FUNGAL NUTRITION
AND PHYSIOLOGY

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PREFACE

The motivation for writing this book came with the insights gained from 10 years of teaching a one-quarter graduate level course in fungal physiology to students in botany, mycology, microbiology, and plant pathology at The Ohio State University. During that period many excellent books were published on all facets of fungal physiology; they included monographs, symposium volumes, and long treatises. But none of these books presented the subject in a manner adequate for use in a fungal physiology course. Books on fungal physiology either provide more detailed coverage of topics than students need or emphasize molecular and biochemical aspects without commensurate coverage of nutritional aspects. Our experience has shown that students of fungal physiology are primarily interested in fungal nutrition and its implications for growth, development, and metabolite production. Our book is written primarily for seniors and undergraduate students who seek this kind of information and knowledge, but we believe that our approach to the subject will also make the book appealing to academic and nonacademic professionals in biological, agricultural, and medical sciences.

The aim of this book is to present in one volume the principles and concepts relating to nutrition and physiology of fungi with supporting evidence from the current literature. In pursuing this goal we have tried to impart a level of coherence and uniformity of style often lacking in similar texts. We think this approach will help make the book useful and interesting to individuals with varying levels of competence in the fundamentals of physiology, biochemistry, and molecular biology. Also, we have tried to present the material with sufficient rigor and detail for it to be useful in explaining and interpreting fungal nutrition and physiology even after the literature sources are no longer current. These features should make the book appealing to a diverse group of individuals with an interest in nutrition and physiology of fungi.

We have also attempted to select chapter themes that are of current interest on the one hand and of lasting importance on the other. The first chapter emphasizes the nutritional theme of the book and points to its evolutionary, ecological, and morphological implications for fungi. The chapter concludes with a discussion of the historical approaches to the study of fungal physiology.

Chapter 2 describes the principal components of fungal cells and some of the ways in which they function. Such knowledge is indispensable to a study of fungal nutrition and physiology.

Chapters 3, 4, 5, and 6 relate to the central theme of the book, fungal nutrition. Here we use numerous examples from the recent literature to illustrate the diverse roles of carbon, nitrogen, inorganic, and vitamin nutrition not only in growth, development, and reproduction but also in metabolism and metabolite production.

The chapters on fungal development include physiological aspects of spore dormancy and germination (Chapter 7), growth (Chapter 8), and reproduction (Chapter 9). The writing of these chapters presented a special challenge to us. We had to reconcile the voluminous literature dealing with the many facets of each topic with our self-imposed mandate to be concise. Moreover, we sought to present the judiciously selected topics in sufficient depth to stimulate the curiosity of interested readers.

The concise reviews of intermediary metabolism (Chapter 10) and of nucleic acid and protein synthesis (Chapter 11) serve at least two purposes. They provide basic information helpful in interpreting and explaining aspects of fungal nutrition and development, and they point to the potential usefulness of fungi in basic research in biochemistry and molecular biology.

As with other themes of this book, metabolite production in fungi is an enormously complex subject about which there is a voluminous literature. Therefore, the presentation on secondary metabolism (Chapter 12), even when read in conjunction with Chapter 10, is merely an introduction to the subject. Nevertheless, these two chapters should provide the reader with a sense of the strategies available to fungi for the synthesis of a wide array of compounds that are potentially harmful or beneficial, either to the fungi which produce them or to other organisms including humans.

Many thanks to our wives, Marie Garraway and Sue Evans, for their encouragement and support over the years. We are also grateful to our colleagues and friends in our respective departments for their interest and their support, tangible and intangible, during the period of preparation of the manuscript. We are especially indebted to Dr. Henry Stempen, Biology Department, Rutgers University, Camden, whose artistic skills have greatly improved this book. To friends and colleagues who have assisted us by contributing original photographs of their published or unpublished works we say many thanks.

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NUTRITION AS A BASIS FOR THE STUDY OF FUNGI

The manner in which fungi obtain nutrients from the environment distinguishes them from plants and animals and is largely responsible for their growth habit. Fungi lack chlorophyll and thus are not capable of utilizing the sun's radiant energy to manufacture organic molecules as plants do. In terms of their mode of nutrition fungi are similar to animals in being heterotrophic: they must obtain organic substances ("food") preformed from the environment. Animals, however, make use of a mouth to ingest food particles, which are then digested internally; fungi digest their food externally by releasing hydrolytic enzymes into their immediate surroundings. These enzymes break down the substrate into smaller subunits, which are then absorbed by the fungus. This absorptive mode of nutrition is one of the bases on which Whittaker (1969) places the fungi in a kingdom all their own (Figure 1.1). In this kingdom, the Kingdom Fungi, are grouped all those eukaryotic multicellular organisms that obtain nutrients by absorption rather than by photosynthesis (Kingdom Plantae) or ingestion (Kingdom Animalia).

There is enormous variety in the types of organic substances which fungi as a group can utilize as energy sources. Individual species, however, are often quite selective in their nutritional requirements and will grow only on certain substrates. In order for a substrate to be utilized as a nutrient three criteria must first be met: (1) the fungus must be able to synthesize and secrete the enzymes necessary to hydrolyze the substrate into molecules of relatively small size, (2) the fungus must possess the uptake mechanisms necessary to transport these small molecules into the cell, and (3) the fungus must possess the metabolic machinery necessary to convert these molecules into cellular energy as well as into building blocks for growth and development. Thus a fungus may well starve in the midst of plenty if it lacks an enzyme required for the hydrolysis, uptake, or metabolism of a particular substrate. Utilization may also be prevented if environmental conditions cause any of these systems to be operating at a critically low rate.

These three criteria, which underlie the absorptive mode of nutrition, determine

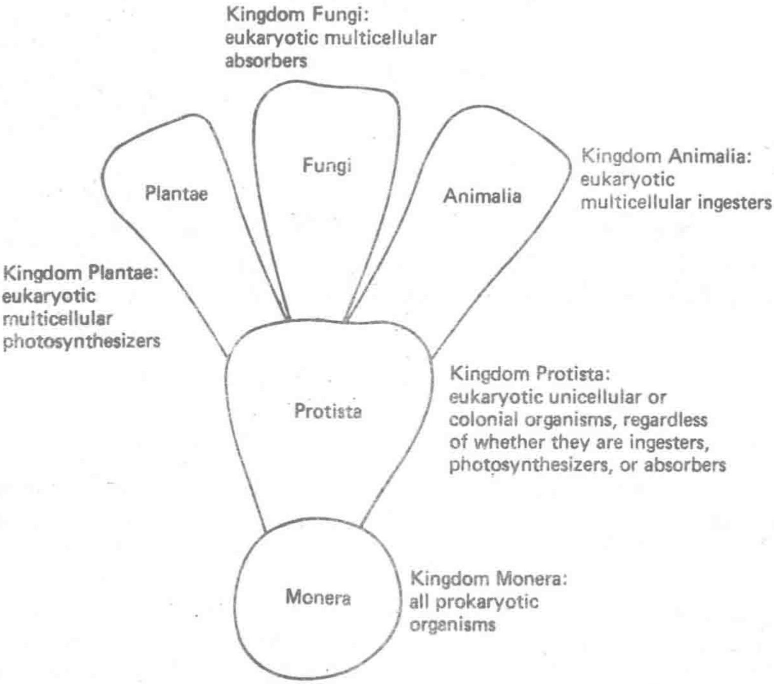

Figure 1.1 The five-kingdom classification scheme of Whittaker (1969).

to a large extent the habitats and ecological niches that a fungus can exploit. For example, many types of fungi are saprophytes; they obtain their primary sources of energy from nonliving organic materials of plant or animal origin. As fungi degrade these materials the nutrients are recycled into the biosphere for use by other organisms. In terms of human needs this decay may be deleterious, as in those cases where fungi cause the spoilage of food, the "rotting" of wooden houses and boats, the deterioration of paint, and the mildewing of clothing and other fabrics.

Other types of fungi possess the enzymes necessary to obtain nutrients directly from living organisms and are then called parasites. There are varying degrees of parasitism. In certain instances the absorption of nutrients by the fungus from the host organism is not detrimental and may even by beneficial to the host. This type of symbiotic relationship is found in associations between fungi and algae in a lichen and between fungi and the root of a vascular plant in mycorrhizae. Other fungal parasites cause significant damage to the host and are then called pathogens. The diseases of plants and animals, including humans, caused by fungal pathogens range from debilitating to deadly and are the subject of worldwide concern.

An understanding of fungal nutrition and of the ways nutrients function at the biochemical level offers a means of controlling the activity of these organisms, thereby maximizing their beneficial effects and minimizing their harmful ones. In addition, fungi serve as ideal experimental organisms for studying the biochemical bases for certain types of developmental and physiological processes because the metabolism of fungi is not additionally complicated by the presence of photosynthesis. Such information may be important not only to fungal physiologists but also to mycologists, plant pathologists, microbiologists, and others.

MORPHOLOGICAL ADAPTATIONS TO THE ABSORPTIVE LIFE—THE EXTERNAL FEATURES OF FUNGI

Over many millions of years of evolutionary time fungi have become well adapted to the absorptive mode of life. Fungi grow within their food supply, and structures have evolved that facilitate the exploitation of the substrate. Parasitic fungi, in addition, have developed a wide variety of structural features that aid in the penetration of the host and the subsequent ramification through its tissues. The fungal physiologist is interested in understanding the manner in which these various structures are formed, the mechanisms by which they function, and the ways in which changes in the fungal environment trigger their development. In preparation for examining these aspects of fungal physiology let us briefly review some of the principal morphological features found in fungi.

The Fungal Thallus

The majority of fungi are composed of long, branching filaments called **hyphae** or hyphal strands. A mass of hyphae make up a **mycelium**. A hyphal strand is actually an elongated tubular cell consisting of a cell wall and a membrane-bound cytoplasm in which are embedded numerous organelles and one or more nuclei. In some species the hyphae are partitioned by cross walls called **septa.** Because most septa are interrupted by one or more pores, these cross walls are not an appreciable barrier to the passage of cellular materials and are thought instead to aid in mechanically strengthening the tubular filament. Apparently, the only solid septa found in fungi are those that delimit reproductive or resistant structures. The delicate, filamentous nature of the hyphae provides an extensive surface area over which enzymes can be released and substrates absorbed. The growth of hyphal strands takes place at the tip, and branches usually arise at some distance behind the apex. By a combination of tip growth and subsequent proliferation of branches, a mycelial fungus can quickly ramify throughout a substrate.

A number of fungi do not form hyphae, and the thallus consists of only a few cells. A yeast, for example, is a nonmycelial fungus that is unicellular. Such an organism is placed in Whittaker's Kingdom Protista, but we shall include such eukarotic, unicellular absorbers with the fungi because mycologists have traditionally done so. Certain fungi have the capability of existing in two alternate forms, depending on the environment, and can switch back and forth between a mycelial and a nonmycelial phase. Figure 1.2 illustrates the growth habit of *Mucor rouxii*. In air this species grows as a filament, but it changes to a yeastlike form under elevated

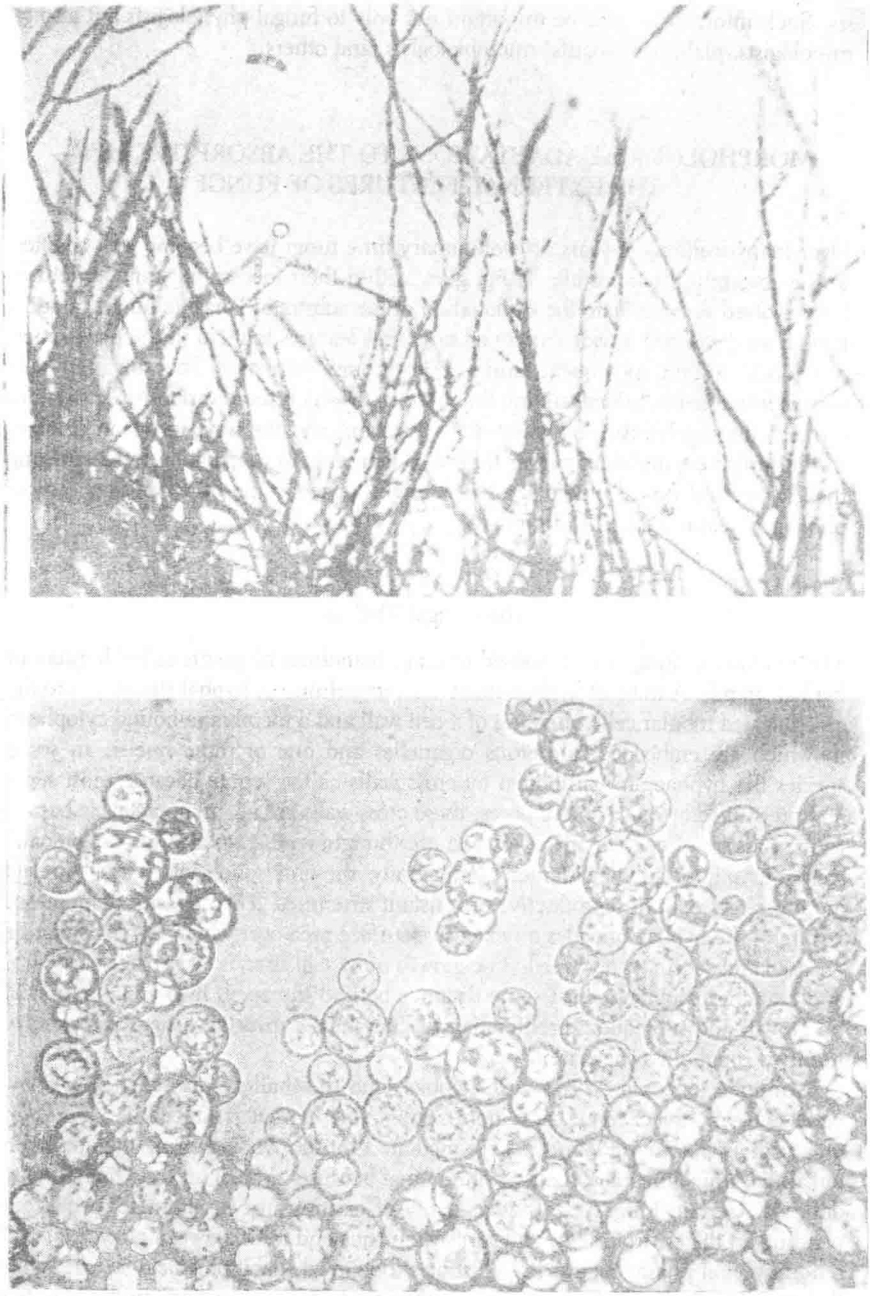

Figure 1.2 Dimorphism in *Mucor rouxii*. Top: filamentous growth form in air. Bottom: yeastlike growth form under CO_2. ×625. (Bartnicki-Garcia and Nickerson, 1962)

CO_2 levels. Such dimorphic fungi are important pathogens of animals, including man.

Still other fungi, the plasmodial slime molds, are unicellular and motile for part of their life cycle but fuse to form a multinucleate, wall-less mass of protoplasm called a plasmodium. The plasmodium creeps along the surface of the substrate and eventually develops fruiting bodies. The stages in the development of the fruiting

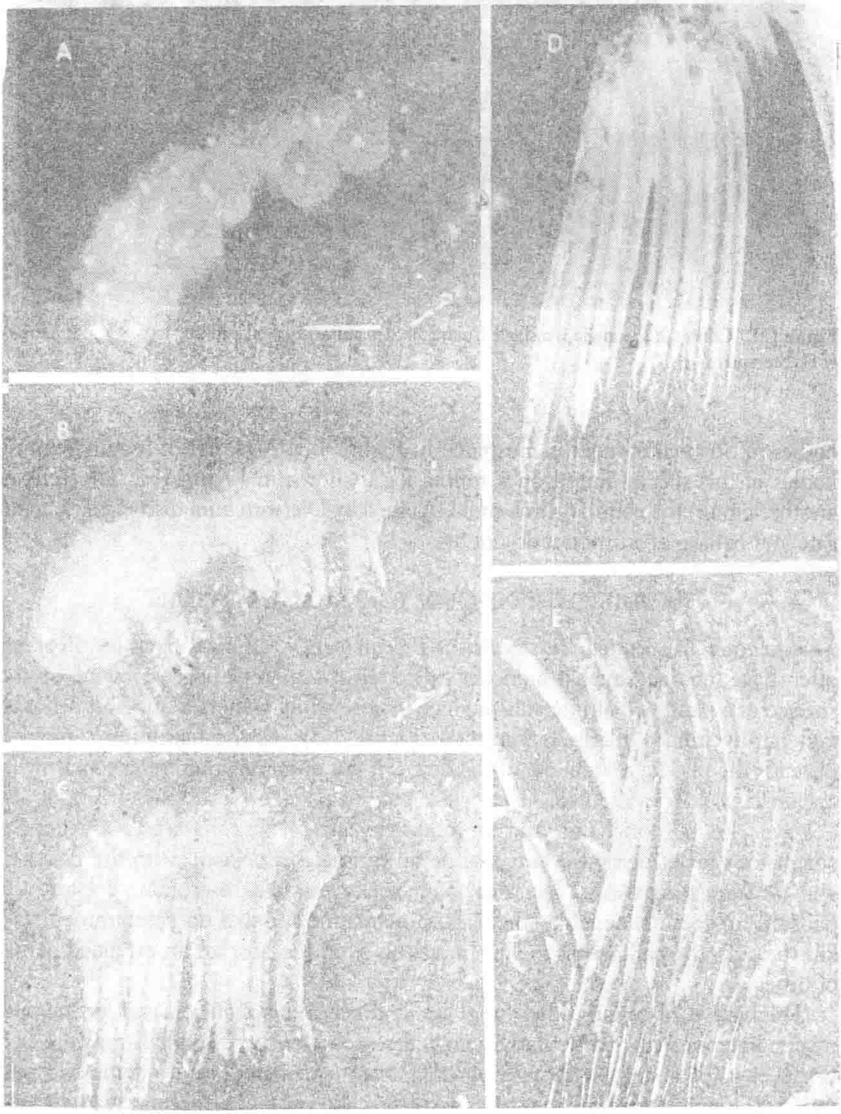

Figure 1.3 Development of fruiting bodies (sporangia) of *Stemonitis fusca*. Time sequence is as follows: (A) 0 time, (B) 2.25 h, (C) 3.25 h, (D) 6 h, (E) 48 h. Bar equals 1 mm. (Courtesy of H. Stempen.)

Figure 1.4 Cluster of sporangia is a single fruiting of *Stemonitis fusca* on a rotting log. ×5.5. (Courtesy of H. Stempen.)

bodies of *Stemonitis fusca* is illustrated in Figure 1.3. A cluster of mature fruiting bodies of this species found on a rotting log is shown in Figure 1.4. The cells of another group, the cellular slime molds typified by *Dictyostelium discoideum*, aggregate and behave as a unit but do not fuse.

MODIFICATIONS OF MYCELIA AND HYPHAE

Modifications Associated with Nutrient Procurement. Fungal parasites of plants often have modified hyphal branches called **haustoria.** A pathogen growing on the surface of a plant will at intervals produce hyphae which penetrate the host cell wall and ramify intracellularly as haustoria (Figure 1.5). As the haustorium grows it distends the plasma membrane of the host cell but does not enter the cytoplasm. In response to this invasion the host cell secretes a polysaccharide sheath which lies between the haustorial wall and the host cytoplasm. Haustoria are thus structural adaptations for bringing the fungus into an intimate association with the host cell and for increasing the surface area available for nutrient absorption. Because the haustorium does not penetrate the plasma membrane and thus does not immediately kill the cell, the pathogen can "tap" nutrients from the host for an extended period of time.

The hyphae of certain other fungi are modified into structures used for trapping invertebrate animals, particularly nematodes. Some of these **hyphal traps** are networks of loops and constricting rings that snare a nematode as it attempts to pass through. Figure 1.6 shows the steps in the formation of a constricting ring trap, and Figure 1.7 illustrates the results of the trapping. In this type of trap the inner surface

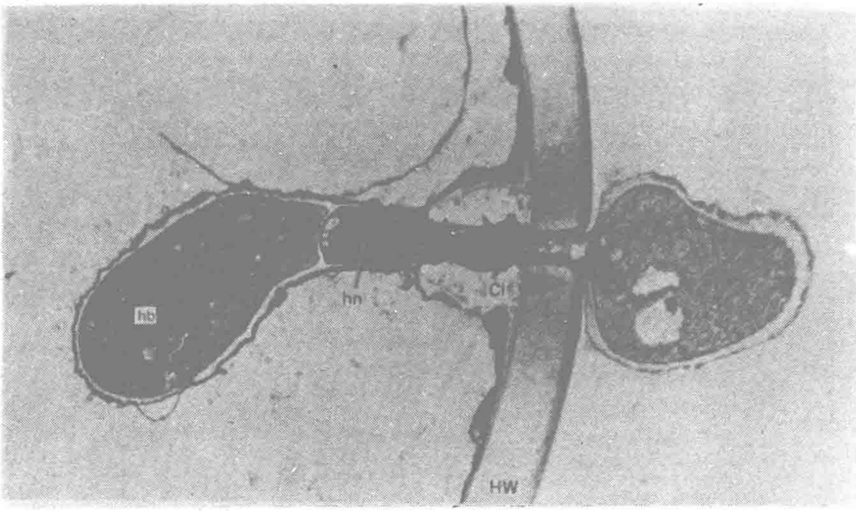

Figure 1.5 Haustoria of *Erysiphe graminis* infecting a wheat leaf epidermal cell. In this transverse section the fungus has penetrated the host cell wall (*HW*) and once inside has formed a haustorial neck (*hn*) and a haustorial body (*hb*) cell. A ring of electron-transparent material associated with the inside of the host cell wall surrounds the proximal end of the neck to form the collar (*Cl*). (Bracker and Littlefield, 1973.)

of the ring is sensitive to touch; the presence of the nematode causes the ring cells to become inflated and prevent the nematode from escapin· Other fungi secrete a sticky fluid from portions of the hyphal strands; when nematodes come into contact with the fluid, they become stuck to the hyphae. Once a nematode is trapped, infection hyphae penetrate the cuticle of the animal and grow through the body, absorbing the contents.

Modifications Associated with Nutrient Transport. Strands and rhizomorphs are aggregations of parallel hyphal strands that aid in the transport of nutrients over relatively long distances. In a strand the hyphae are loosely associated but may fuse at intervals in an anastomosing pattern. Such strands grow in diameter by the addition of hyphae at the base. Rhizomorphs, though similar in appearance to strands, are much more highly organized and grow from an apical meristem in a manner resembling the growth of a plant root. The rhizomorph is thus an autonomous structure that involves a greater degree of coordination among component hyphae than that found in strands. Strands and rhizomorphs are usually subterranean structures that infect roots of higher plants. The rapid rate at which nutrients are transported via these structures enables a pathogen to infect a host at some distance from its original food supply.

Modifications Associated with Survival of Environmental Extremes. At the onset of harsh environmental conditions such as dessication, extremes of temperature, or

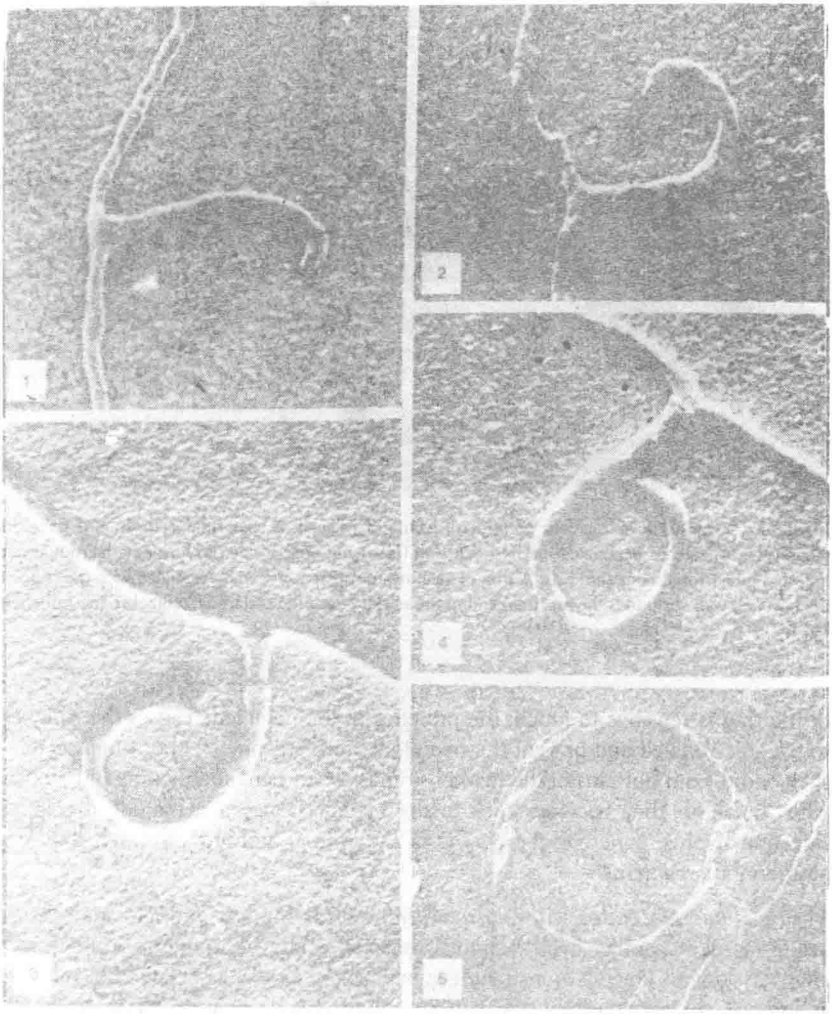

Figure 1.6 SEM micrographs depicting the formation of a constricting ring trap in *Dactylaria brochopaga*. (By permission of Dowsett et al., 1977.)

nutrient deprivation certain fungi form resistant structures such as **chlamydospores** and **sclerotia**. Both structures arise from either terminal or intercalary cells of the hyphal strand. In the formation of chlamydospores, an individual cell enlarges, rounds up, and develops a thick wall. When sclerotia form, one or more cells begin to branch profusely and develop into a tightly interwoven mass of hyphae surrounded by a thick rind. Both chlamydospores and sclerotia contain stored nutrients and may survive for years under unfavorable conditions. Once the environment becomes suitable for growth, germination occurs and new hyphae emerge.

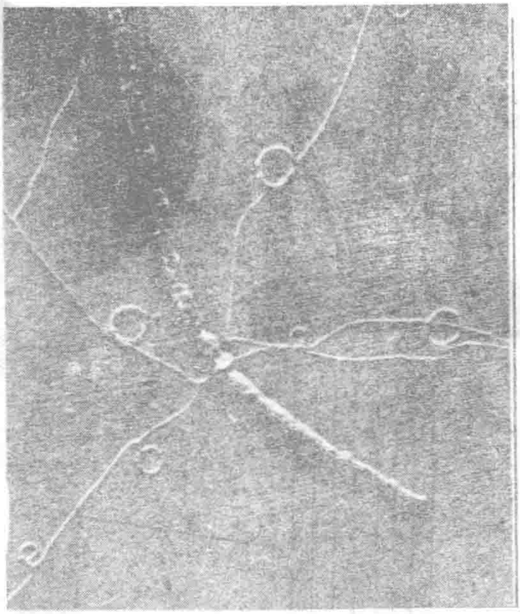

Figure 1.7 SEM micrograph of a nematode trapped by *Dactylaria brochopaga*. Notice the regular arrangement of rings along the vegetative hyphae. ×825. (By permission of Dowsett et al., 1977.)

Modifications Associated with Entry. Frequently when a spore from a plant parasite lands on a host and germinates, a localized swelling called an appressorium develops at the end of the germ tube in response to contact with the host cell. Appressoria secrete a mucilaginous substance that firmly attaches the fungus to the plant. Once this attachment is made, one or more infection hyphae (or infection pegs) arise from the lower surface of the appressorium and penetrate the host cell. The formation of appressoria and infection hyphae are often crucial steps in the establishment of the disease, and factors influencing the development of these structures are of particular interest to plant pathologists.

Modifications Associated with Reproduction. Sexual and asexual spores are frequently produced on an aggregation of mycelial tissue that has a different organization from that of the vegetative mycelium yet does not have a direct role in the reproductive process. The component hyphae of these structures range from loosely woven and poorly differentiated to closely packed and highly differentiated. The simplest of these structures is the stroma (pl. stromata), which is a compact mat of interwoven hyphae. In some species, spores are borne on the surface of the stroma; in others, the spores are embedded within. Figure 1.8 illustrates a stroma of the latter type. Stroma are not only important in reproduction; they may also function as resistant structures for the survival of the fungus during adverse conditions.

A more highly differentiated hyphal aggregation that is analogous to stromatic

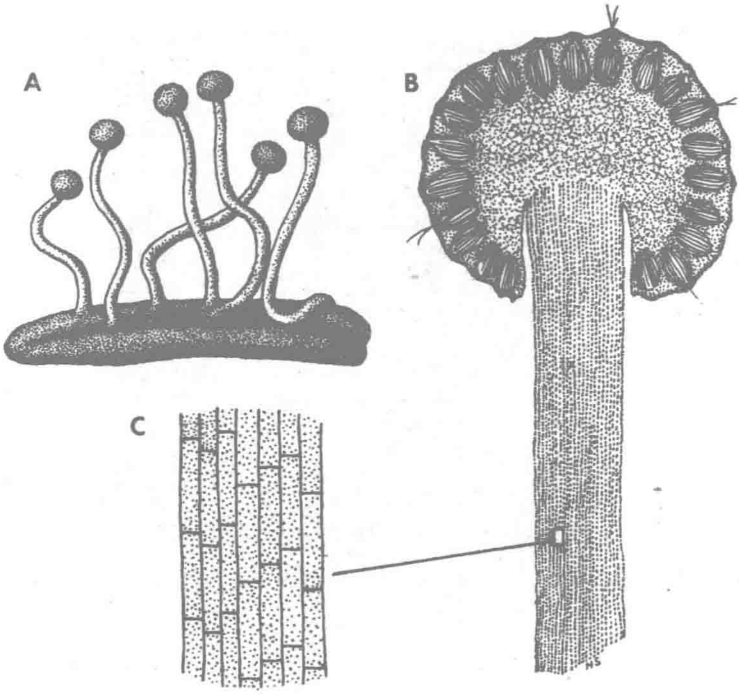

Figure 1.8 Stroma of *Claviceps purpurea*. (A) Several stromata developing from a sclerotium. (B) Longitudinal section through the head and upper portion of the stalk of a stroma. Perithecia lie just beneath the surface of the head. (C) Enlargement of stalk section to show hyphae. (Drawing by H. Stempen.)

tissue is found in the **fruiting bodies** or **fructifications** of certain Ascomycetes and Basidiomycetes. These structures, such as those found in mushrooms, puffballs, and morels, can be quite large and diverse in morphology. As with stroma the vast majority of the tissue in a fruiting body serves to support the actual spore-forming apparatus.

The Fungal Spore

Spores are minute propagating units of various shapes, sizes, and colors, produced by almost all fungi in at least one phase in their life cycle. Spores are usually dispersed by wind, water, or insects or other animals and provide a mechanism for establishing the fungus in new habitats. Although a considerable terminology has built up around the multitude of spore types, in general there are two basic kinds of spores depending on whether they are produced by sexual or asexual means. Sexual spores are formed following the fusion of compatible nuclei from two individuals; asexual spores are formed in the absence of such fusions.

During the fungal life cycle, asexual spores are typically produced in greatest numbers when environmental conditions are favorable and nutrients freely available. Under such conditions several generations of asexual spores may be produced; these spores are usually capable of germinating immediately after being released. For saprophytic fungi these repeating cycles of spore production, dispersal, and germination increase the chances of colonizing a wide variety of substrates. Similar patterns of asexual spore production in plant-parasitic fungi result in repeated cycles of infection that increase the severity of the disease in a particular area and accelerate the spread of the pathogen to other locations.

The production of sexual spores is usually associated with the onset of adverse environmental conditions such as extremes of temperature or the depletion of water or nutrients. Accordingly, sexual spores frequently exhibit adaptations such as thick, resistant cell walls, reserves of lipid and carbohydrate, and dormancy mechanisms which enable them to remain viable until conditions are favorable.

The following is a brief summary of the principal types of fungal spores.

ASEXUAL SPORES

Some spores are formed within a modified fungal cell called a **sporangium** and are thus called **sporangiospores.** Motile sporangiospores are termed **zoospores;** motility is provided by one or two flagella, depending on the species. When zoospores are formed, the multinucleate contents of the sporangium are cleaved into uninucleate portions that develop a surrounding membrane, a cell wall, and finally flagella. Eventually one or more pores develop in the sporangial wall and the zoospores are released. Nonmotile sporangiospores are called **aplanospores** and are also formed by cleavage of the sporangial cytoplasm. These spores may be released through pores or by disintegration of the sporangium. However, some fungi have evolved rather elaborate mechanisms for the dispersal of aplanospores. For example, *Pilobolus* (the "hat-thrower") forcibly ejects the entire sporangium from the underlying stalk or sporangiophore. This process is powered by a squirt of vacuolar sap and can propel the sporangium as far as two meters.

Asexual spores that are not produced in a sporangium are called **conidia**. Conidia are thought to have evolved from a sporangium containing only one spore whose wall is fused to the sporangial wall. Conidia may be formed directly at the surface of a hyphal strand or at the tips of stalks called **conidiophores.** Figure 1.9 illustrates some of the many different types of conidia produced by fungi. In some species the conidiophores are clustered on a stroma forming a **sporodochium;** in others the conidiophores are cemented together resulting in a **synnema** (Figure 1.10). Lastly, conidiophores may occur within discrete fruiting bodies such as a flask-shaped **pycnidium** or a saucer-shaped **acervulus.** The morphology and arrangement of conidia and conidiophores are important taxonomic criteria.

SEXUAL SPORES

In general, sexual spores are those associated with the meiotic divisions that follow the sexual fusion of nuclei. The differences in the morphology and manner of development of sexual spores are the principal criteria for distinguishing the major