapted to colonize senescent bark, may be sampled (see Chapter 12).

Field Observations

Detailed field notes are an essential part of objective and reproducible sampling. Information to be recorded includes identity of the associated plant species, the precise plant part colonized, date of collection, location within the sample site, and microclimate (e.g., sun or shade, aspect). Prevailing weather conditions also should be recorded. Samples should be defined precisely and separated or cut into appropriate portions if needed to achieve internal homogeneity.

Notes on the population structure of the host and its position within the ecosystem also may be valuable; some fungi may be present only where there is a critical mass of the associated plant. An assessment of the general health of the host also may be important, although less so than with sampling protocols for biotrophic fungi inhabiting living plant tissue.

Field Collections

Samples should consist, where practical, of several individual, defined plant parts with visible signs of fungal growth. Asymptomatic dead tissue probably can be ignored because additional species presumably will be sampled using endophyte techniques, although no studies have been carried out to confirm that. Multiple collections should be taken of tissues in various states of decay, especially when the dead tissues differ in appearance as a result of the presence of different fungi. Tissues affected by other organisms (e.g., beetles) should be treated as separate samples. Samples collected from fallen or from dead, attached plant parts should be separated.

Voucher material of the host should be gathered along with the fungal specimens if the identity of the host plant is not obvious. Unrecognizable hosts can be identified based on wood anatomy and by comparison with herbarium specimens, but such methods should be used only in the absence of an alternative.

An investigator sampling a given plant population needs to specify the part of the plant surveyed (e.g., leaf, petiole, stem, branch, trunk, wood, bark, root) and record its dimensions. In addition, its size should be taken

into account. Fallen flowers, fruits, and seeds will have specific mycotas. Specialized plant structures, such as aerial roots, pneumatophores, and cladodes must be sampled separately using protocols adapted from those used for more standard plant parts. Some standardization of sample sizes may be appropriate, although this will depend on the fungal structures present and the heterogeneity of the plant material. Collectors should cut samples into suitable sizes for specimen packets or moist chambers on-site to avoid carrying extra material to the laboratory, but samples should be large enough to allow analysis and division into multiple specimens. The size of the organ collected also will influence decisions of sample size. Standardization of terminology for the degree of host-plant decay also may be valuable for sampling purposes, but generalization can be difficult because decay rates and fungal agents of decay vary among plant species.

Collectors should be trained to recognize mature fruiting material of at least the medium-size pyrenomycetous Ascomycota and coelomycetes to prevent the uncontrolled gathering of senescent specimens. Samples apparently containing only senescent and dead fungi still may be valuable, however, because small, secondarily saprobic species, difficult to observe in the field, can be found by microscopic inspection in the laboratory.

Collections are placed in paper bags or glassine packets (plastic bags result in an uncontrollable buildup of humidity). Tiny or fragile specimens are put or pinned into small boxes (e.g., those sold for fishing tackle) to protect them from physical damage. Collecting equipment includes a sharp knife, clippers (secateurs), small axe, wood chisel, folding saw, and a good-quality hand lens. *In situ* photography may be appropriate for larger specimens using a camera with a ring-flash attachment, but for microfungi in general this is best carried out in the laboratory with dedicated equipment.

Laboratory Analysis and Specimen Processing

As soon as practical after returning from the field, the investigator should examine the material collected using a dissecting microscope for preliminary identification and culturing. Slides of fungal structures made with a range of mounting media also will be needed (Appendix II), along with materials for standard tests such as those for reactions to iodine. The choice of a mountant is part personal preference and part taxon dependent, but observations in aqueous preparations as well as the preparation of permanent mounts is essential. Baral (1992) vividly illustrated the benefits of water mounts, in which cells are turgid and gelatinous sheaths and appendages are more obvious. Material that is overmature should be discarded, but immature collections should be placed in moist chambers to encourage further development. Once collections have been processed, representative samples should be dried and fumigated or frozen to kill animals such as mites and beetles, placed in labeled packets, and deposited in an herbarium. Specimen data (i.e., field notes, identification, laboratory observations) should be entered into a database from which packet labels can be produced. Air-drying is appropriate for most microfungi, but large and fragile specimens may benefit from freeze-drying. Some physical protection may be need to prevent crushing, especially of large hyphomycetes and long-necked ascomycetes but also for some small specimens in herbarium packets. Noting the appearance and precise position of fruit bodies on a specimen (e.g., in pale patches on both surfaces of the leaf laminae, along the abaxial surface of the midrib) and any apparent interactions with other organisms (e.g., saprobic on another fungus) is valuable, particularly for substrata such as large slowly decaying leaves, which may harbor a number of different fungal species. Such data are critical for type specimens to prevent unnecessary depletion of the original material by other mycologists.

We recommend that investigators photograph both whole fungi and relevant details of microscopic structures, especially for unknown or critical taxa. Line drawings made with a microscope and camera lucida are also valuable and ideally should be made in addition to photographs. The value of both photographs and drawings in characterization and subsequent recognition of species is obvious from the work of Matsushima (e.g., Matsushima 1995).

Cultures

It may be necessary to culture many microfungi to characterize and elucidate their life histories properly and to confirm their identities when the morphologies of field collections are not optimal. Investigators should transfer spores to suitable media in culture plates using a sterile needle or, for particularly small fungi or sparse samples, a micromanipulator. With care, hyphal fragments can be treated similarly. Some pyrenomycetes with forcible spore discharge can be attached to the lids of the Petri dishes so that they eject their ascospores onto the media surfaces. Tap water agar is an effective culture medium for initial isolation and often for stimulating sporulation. Low-strength cornmeal-dextrose or potato-carrot agars are used widely (Appendix II). We recommend use of low-nutrient media and moderate incubation temperatures to discourage weedy mold contaminants from overgrowing new colonies before they can be subcultured. However, optimal temperatures, media, and germination

times will depend on the kinds of fungi; in the absence of prior knowledge, investigators should approximate conditions at collection sites. Antibiotics discourage bacterial contamination, but anecdotal evidence suggests that some fungi are sensitive to such compounds, so they should not be used universally. Sterilized fragments of a substratum from which propagules have been isolated may be placed on a culture plate to provide both a physical structure on which colonies can develop and basic nutrients to which the species are adapted.

Handling Data

The most intractable problem with laboratory analysis of collections, especially in tropical regions, is how to collect data on unknown taxa so that further collections can be grouped correctly. A sophisticated, well-planned, computerized database with well-defined fields that contain homogeneous information is essential. Drawings and photographs are useful for rapid confirmation of similarity and can be included within a database or stored in file folders. Two linked databases are required. A specimen database gives details of places of collection, associated organisms, field and laboratory notes, and (where known) an identification. A species database organizes information from the specimen database into standardized species accounts, which then provide descriptions. The two databases may be part of a formal relational structure (see "Table Relationships" in Chapter 4), or they may be linked using a descriptor field (typically the accepted name of the species) rather than a numeric value. The interface between the databases is particularly important, and automated transfer of data from one to the other presents a considerable challenge. Collection data also may be combined for statistical analysis of sampling, design, and data. Other databases may be appropriate also, covering such details as nomenclature and bibliography. Minter (1996) provided a comprehensive description of one computerized data-gathering system for fungal collections (see also Chapter 4).

SAMPLING MICROFUNGI ASSOCIATED WITH DEAD WOODY PLANT MATERIAL

Choice of Host Species and Substrata

The size and life form of the host species and the selected substratum components will affect significantly the number and profile of associated fungal species recovered. Woody plants offer a much wider range of microhabitats to colonizing fungi than do herbaceous species. In addition, the long life span of a woody plant and its parts and its extended decay period promote the diversity of associated organisms. The ultimate choice of species may be largely personal or project driven, but ease of identification (both in living and decayed states), ecological role (dominance in the community), and conservation status all may play a role in the decision.

Life-Form Analysis

The variety of microhabitats provided by a woody plant must be analyzed prior to the sampling program. Dead plant parts are likely to include the following:

Leaves: fallen; buried; dead attached; suspended; dead parts of leaf laminae affected by necrotrophic fungi, action of other organisms, or physical damage; petioles and rachides; upper and lower lamina surfaces; leaf hairs, spines and glands; extrafloral nectaries

Twigs, stems, branches and trunks: fallen; buried; dead attached; suspended; killed by die-back or disease; bark surfaces; decorticated sections; cut or fractured surfaces; areas damaged by rodents, bark beetles, or other animals; natural cavities and bark fissures; spines or other special morphological features

Roots: cortex; exposed or covered by soil; physically damaged sections with decay caused by fungi or other organisms

Flowers: fallen, either complete or as component organs; attached but damaged as a result of other organisms; nectar; pollen

Fruits and seeds: fallen; buried; dead attached; decaying fleshy tissues; damage as a result of animal action; spines, wings; seeds decaying as a result of physical damage or action from other fungi; unfertilized ovules

The substratum descriptors are not strictly comparable, and the list is not exhaustive.

Temporal and Successional Issues

The age at death and state of decomposition of the plants sampled must be taken into account. For some woody plants such as conifers, leaves and young stems are formed in a precise sequence, which allows their ages to be ascertained whether attached to a living tree or to fallen branches. For other trees, age of lignified parts can be estimated by counting annual rings or can be estimated roughly from diameter. Ideally, all substrata should be sampled from trees representing a range of

ages at death and various degrees of decomposition. Speed of decay varies considerably depending on the plant part, environmental conditions, and the spectrum of accompanying organisms. In ideal circumstances, local rates of decay are determined before the sampling begins, so that fungi can be collected at regular intervals in the decay process under completely natural conditions. This is rarely possible, for practical reasons, but long-term observation of material in moist chambers offers a reasonably effective alternative.

Sampling Recommendations

We already have emphasized the importance of comparability of biodiversity sampling. Because many of the substrata described previously (see "Life-Form Analysis," earlier in this chapter) may be encountered infrequently, we recommend that a minimum set of substrata that is widely reproducible be sampled, even if those substrata do not yield maximal measurement of diversity. The minimum set will vary to some extent among plant life forms and major taxonomic groups—for example, between deciduous and evergreen trees, herbs and shrubs, and angiosperms and gymnosperms. For a deciduous oak species (*Quercus*), the minimum set might include:

Leaves: newly fallen, partly decayed (with the lamina having lost most of its strength), fully decayed (reduced to petiole and midrib)
Twigs (<20 mm in diameter): dead attached; recently fallen, with bark intact; well rotted with bark lost
Branches (>50 mm in diameter): dead attached; recently fallen, with bark intact; well rotted with bark lost
Fruits: recently fallen cupules, well-rotted cupules, recently fallen seeds, well-rotted seeds

A minimum of 20 collections of each of those categories of plant parts should be obtained from each of 10 trees that are widely spaced within the sample site, including young, old, isolated, and clustered individuals. The collections should be spread evenly throughout the available collecting seasons; for the oak species of the example, leaves and fruit would be sampled throughout autumn, winter, and early spring, whereas woody tissues could be collected over the entire year. Some compromise may be appropriate to restrict the total physical volume of the collections. Individual parts should be studied using a hand lens as they are collected and selected to maximize the range of physical appearances included, with special attention to evidence of external fungal growth.

In the laboratory, each individual plant part should be examined under a dissecting microscope. Samples of apparently sporulating fungal material should be removed with a scalpel or wetted needle and mounted on glass slides in water and lactofuchsin before examination using a compound microscope with oil immersion and, ideally, Nomarski optics. If recognizable sporulating structures are present, identification can proceed. Multiple voucher collections of each species should be preserved along with permanent microscope mounts. Ideally, cultures should be prepared by transferring spores or hyphal fragments to culture plates. We recommend use of tap water agar with sterilized pieces of the plant being studied and half-strength cornmeal or potato-carrot agar (Appendix II).

A portion of at least 10 collections of each component of the minimum sample set should be placed in a moist chamber (Appendix I) and observed at regular intervals (weekly) until the plant tissue has decayed completely or no additional fungal species are recorded. That process may take 6–9 months, even with leaves. It will provide information on succession and the occurrence of additional species that sporulate at intermediate stages of the decay cycle.

An additional portion of at least five collections of each component of the minimum sample set should be cultured using the particle filtration technique described by Bills and Polishook (1994) and Bills and colleagues in Chapter 13 (see "Particle Filtration," in the Appendix) after being washed thoroughly to remove surface contaminants (Tokumasu et al. 1997). Investigators should excise the rapidly growing colonies from all but one or two isolation plates with a sterile scalpel or kill rapidly germinating colonies with a soldering iron so that more slowly growing species can develop.

In areas where the fungal biota is poorly known (e.g., in tropical regions), each individual strain should be described and recorded and features of each recognized species (whether named or not) should be drawn and photographed. Such records are helpful for subsequent identifications and document the sampling process. A computerized database (see Chapter 4) will facilitate use of the information.

Complete Survey of a Small Wood

A complete survey of a small area largely involves repetition of the process described for a single plant species, although for practical reasons, the numbers of samples studied in culture rather than by moist chamber observation is likely to be substantially reduced. The culturing process (if carried out carefully) is used to isolate species from one another, thereby eliminating the competitive interactions operating among species in moist chamber environments. As a result, dormant propagules

dispersed onto a host plant from surrounding vegetation that might not be expected to develop in the presence of species well adapted to the host material will grow. Once the initial site survey is complete, fungi on dead tissues of at least one representative of each plant genus present should be studied, taking special notice of overall dominant taxa and those occupying restricted environmental niches. That process inevitably will be protracted and result in considerable duplication of species collected as a result of the absence of detailed information on host specificity for most fungal groups. In the absence of information from well-documented inventories of similar ecosystems, it is valuable to plot rarefaction curves (see "Richness" under "Quantitative Indices," Chapter 5) in the course of the sampling process to assess its efficiency.

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