SECTION 2 PATHOGENS: FUNGI, BACTERIA AND VIRUSES

reading

Read Chapters 6, 7 and 8 of Manion (1991) that deal with viruses, bacteria and fungi, respectively.

Because most pathogens of western forest trees are fungi, you need to be much more familiar with these organisms. As you read Chapter 8, examine the various illustrations carefully, especially those dealing with reproductive structures. Make sure you can relate the photographs to the diagrams for the various groups.

Study the material in the text, then read the discussion that follows in this manual. To test your understanding of the material in this section, answer the self-testing/review questions before proceeding to the next section.

VIRUSES AND BACTERIA

The two groups of pathogens, viruses and bacteria, cause many serious diseases in various agricultural crops, but they are not known to cause serious diseases in west coast coniferous forests. However, as you study the chapters in Manion, you will discover that there is considerable uncertainty about this. Particularly for the viruses, the problem may be that no one has actually looked carefully enough in coniferous trees to detect them.

FUNGI

Structure

Because of the relatively greater importance of fungi in forest tree diseases, we will discuss them in more detail. The basic body structure of most fungi is the **hypha** (plural, **hyphae**); a microscopic tube consisting of a wall of chitin (usually), surrounding a living cell. Hyphal diameter varies, commonly within the range of 1 to 10 microns (average of 3). But for some species or stages, hyphal diameter can be larger or smaller. Hyphae are often divided into segments (cells) by cross walls called **septa** (singular, **septum**). These septa may have special pores in their centers that allow movement of materials from segment to segment. Hyphae grow only at their tips; they do not "stretch" once formed. Branches may arise at the tips or, more commonly, at septa. The collective term for the mass of hyphae constituting a fungus is **mycelium** (plural, **mycelia**). Individual hyphae cannot usually be seen with the naked eye. Masses of hyphae may form white or variously coloured sheets or strands.

Most of the cytoplasm (and hence most of the metabolic activity) occurs at the growing tip. Further back, hyphal cells consist of large vacuoles. Thus the description of fungi as "simple animals in a tube" helps to visualize the manner in which fungi function. However, some fungi (e.g., yeasts) do not produce hyphae, but grow as single cells surrounded by a cell wall.

Requirements for Growth

All fungi are heterotrophs; since they lack chlorophyll, they must obtain their energy from organic materials, such as carbohydrates, fats and oils, produced by green plants. Most fungi require oxygen, though not necessarily in atmospheric concentrations. Some, such as yeasts, can grow anaerobically. All the normal mineral elements (N, P, K, Ca, Mg, Mn, S, Fe, Cu, B, Mo, Zn, and perhaps others) required by living organisms are also required by fungi, mostly in simple inorganic soluble form. Most fungi can grow provided these minimal requirements are supplied in a suitable aquatic environment. Some fungal species have special requirements for certain amino acids, vitamins, and other growth factors.

Habitat

Hyphal fungi cannot ingest solid materials. All the above requirements must be met by absorption through the fungal cell wall and the outer plasma membrane. Hence all such materials must be dissolved in water. Also, wastes are eliminated in the surrounding aquatic medium. Thus fungi are essentially aquatic although parts of a mycelium, particularly reproductive structures, may grow in air. Such parts get their nutrition by translocation along hyphae from parts of the mycelium that are in contact with water.

Since the substrate (e.g., wood) is often insoluble, fungi must produce extracellular enzymes to digest such materials. Enzymatic function is strongly affected by pH. The three-dimensional structure of proteins changes with pH, so that enzymes are active over a relatively narrow pH range, (reversibly) inactivated over a wider pH range, and permanently denatured at extreme pH levels. Hence there is a requirement for a fairly narrow pH range (about 4 to 6.5 for many fungi, but either higher or lower for certain specialized ones). Of course, if energy sources and minerals are supplied in soluble form, a fungus is much less sensitive to the pH of the aquatic medium. The pH within the cytoplasm is very closely controlled by the fungus, and so long as energy sources are sufficient, it can be maintained even if the pH of the immediate environment is different.

Most fungi occurring in the forest are low-temperature organisms. Many become active just a few degrees above 0°C, and reach their maximum rate of growth between 20 and 30°C. Temperatures in excess of 40°C are often lethal. Not surprisingly, different fungi have different optimum temperature ranges; different species, and even different isolates of the same species, are often adapted to the conditions (warm or cold) from which they have been isolated.

Ultra-violet light in sunlight is destructive to all living things, and those that are exposed to sunlight have various protective devices. Vegetative fungal hyphae, as a rule, do not have sunlight protection (they are too small to have the required pigments in sufficient quantities), and hence they cannot grow in locations exposed to full sunlight.

To summarize, fungi have the following requirements for vegetative growth:
• an energy source;
• a supply of mineral nutrients;
• oxygen;
• a suitable temperature;

- protection from ultraviolet light; and
- liquid water at a suitable pH.

Some have additional requirements for special organic compounds, such as amino acids or vitamins.

Reproduction

Fungi reproduce both sexually and asexually. Asexual reproduction can occur by fragmentation of hyphae and by spores. Living hyphal fragments can be dispersed in soil particles or organic materials and establish new fungal colonies at distant locations. This is the only known method of reproduction for a few fungi. Asexual reproduction by spores is much more common. Asexual spores that are produced at the tips of hyphae, either singly or within specialized structures, are known as **conidia.** In some groups of fungi, asexual spores are formed within a container-like structure (the sporangium) and released at maturity. Such spores are called **sporangiospores.** Asexual reproduction can be very quick. A conidium landing on a suitable substrate can germinate, penetrate, and establish a new fungal culture that begins to produce more conidia, all within a week. Thus a single spore can lead to the production of millions of new spores within a month. This tremendous reproductive potential makes a mockery of attempts to control many diseases by sanitation (although, as we shall see, there are some exceptions). Generally, control is most often achieved by manipulating the environment so that it becomes less suitable for the pathogen in question.

Many species of fungi reproduce only asexually. Others reproduce both sexually and asexually, or only sexually. A discussion of sexual reproduction requires a look at nuclear cycles. In most fungi the nuclei in **somatic** (i.e., vegetative) hyphae are haploid. In some groups these haploid nuclei may occur in pairs, called **dikaryons**, consisting of nuclei of compatible sex types. **Karyogamy** (fusion of nuclei to form a diploid) and meiosis usually occur in rapid succession within specialized structures, but sometimes there may be a significant diploid stage. Thus there are three separate phases in a fungal life cycle, namely the haplophase, the dikaryophase, and the diplophase. These stages are separated by the processes of **somatogamy** (fusion of haploid hyphae to form a dikaryon), karyogamy, and meiosis, respectively.

Sexual reproduction results, among other things, in the recombination of genetic material. However, that recombination can also occur within somatic hyphae via what are known as **parasexual processes.**

In those fungal species that exhibit regular sexual stages involving meiosis, there may be one sex (so that karyogamy can occur with identical nuclei, although most often it will in fact involve different nuclei); two sexes (in which case there may or may not be morphologically distinguishable sexes — if not, the sexes are called + and –); or more than two sexes. In some decay fungi for instance, if one starts with four different haploid mycelia A, B, C and D, dikaryons can form in all combinations: AB, AC, AD, BC, BD and CD, but not as AA and so on. (If

A is male and B female, what then is the sex of C and D?) The genetic explanation is that the locus that determines sexual compatibility has many alleles, and that compatibility requires only that the two alleles be different. This is known as bipolar sexuality. In tetrapolar sexuality there are two loci, and again several to many possible alleles at each of these. Compatibility occurs when the alleles at both loci differ. The advantage of this arrangement is that almost any pair of randomly selected haploid mycelia can fuse, form dikaryons, and eventually reproduce sexually, rather than only half (or, in the case of tetrapolar species, one quarter) of such pairs.

Taxonomy and Nomenclature of Fungi

Taxonomy is the process of dividing living organisms (in this case the fungi) into a hierarchical system of groups, the basic group being the species. Species are then grouped into genera, genera into families, and so forth. **Nomenclature** is the process of naming such groups. A valid Latin name of a species consists of three parts: the **genus**, the **specific epithet** (the species name), and the **author** who described and named the species and placed it in that genus. There are strict international rules of nomenclature. These include rules of priority (the name given by the first person to describe the species is the valid one), rules about the allowable form of names, and so on. Sometimes changes in Latin names are mandated by the rules of nomenclature. It is not uncommon, for instance, that two fungi, described by different names in different parts of the world turn out to be one and the same species. When that is discovered, the earlier name applies, and the later name becomes invalid.

Taxonomy, however is not governed by a set of explicit rules. Rather, it follows to some extent current scientific fashions, but more important, as our understanding of fungal structures and function improves, it is often realized that old groupings are artificial and that new, more meaningful arrangements are possible. There is no doubt that there are natural groupings of fungi (taxonomy isn't an arbitrary process), but no taxonomic system captures that grouping completely, in part because we don't know enough about the fungi to see all the relationships. And so situations commonly arise where there are two or more valid Latin names for the same species. A good example is found on pages 261–262 of the text which discusses classification of decay fungi. Sometimes two different taxonomists working on the same group of fungi end up with quite different ways of dividing them into genera and species. When this happens, there are two valid Latin names for each of the species involved. Confusion is avoided by listing the author. When one uses such a name, one in fact says: "The fungus I am speaking of is the one named *abc* by *xyz*." Such a situation is not a case of one being right and the other wrong; the differences arise because the criteria to be used to define species, and to group species into genera are not (and probably cannot be) unambiguously specified. Different taxonomists use such criteria in somewhat different ways, and consequently end with different names. The issue is usually decided by popular demand — the majority of scientist will use one of the two possible names, and eventually the other is more or less forgotten.

How does all this affect the forest practitioner? First, we have to live with the situation, and (hopefully) understand why some species have synonyms (two or more valid names). Since practitioners are seldom in a position to make a judgment about the issues involved, they will typically use the name that is commonly used in their region. However, when accessing the literature on a species, it is important to search under all the names, both old and new, popular and unpopular. For instance, the pathogen that causes Annosus root and butt rot was first described by Fries as *Polyporus annosus* Fr. in 1821. In 1881 the Finnish mycologist Karsten placed the species in his new genus *Fomitopsis*, and the name became *Fomitopsis annosa* (Fr.) Karst. However that name (and that system of classifying the Polypores) never become popular and was seldom used. Instead the name *Fomes annosus* (Fr.) Cke., first published in 1885, was almost universally adopted and used for a century. More recently the whole group of Polypores has been re-examined and divided into new genera, and that recent revision has now been widely accepted. Hence the name in common usage today is *Heterobasidion annosum* (Fr.) Bref.

Classification of fungi is based largely on the microscopic structures associated with sexual reproduction. For those that do not form sexual stages, the asexual stages are used. Fungi that produce both sexual and asexual stages are therefore sometimes known by two names, one for each, although in such cases, the name for the sexual stage is always the valid name for the species. For such fungi, the sexual stage is known as the **teleomorph** and the asexual stage as the **anamorph**. For the purpose of this course we will recognize five major subdivisions in the Kingdom Fungi. Below is a simple key to these; a more detailed one is presented on pages 121–122 of Manion (1991).

I Hyphae aseptate, asexual spores borne in endogenously in sporangia.

- **A.** Male and female sexual structures dissimilar; asexual spores have flagella, and are dispersed in water.
  (Class) Oomycetes
- **B.** Male and female sexual structures indistinguishable; asexual spores nonflagellate and usually dispersed in air.
  (Class) Zygomycetes

II Hyphae septate, asexual spores borne on hyphal tips

- **A.** Sexual reproduction involves the formation of a basidium bearing haploid basidiospores.
  (Subdivision) Basidiomycotina
- **B.** Sexual reproduction involves the formation of an ascus containing (usually) eight haploid ascospores.
  (Subdivision) Ascomycotina
- **C.** Sexual reproduction does not occur (or is as yet undescribed)
  (Subdivision) Deuteromycotina or **Fungi Imperfecti**

I A Oomycetes

This group contains several parasites of young roots and leaves of trees or agricultural plants. Life cycles usually consist of a brief asexual cycle (10 days from infection to spore production) and an annual sexual cycle usually involving a resting stage to tide the fungus over unsuitable conditions.

I B Zygomycetes

This group, mostly saprophytes, contains the vesicular arbuscular (VA) mycorrhizae.

II A Basidiomycotina

This is a homogenous group characterized by the production of a **basidium** (plural, **basidia**) bearing four external **basidiospores** at maturity. These basidia are usually organized in a layer called the hymenium which lines the gills of mushrooms or the pores of bracket fungi. This class contains most of the decay fungi, most of the important root diseases, and a special group of obligate parasites, the rusts. Most ectotrophic mycorrhizal fungi also belong to this group. Basidiospores are usually small, thin-walled, and therefore short-lived and easily killed by adverse conditions. On the other hand, they are produced in very large numbers.

II B Ascomycotina

This is a homogenous group characterized by the production of an ascus (plural, **asci**) containing (mostly) eight **ascospores**. These asci are arranged in various macroscopic structures. Most pathogens of foliage and bark, wilt diseases, and a few decay fungi belong to this group. Some species reproduce only sexually, and those usually have a short spore-producing period each year; others produce both sexual and asexual spores, in which case the latter usually consists of a brief cycle summer stage while the former is produced early in the season following a dormant over-wintering period. Ascospores are usually large and thick-walled. They can be carried long distances under adverse conditions.

II C Deuteromycetes

This group contains all the fungi in which sexual reproduction does not occur or is unknown. Thus the Deuteromycetes are an artificial group in the sense that it contains many species that clearly are closely related to species in the Ascomycotina in terms of their asexual reproductive structures as well as their physiology. It contains several pathogens of foliage, bark, and a variety of other tree tissues.

SECTION ASSIGNMENT

SELF-TESTING/REVIEW QUESTIONS

Test your understanding of the material in this section by attempting to answer these questions. Do not proceed to the next section until you are satisfied with your proficiency in this section.
Do *not* send your answers to the tutor for marking. If you continue to have difficulty with a question after you review the relevant material, you may wish to discuss it with your tutor.

A. Viruses

1. What are the basic components of a virus particle?
2. How are viruses classified?
3. What is the host specificity of viruses?
4. What are the common symptoms of virus infection?
5. Distinguish between local and systemic infection.
6. How are viruses transmitted from plant to plant?
7. Describe the ELISA technique as a sensitive assay for viruses.

B. Bacteria

1. List the major differences between the prokaryotic and the eukaryotic cell.
2. What types of plant tissue are most easily invaded by bacteria?
3. What are FXLBs and MLOs?
4. How are bacterial diseases transmitted?

C. Fungi

1. Fungi are essentially aquatic organisms. Explain.
2. Distinguish between taxonomy and nomenclature, and explain why a particular fungal species may have more than one valid Latin name.
3. How do fungi obtain their energy supply? Their essential nutrients?
4. Define and explain the following terms: *somatogamy, karyogamy, meiosis, diplophase.*
5. Sketch a typical ascus, basidium, apothecium, cleistothecium, perithecium, conidiophore bearing conidia, and a pycnidium.