AAAAI
2021 Basic Aeroallergen Course

Airborne Fungal Spores

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Introduction to Fungal Spores

The atmosphere contains an incredible variety of fungal spores. To those just beginning the study of aerobiology, the diversity may appear overwhelming. However, after learning the basics about airborne fungi, the air samples can become comprehensible. Part of the basics encompasses understanding spore dispersal; this includes knowledge of the methods of spore formation and discharge, the influence of meteorological conditions, and the diurnal rhythms of discharge. These principles help aerobiologists understand how and when spores are entrained into the atmosphere; however, knowledge of the microscopic features of spore morphology is necessary for spore identification. Spore shape, number of cells, wall thickness, surface ornamentation, attachment scars, and color are all important features for identification. This discussion will start with a brief introduction to the fungi.

The Fungi

The Kingdom Fungi contains a large, diverse group of eukaryotic organisms that include molds, yeasts, mushrooms, bracket fungi, and puffballs. Fungi are a distinct lineage from animals, plants, and bacteria, although molecular data indicate that they are more closely related to animals than the other groups. Nutritionally, fungi are absorptive heterotrophs that exist as saprophytes, symbionts, or pathogens, relying on preformed organic compounds as energy sources. Fungi secrete enzymes into their surroundings and absorb the breakdown products of enzyme action. Fungi may be unicellular such as yeast, but typically have a thread-like or tube-like body composed of hyphae. Hyphae frequently branch resulting in an interconnected network of hyphae called a mycelium. In addition, interwoven hyphae also make up the complex reproductive structures of higher fungi, such as mushrooms and bracket fungi.

Fungi reproduce by spores, and the majority of spore types are adapted for airborne dispersal. They are discharged from the fungus either passively or through active mechanisms. Spores may be produced by asexual processes or may be the products of sexual reproduction. In some fungi, asexual spores are formed enclosed within a sporangium and, therefore, are technically called **sporangiospores**. There may be as many as 10,000 sporangiospores enclosed by the sporangial wall (Fig 1a). When the spores are mature, the wall breaks down and the spores are dispersed by wind. In the majority of fungi the asexual spores are called conidia (sing. conidium), and these do not form within a sporangium. Conidia develop either on undifferentiated hyphae or on specialized branches known as conidiophores (Fig. 1b); however, other conidia develop from fragmentation of ordinary hyphae. On many days, the majority of spores in the atmosphere are asexual conidia formed by fungi growing on leaf surfaces and other substrates (Gregory, 1973; Lacey and Venette, 1995). The types of sexual spores are varied and are described below for various groups.

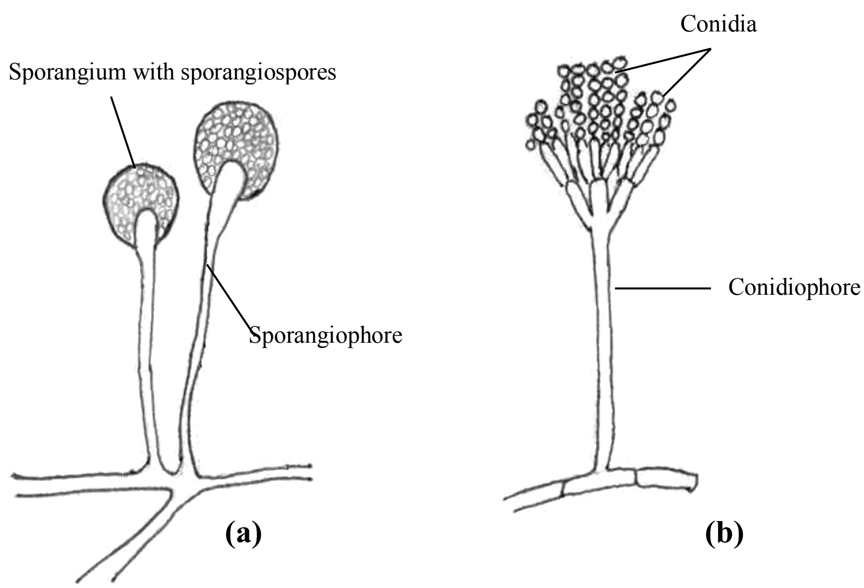


Figure 1. Asexual Spores (a) *Rhizopus* sporangia containing sporangiospores, (b) *Penicillium* conidiophore, a specialized structure bearing chains of conidia.

For centuries the phylogenetic relationship of the organisms in the Kingdom Fungi was unclear. Classification had been based primarily on the morphology of the sexual stage; yet thousands of fungi lacked an obvious sexual stage making accurate classification impossible. Consequently, these fungi were previously assigned to an artificial category called the Deuteromycetes or Fungi Imperfecti. During the last 25 years DNA sequencing has provided a well-documented classification system for fungi. Based on phylogenetic analyses, fungi are now classified into eight phyla. As a result, the artificial category Deuteromycetes is no long recognized and the term should be discarded. In addition, other fungus-like organisms such as slime molds (myxomycetes) and water molds (oomycetes) are now classified in other kingdoms, since it is now recognized that they are not in the main line of fungal evolution.

Methods of sexual reproduction form the basis for classification in the fungi. The two fungal phyla that are most important in aerobiology are the Ascomycota and Basidiomycota. The fungi in these phyla are commonly called **ascomycetes** and **basidiomycetes**, respectively. The **zygomycetes** are another group of fungi that produce airborne spores. Although previously classified as the phylum Zygomycota, molecular data suggest that zygomycetes should be split into two or more separate phyla. Until the evolutionary relationship of these fungi is resolved, we will simply refer to these fungi as zygomycetes.

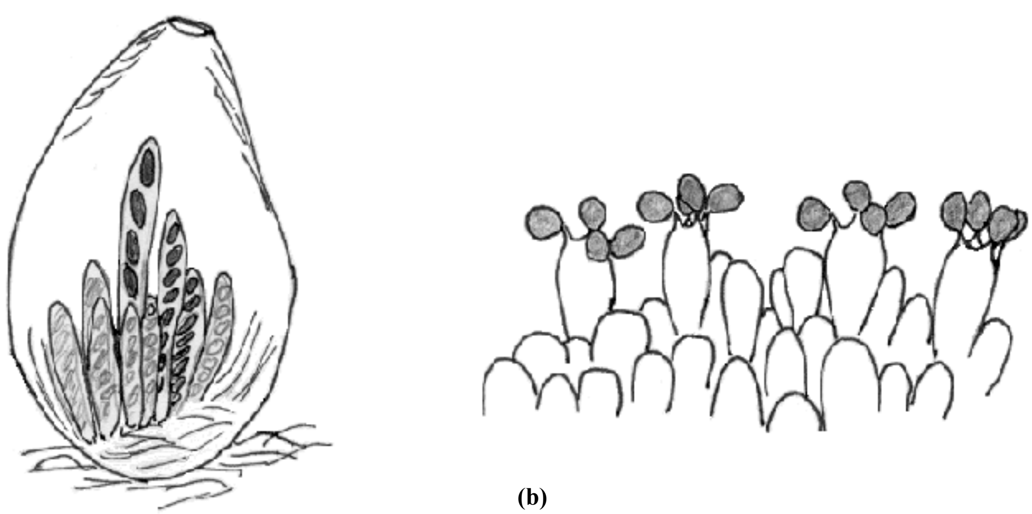
Zygomycetes are a small group of simple fungi with non-septate, multinucleate hyphae. During asexual reproduction, members of this group form sporangia as described above. Sexual reproduction occurs when compatible hyphae, of opposite mating types, meet in the environment and produce zygospores. Zygospores are dormant structures that are able to survive long periods of adverse environmental conditions. These are not usually dispersed from the point of formation.

Ascomycetes and basidiomycetes are often referred to as higher fungi and constitute over 95% of all fungi. The hyphae are septate, and the cells are initially uninucleate. The fusion of compatible hyphae early in the life cycle results in binucleate cells; however, the union of the two nuclei is delayed.

The Phylum Ascomycota (ascomycetes) is the largest group of fungi with recent estimates indicating over 90,000 species. Sexual reproduction in this phylum results in the development of sac-like structures called asci. Within each **ascus**, the compatible nuclei fuse producing a diploid zygote. The zygote undergoes meiosis followed by mitosis resulting in 8 haploid **ascospores** within the ascus. Asci typically form within fruiting bodies. The fruiting bodies of most ascomycetes are small; in fact, many are microscopic (Fig 2a). By contrast, other species form large fleshy fruit bodies, such as morels. Ascomycetes also reproduce asexually by forming conidia. There are frequently many generations bearing conidia during the life cycle of many ascomycetes; however, the sexual stage may occur infrequently or only once.

These **asexual stages** of ascomycetes (also called **anamorphic stages**) constitute a large and familiar group of fungi. As indicated above, many fungi were previously considered members of the deuteromycetes or fungi imperfecti when their sexual stages were not obvious. **These categories are no longer valid and should be eliminated**. DNA sequencing has allowed mycologists to place these fungi in the correct ascomycete families, although a small percent are basidiomycetes. The asexual stages of these fungi are very successful, and the majority of microfungi in the environment are asexual stages of ascomycetes.

The Phylum Basidiomycota (basidiomycetes) includes about 50,000 species based on recent estimates. Sexual reproduction results in the development of club-like structures called basidia. Within each **basidium**, the two compatible nuclei fuse producing a zygote that immediately undergoes meiosis. The four haploid nuclei resulting from meiosis enter developing **basidiospores**, which are produced externally by each basidium (Fig 2b). Most basidiomycetes form large conspicuous fruiting bodies. Basidia line the gills of mushrooms and the pores of bracket fungi. In puffballs, the basidia and basidiospores form within an enclosing wall of the fruiting body. Some basidiomycetes also reproduce asexually by forming conidia.



(a) **(b)**

Figure 2. Sexual spores. (a) Ascomycete fruiting body with asci. Eight ascospores are produced within each ascus. (b) Portion of a basidiomycete fruiting body with four mature basidia. Each basidium produces four external basidiospores at specialized attachment points.

Two important groups of basidiomycetes lack fruiting bodies; these are the rust fungi and the smut fungi, which are important plant pathogens worldwide. These pathogens form specialized asexual spores known as uredospores and teliospores.

Two additional groups of fungal-like organisms to be aware of are the Phylum **Myxomycota** (the slime molds or **myxomycetes**) and the Phylum Oomycota (the water molds or **oomycetes**). Slime molds have an animal-like vegetative stage that consists of a plasmodium, which slowly creeps over substrates engulfing bacteria or other unicells. The reproductive phase of these organisms is fungal-like and consists of sporangia or similar fruiting bodies. The dry, powdery spores produced by myxomycetes are readily airborne and often seen on spore-trap slides. Many oomycetes are true water molds and occur in bodies of water as saprobes of fish pathogens. However, other oomycetes are important plant pathogens and are found on land. Many of these produce sporangia that become airborne. Although these two groups of organisms appear fungal-like, they are not in the main line of fungal evolution. Because they may produce airborne spores, we need to be aware of them.

Spore Discharge Methods - Passive Discharge

Passive mechanisms that entrain spores into the atmosphere include dispersal by wind and rain, with wind dispersal the best understood method (Aylor, 1990; Lacey, 1996; Deacon, 1997). The most abundant airborne spores in temperate areas of the world are wind-dispersed. For many of these spores, the atmospheric concentration depends upon the ease with which the spores are detached from the parent hyphae or spore-bearing structure. Many wind-dispersed spores are borne on erect sporangiophores or conidiophores that elevate the spores above the substrate.

Warm dry weather conditions promote passive dispersal, and the entrained spores are typically referred to as the ***dry air spora***. Components of the dry air spora include conidia of *Cladosporium*, *Alternaria*, *Drechslera, Curvularia, Pithomyces, Epicoccum*, *Botrytis,* smut teliospores, and rust uredospores (Fig. 3). Diurnal levels of these spores usually have peaks during the afternoon hours under conditions of low humidity and maximum wind speeds and low points during the still, humid conditions of early morning (Hamilton, 1959; Gregory, 1973). Members of the dry air spora commonly have thick, pigmented walls, which protect the spores from desiccation and UV-light. Minimum wind speeds needed to entrain spores into the atmosphere range from 0.2 and 2.0 m/sec (Lacey, 1996; Hau and de Vallavielle-Pope, 1998). Wind gusts promote dispersal even when average wind speeds are too low to accomplish this.

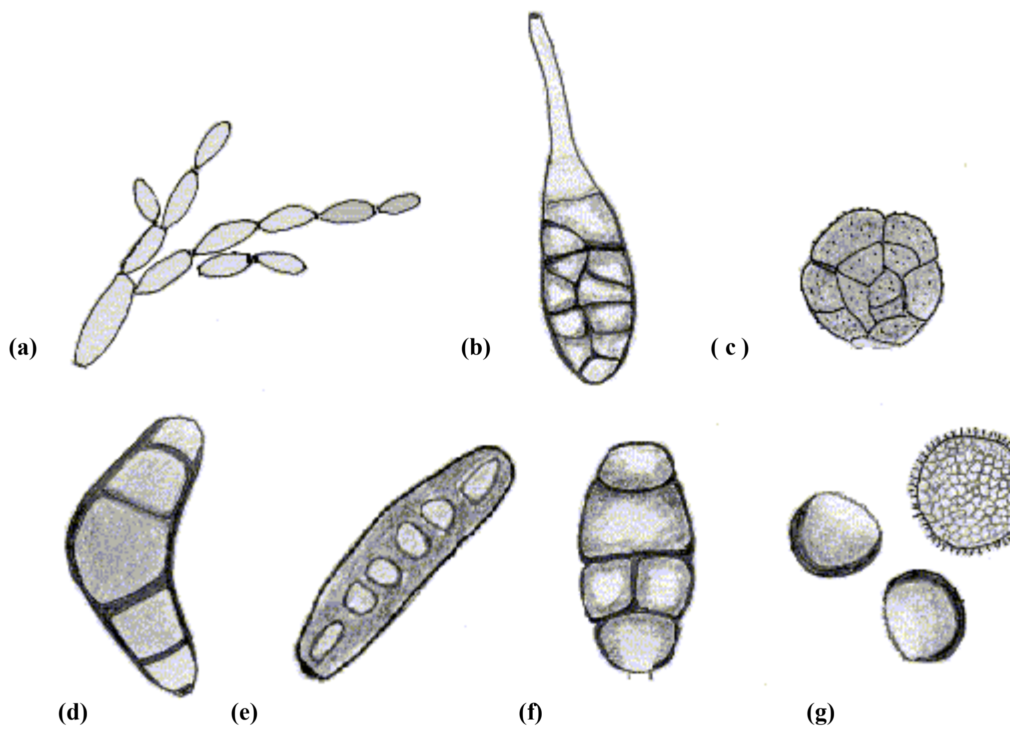


Figure 3.Dry Air Spora. (a) *Cladosporium* (b) *Alternaria* (c) *Epicoccum* (d) *Curvularia* (e) *Drechslera* (f) *Pithomyces* (g) Smut teliospores (two different species)

Spores are also passively discharged by rain through several different mechanisms. Members of the dry air spora may be dispersed by raindrops striking a substrate with fungi growing on the surface. The vibration and shaking caused by the raindrops propel the spores into the air. This mainly occurs with the first drops of precipitation and may explain the increased concentrations of *Cladosporium* and *Alternaria* reported during some rain events (Hirst, 1953; Venables, 1997). Release of basidiospores by puffballs occurs by a similar puffing mechanism when a raindrop strikes the peridium of a mature fruiting body.

Splash dispersal occurs in some fungi. Splash-borne spores are usually surrounded by a mucilage layer, which protects the spores from desiccation but also prevents their removal by wind. Raindrops dissolve the mucilage and leave a spore suspension free for subsequent dispersal by additional raindrops. Overhead irrigation can also disperse fungal spores through this mechanism. Splash dispersed spores typically have thin, colorless walls and elongate shapes, such as *Fusarium* conidia. Splash dispersal is second to wind in importance for the dispersal of fungi that are important plant pathogens, but it is only significant for local dispersal (Fitt et al, 1989). Raindrops can also be efficient spore collectors and even a light rain can scavenge some spores from the atmosphere.

Spore Discharge Methods - Active Discharge

Active mechanisms that propel spores into the atmosphere are common in fungi and the ballistics of some species are quite spectacular. In many ascomycetes, the explosive release of ascospores from the ascus is tied to atmospheric moisture. Hygroscopic material within the ascus absorbs available moisture causing the ascus to swell and develop high osmotic pressure. The pressure induces the ascus to burst, explosively shooting the spores into the turbulent layer of the atmosphere. The need for atmospheric moisture generally restricts dispersal to periods during and after rainfall or times of high humidity. The response to moisture varies among different species: (1) some releasing spores within minutes after the onset of rain; (2) other species require a longer period of rain; (3) others are not released until the fruiting body begins to dry; and (4) in others rain is not required, sufficient moisture is obtained from dew. Those species that obtain moisture from dew typically show peak concentrations in early morning hours. Although no obvious diurnal rhythm is apparent in species requiring rain, some taxa release spores only in daylight while others release spores only during darkness (Ingold, 1971).

The active release of basidiospores from mushrooms and bracket fungi also depends on moisture through a unique mechanism described as a surface tension catapult. Each basidiospore is attached to the basidium by a hilar appendage, and moisture from the atmosphere condenses around sugars excreted by the mature spore at the hilar appendage

(Webster et al, 1989; Money, 1998). The drop of fluid, known as Buller's drop, continues to enlarge until it fuses with a thin film of moisture that surrounds the spore. This suddenly shifts the center of gravity and results in the spore being shot from the basidium. Although this mechanism only ejects the spores a fraction of a millimeter, the spores are released from the gills (or pores) and are able to reach the free atmosphere. The moisture requirement confines basidiospore discharge to periods of high humidity, and they typically have daily peak concentrations during pre-dawn hours with lower levels during the afternoon (Hirst, 1953; Hamilton, 1959; McCracken, 1972; Craig and Levetin, 2000). However, Haard and Kramer (1970) found three patterns of basidiospore discharge. In addition to species with a pre-dawn peak, certain species exhibited a double peak, in the early morning and again in the evening. A third pattern was found in some small mushrooms, which show continuous spore release with the peak 24 to 48 hours after the beginning of spore release. Because of the need for atmospheric moisture, both the ascospores and basidiospores can be referred to as members of the **moist air spora** (Fig. 4). It should be remembered that certain rain-splash fungi might also occur in the moist air spora.

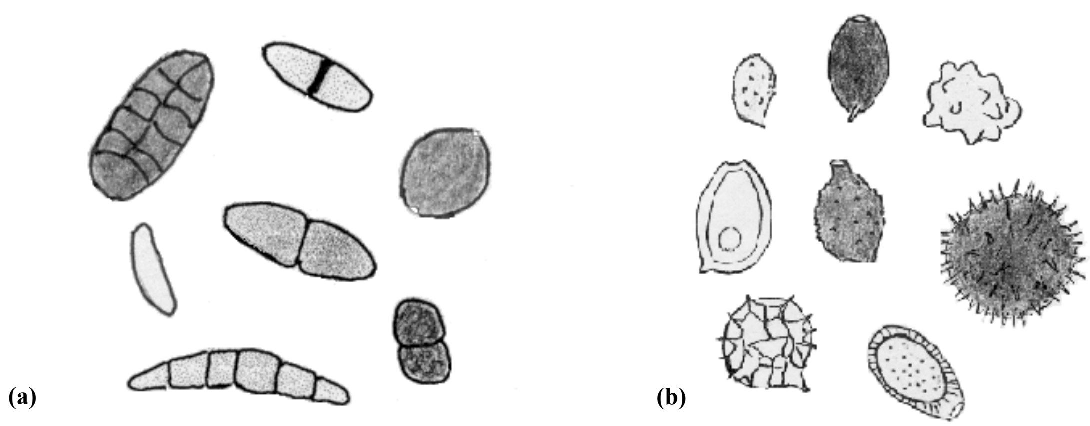


Figure 4. Moist Air Spora. Both ascospores and basidiospores are commonly found in moist air. (a) Various ascospores. (b) Various basidiospores.

Indoor Aeromycology

The air in most indoor locations also contains fungal spores, and the outdoors is typically the origin of these spores. Anytime that outdoor air is introduced, spores entrained in the air will be introduced as well. Air filters in central heating, ventilation, and air condition (HVAC) systems will reduce the number of spores in the ventilation system but will not eliminate all spores (Levetin, 1995). Spores can enter (1) when doors or windows are open; (2) through tiny cracks in the building; and (3) on the person or clothing of building occupants or visitors. In addition, many fungi can grow indoors, and the spore levels can, therefore, be amplified indoors. Any time moisture is available, spores can germinate and fungi can grow on many indoor substrates with the production of new generations of spores. While many fungi are able to grow, *Penicillium*, *Aspergillus*, and *Cladosporium* are typically the most common genera identified. In buildings with water damage and extensive fungal contamination, there has been a great deal of concern and research focused on the possible health effects of exposure to the spores and to any mycotoxins that may be present.

Spore Morphology

A tremendous range of spore morphology is seen in air samples. Spore size, shape, number of cells, wall thickness, color, surface ornamentation, and attachment scars are features used in the identification of spores.

Size: Spores range from small spores that are 2 to 3 µm in diameter to greatly elongated spores that are over 100 µm in length. A great many spores are in the size range of about 5 to 15 µm. Although size is a helpful characteristic in identification, it must be remembered there may be variability in spore size even from a single fruiting body.

Shape: Many spores are spherical, oval, or elliptical; however, other spore shapes are common. Some spores are curved, coiled, barrel-shaped, cylindrical, fusiform, or filiform (thread-like). Others like *Alternaria* are club-shaped, while some have an irregular shape that is difficult to describe. Spore shape may also be modified by appendages. Conidia of *Pestalotia* and *Tetraploa* are two spore types with prominent appendages

Number of Cells: Many spores are unicellular; these are sometimes called non-septate. Other are characteristically composed of two, three, or many cells. The cross-walls (septa), which divide the spore into cells, may be only transverse (such as *Cladosporium*) or both transverse and longitudinal (such as *Pithomyces* and *Alternaria*), or irregular (such as *Epicoccum*). The number of cells can sometimes be a useful characteristic for identification. Sporangiospores from the zygomycetes, basidiospores, and smut teliospores are all unicellular spores. By contrast both ascospores and asexual spores are tremendously variable ranging from unicellular to multicellular types. Also, some conidia like *Cladosporium* may have one, two, or three cells.

Spore Wall Characteristics: Wall features useful for identification include, color, thickness, ornamentation, and apertures. Spores may be colorless (often referred to as hyaline) or pigmented. Spore color often ranges from pale yellow, to golden, tan, brown, or even black. Other colors such as gray, green, and even blue-green are far less common. Colors can be variable and gradations in color are common from a single specimen or culture. In addition, colors may be influenced by the mounting medium and microscope light source.

Some spores have characteristically thin or thick walls. Myxomycete spores are typically thin-walled and may even show signs of having collapsed when caught in spore traps. Smut spores usually have thick wall, although some smut species have unevenly thickened spore walls with a thin wall area on one side. The thin wall area usually has differences in pigmentation and ornamentation as well. For other groups of fungi, wall thickness varies with the species; however, many colorless spores are frequently thin-walled.

Spores may have smooth outer walls or various types of ornamentation. The surface of ornamented spores may appear spiny, punctate, warted, striated, ridged, or reticulate. Examination of the ornamentation often requires careful focusing with an oil immersion objective lens (1000x magnification). Although the ornamentation normally occurs on the outermost surface, basidiospores of *Ganoderma* spp. have an unusual type of ornamentation. These spores have a transparent outer wall and a golden inner wall with interwall pillars connecting the two layers.

The spore wall may be interrupted by various types of apertures; the most common are germ pores. Germ pores commonly occur in many basidiospores, and several families of mushrooms are characterized by spores with prominent germ pores. Some rust spores also contain germ pores, and a few genera of ascomycetes have ascospores with germ pores. *Chaetomium* is one of the ascospores with a pore. Other ascospores are characterized by germ slits. Slits also occur on some conidia, such as those of *Arthrinium.*

Attachments: The presence or absence of attachment structures is an important feature in identification. Since conidia form externally on a hypha or conidiophore, they frequently display prominent attachment scars when they separate. Some spores that form in chains (or branched chains) show two or more attachment scars, at opposite ends of the spore. Multiple attachment scars are common on *Cladosporium* conidia, while other spores typically show only one two attachment scars when carefully examined at 1000X magnification. Attachment scars in *Alternaria* are particularly noticeable at the end of the beak. Both *Pithomyces* and *Epicoccum* display colorless attachment structures at the base of the spores.

Basidiospores usually display a prominent attachment peg, termed a hilar appendage (also called an apiculus), which is asymmetrically placed at the base of the spore. The hilar appendage is especially prominent in some genera, such as *Chlorophyllum, Russula*, and *Conocybe,* and barely visible in others. By contrast, ascospores, which are produced within the protoplasm of the ascus, never show an attachment peg or scar.

The features described here are all useful for identification. To further assist in spore classification, students in aerobiology are referred to a number of useful keys, books, and articles (Table 1). Some of these references have excellent color photos, while others have detailed line drawings. In addition to this literature, a set of permanent reference slides is a valuable asset for the beginner. These can be prepared from known cultures and fresh specimens. Suitable mounting media for making permanent slides are lactophenol with polyvinyl alcohol (Levetin, 1989) or Gelvatol. Both mounting media provide clear backgrounds and permit the study of surface features.

Summary

The topics presented will help understand the basics of fungal aerobiology. Knowledge of spore morphology and spore dispersal will permit participants to become familiar with the major components of the air spora and when these spores are typically found in the atmosphere. Insight into the methods of spore discharge will help provide a general understanding of the influence of environmental conditions on airborne concentrations. However, research is still needed on many aspects of fungal biology to fully understand the effects of meteorological conditions on dispersal, transport, and survival in the atmosphere. In addition, a tremendous variety of spores occurs in the atmosphere, and only the major categories have been described here. Within each group, there are individual differences in the way particular species respond to the environment. While many airborne spores originate from saprobic fungi, spores of plant pathogens are often a significant component of the air spora. Fully understanding the aerobiology of these pathogens will require knowledge of the interaction between the pathogens and host plants. Research is also needed in the clinical area to understand the risk and circumstances of sensitization to allergenic species.

Table 1. Useful Reference Material for the Identification of Airborne Spores

Spore Type	Author	Title
All	Smith (1990)	Sampling and Identifying Allergenic Pollens and Molds
	Ellis and Ellis (1985)	Microfungi on Land Plants
	Ellis and Ellis (1988)	Microfungi on Miscellaneous Substrates
	Lacey and West (2006)	The Air Spora
	Wang and Zabel (1990)	Identification Manual for Fungi from Utility Poles in the Eastern United States
Asexual Spores	Barnett & Hunter (1998)	Illustrated Genera of Imperfect Fungi
	Ellis (1971)	Dematiaceous Hyphomycetes
	Ellis (1976)	More Dematiaceous Hyphomycetes
Ascospores	Hanlin (1990)	Illustrated Genera of Ascomycetes, Vol I
	Hanlin (1998)	Illustrated Genera of Ascomycetes, Vol II
	Allitt (1986)	Identity of Airborne Hyaline, One-Septate Ascospores and Their Relation to Inhalant Allergy
Basidiospores	Pegler & Young (1971)	Basidiospore Morphology in the Agaricales
	Levetin (1991)	Identification and Concentration of Airborne Basidiospores
Smut spores	Vanky (1987)	Illustrated Genera of Smut Fungi
	Crotzer & Levetin (1996)	The Aerobiological Significance of Smut Spores in Tulsa, Oklahoma

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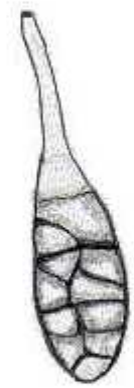
Common Spore Types

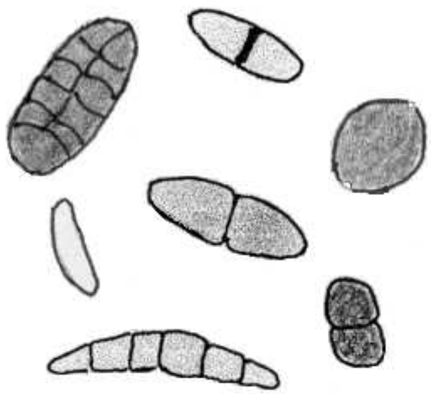
Alternaria - *Alternaria* conidia are frequently the second most abundant component of the dry air spora. The genus is characterized by very distinctive large multicellular spores, which are beaked and produced in chains. Septa (cross walls) are both transverse and longitudinal, although young spores may have only transverse septa. Prominent attachment scars are visible at the apical end (tip of the beak). Spores are deeply pigmented in various shades of brown. Spore sizes vary among species from 7 µm x 18 µm to 15 µm x 75 µm.



Alternaria spores commonly are found in atmospheric concentrations of several hundred to several thousand spores/m^3. In some areas these asexual spores may be present throughout the year; however, peak concentrations usually occur in late summer or fall. *Alternaria* conidia are passively released from infected leaves or other substrates by moderate to strong gusty winds. Dispersal typically occurs during the afternoon when conditions are warm and dry with high wind speeds. High humidity inhibits the release of spores from wet leaves and airborne spores are washed from the atmosphere by rain and irrigation. *Alternaria* species also occur on various indoor substrates.

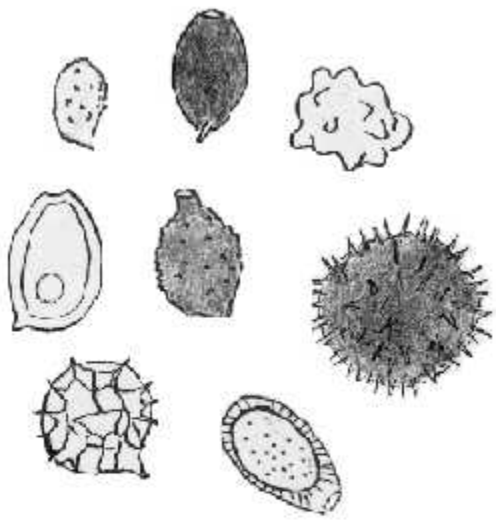
Species of *Alternaria* are pathogens on a number of crop plants, causing early blight, black spots, brown spots, seedling blight, black rots, or leaf spot diseases. They also occur as saprobes on a large variety of organic substrates including leaf surfaces, stored foods, soil, textiles, and indoor substrates. This genus is also prominent in the aerobiological literature because it is recognized as an important airborne allergen. Literature shows an association between *Alternaria* sensitivity and severe asthma.

Ascospores - Ascospores are sexual spores produced by ascomycete fungi. Spores vary enormously in size, shape, color, and wall features. Ascospores may be single celled without any internal septa, two-celled with a single septum, or multi-cellular with many septa. In the multicellular spores, septa may be only transverse or both transverse and longitudinal. Size varies from less than 5 μm in length for some of the small unicellular spores to over 100 μm for some of the large multicellular spores. Color ranges from completely colorless to dark brown and black spores. A transparent mucilaginous layer surrounds some ascospores. Spores may be smooth or ornamented, and germ pores or slits may be present. One common feature is the lack of any attachment scar due to the fact that they are produced within an ascus.

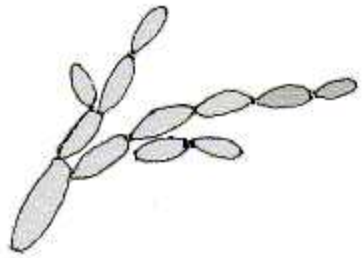


Because many ascospores are released from the ascus through an active mechanism that requires moisture, they are frequently seen in air samples collected during rainy periods. However, some ascospores require less moisture and can be found in the atmosphere during early morning hours or other periods of high humidity. Usually the eight ascospores within an ascus are released at one time. On occasion, the eight ascospores from a single ascus can be caught on an air sample still close to each other. Although generally we associate ascospores with the outdoor air spora, *Chaetomium* is frequently seen indoors on wet cellulose materials. *Chaetomium* has brown, lemon-shaped ascospores with germ pores on both ends.

Basidiospores: Basidiospores are the sexual spores produced by basidiomycetes including mushrooms, bracket fungi, and puffballs. Basidiospores show a wide range in size, shape, structure and color; however, they are always single-celled and are frequently small, in the range of 5-12 μm. The overall shape of basidiospores can be globose,



elliptical, fusiform, nodulose, angular, or irregular. In addition, the basidiospores of many mushrooms and bracket fungi are asymmetrical due to the presence of a hilar appendage, which attaches the spore to the basidium. This attachment structure can be distinct or indistinct. Spore walls may be smooth or ornamented with spines, warts, or ridges. An apical pore is a common feature on many basidiospores and, when present, is generally visible in the light microscope. Basidiospore colors vary from completely colorless to various shades of yellow, golden brown, light brown, and dark brown to nearly black. Some basidiospores, such as those in the genus *Ganoderma*, have a transparent outer wall layer, while the inner wall layer is golden brown with spines. Wall thickness also varies considerably among different species. Some thin-walled basidiospores may collapse when dehydrated, while thick-walled species retain their shape. Since basidiospores are released from the basidium by an active mechanism that requires moisture, they are often most abundant in the pre-dawn hours when the humidity is high.

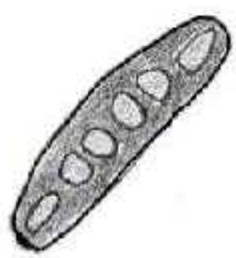


Cladosporium - *Cladosporium* is a large genus of asexual ascomycetes with a wide range in spore size and shape. Spores are typically ellipsoidal to cylindrical in colors from pale yellow to light brown. Spores are produced in chains and may be unicellular or have one or two septa. Spore surface varies from smooth in some species to warty in others. Spore size varies. *Cladosporium sphaerospermum* and *C. cladosporoides* have small spores that range from 3-4.5 µm in diameter for the former to 3-7 x 2-4 µm, for the latter; while the large spores of *C. macrocarpum* average 15-25 µm x 7-10 µm. Spores are characterized by very prominent scars where adjacent spores were attached.

Conidia of *Cladosporium* species are generally the most abundant airborne spore type in temperate areas of the world, and spores are considered important aeroallergens. *Cladosporium* species are common saprobes on leaf surfaces, dead herbaceous and woody plants, soil, foods, fabrics, and paint. These fungi are also common indoors and are often the most abundant indoor spore type. Several species are plant pathogens causing various diseases such as leaf spots, scabs, and fruit rots.

In many areas airborne *Cladosporium* spores can be detected year-round with the highest levels occurring from late spring though early fall. Hourly spore concentrations can exceed 100,000 spores/m^3 in some areas. Atmospheric concentrations of *Cladosporium* spores typically peak in the afternoon. Although generally considered a member of the dry air spora various researchers have also described dispersal by mist droplets in this genus. In addition, studies have shown transient increases in concentration after the start of rainfall.

Curvularia – *Curvularia* is a genus of asexual fungi with distinctive spores; conidia have three or more transverse septa and are typically curved due to an enlarged central cell. Spore color varies from tan to dark brown; the end cells are frequently lighter in color, while the septa appear very dark. The attachment scar at the base is often protuberant. Spore size varies among species from 25 μm to over 50 μm in length. Spore surface is smooth in most species. *Curvularia* is responsible for a number of leaf spot diseases and is a common component of the dry air spora. Spores are allergenic and have been found to cause fungal sinusitis.

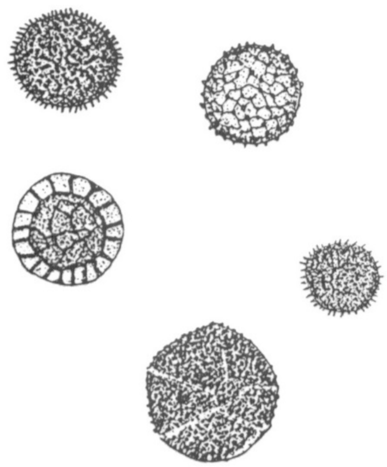


Drechslera/Helminthosporium-type spores - Several genera of asexual ascomycetes form similar thick-walled, cylindrical conidia with rounded ends and a distinctive attachment scar at the base. Conidia either have transverse septa or are pseudoseptate. Spores vary in size and shape with some being very long (up to 160 μm) and tapering, while others are relatively short (10 x 25 μm). Spore color varies from light to dark brown, and the spore surface is usually smooth.

For many years, fungi with this type spore were classified as species of *Helminthosporium*; however, in the past 20 years the taxonomy in this group has undergone revision and several genera are now recognized. The best-known genera in this group are *Helminthosporium, Drechslera, Bipolaris*, and *Exserohilum.* Genera in this group are delimited by the shape of the conidium, morphology of the attachment scar, and the number and location of germ tubes produced by the conidium. Mycologists and plant pathologists acknowledge the separation of these genera, but this is not universally adopted in the allergy community. Some books, journals, and extract companies still use *Helminthosporium* and others use *Drechslera* for all.

This group of fungi is known to cause leaf spots and blights and other diseases on wild and cultivated members of the Poaceae; they are especially important pathogens on cereal crops. In addition to their role as plant pathogens, members of this group are allergenic and are a leading cause of fungal sinusitis. In addition, they are capable of causing fungal infections in livestock and some are toxigenic. Conidia from these fungi are common in the atmosphere as members of the dry air spora.

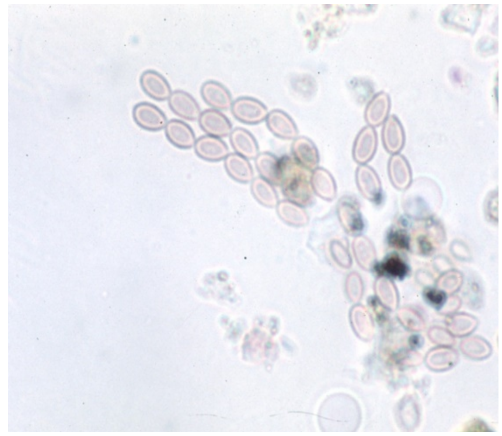
Epicoccum - *Epicoccum* is a genus of asexual ascomycetes common on leaf surfaces and decaying vegetation. Conidia are subglobose to pyriform (pear-shaped) and dark brown. The multicellular spores have both transverse and oblique septa; however, the dark color often makes the septa difficult to see. The spore surface is warty and a broad colorless attachment area may be visible at the base. Spores are typically 15 to 25 μm in diameter, although larger spores often occur. Spores are produced in dense clusters known as sporodochia; however, they occur singly in air samples. Atmospheric concentrations are usually low, but during crop-harvesting levels may become abundant as the conidia are shaken from leaf surfaces. In culture, *Epicoccum* produces a characteristic orange to red pigment that diffuses freely into the agar. Spores are known to be allergenic.



Myxomycete Spores – The myxomycetes, or slime molds, are a group of organisms, which are commonly found on decaying wood, on other organic substrates or on the soil. Myxomycete spores are often seen in air samples. Spores are typically small, single-celled, and globose. The thin-walled spores frequently show evidence of partial collapse. Spores are lightly to darkly pigmented in shades of gray, and the spore wall is ornamented. Sizes range from 8 to 20 µm in diameter. These may be difficult to distinguish from smut spores.

Nigrospora – *Nigrospora* is an asexual fungus that is a saprobe on decaying vegetation. I can also be a pathogen on various grasses and is especially common in warm climates. The distinctive black spores appear spherical, but are actually flattened. Spores are usually 10 to 20 µm in diameter although larger sizes also occur in some species. This genus is common, although usually not abundant on air samples.

Penicillium/Aspergillus – Both *Penicillium* and *Aspergillus* species are very common asexual ascomycete genera, especially in indoor environments. In nature, these fungi typically occur in the soil and the spores are often present in outdoor air samples. Both genera are large with approximately 250 species of *Penicillium* and 150 species of *Aspergillus*. The spores of these fungi are well known allergens. In addition, some *Aspergillus* species can cause infections in immune-suppressed individuals. Many species of *Aspergillus* and even some species of *Penicillium* produce mycotoxins.



Spores produced by both genera are similar; they are globose to subglobose and generally 3 to 6 µm. The colorless or pigmented spores may have smooth or ornamented walls. Spores are produced in chains and have attachments on opposite ends. The two genera can be distinguished in culture but not on spore trap slides. Occasionally chains of spores are visible on air samples.

Pithomyces - *Pithomyces* is a genus of common saprobic fungi that is prevalent on decaying vegetation especially grasses. It is occasionally found indoors as well. Spores of this asexual fungus are produced individual on short conidiophores. The multicellular spores are oval to barrel-shaped with both transverse and longitudinal septa. Spores are dark brown and the spore surface is usually smooth although warty spores are sometimes seen. When the spores are released part of the colorless conidiophore is broken off; often this structure can be faintly seen at the base of the spore.

Rust Spores: Rust fungi are basidiomycete plant pathogens that belong to the order Urediniales. Although these are basidiomycetes, there is no fruiting body produced. There are about 6,000 species of rust fungi that attack a wide range of plants and cause destructive diseases, especially on cereal crops. Many rust fungi have complex life cycles with as many as five different spore types produced. Probably the most important spore stage is the uredial stage, which typically causes reddish (rust-colored) lesions on plants. The common name of these *rust* fungi comes from these reddish lesions. The spores produced in these lesions are called uredospores (also called urediospores or urediniospores in some books). These spores are produced in enormous numbers (up to 10,000 spores per lesion each day) and are easily airborne. This is the repeating stage, rapidly spreading the infection from one plant to another within a crop.

Uredospores can be found in air samples in most parts of the country. Some of the early aerobiological studies in the U.S. focused on the movement of uredospores that cause wheat rust. Uredospores are typically large (commonly 25-35 x 15-20 μm) oval spores. The cytoplasm may show intense yellow pigmentation in some species, but is colorless in others. The outer wall is typically spiny. Teliospores are another type of spore produced by rust fungi. Teliospores are often formed in late summer or fall and can also be seen in air samples. Teliospores are very-thick walled spores that may have one or two cells and are usually more deeply pigmented.

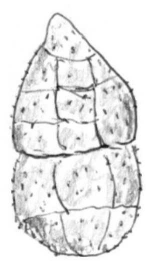
Smut spores: Smut fungi are basidiomycete plant pathogens in the order Ustilaginales; the common name relates to the black, dusty spore masses on the plants they infect. There are approximately 1,200 species of smuts within 50 genera. The majority of species are classified into two large genera, *Ustilago* and *Tilletia*. The characteristic asexual spores produced by these fungi are teliospores, which are readily dispersed by wind. In addition to the significance of these spores as plant pathogens, smut teliospores are also allergenic.

As members of the "dry air spora," low humidity and gusty winds promote spore dispersal. Levels decrease during rain or high humidity and increase during periods of peak sunshine and high barometric pressure. Occurrence of smuts is not uniform during the growing season but is often seasonal and related to the phenology (life cycle) of the host plant. Some smut fungi release spores when the host plant pollinates; however, in other smuts, spores are released when the host plants are harvested.

Smut teliospores are typically globose with smooth, echinate, or reticulate sculpted walls with yellow to brown pigmentation. *Ustilago* spores range in size from 3-4 μm up to 10-11 μm. In contrast, the spores of the other genera are often larger, ranging up to 22-24 μm.

The spore walls of a number of smut species have wall areas, which appear thinner and less pigmented using light microscopy. For some species, the surface morphology of the thin area also appears different from the rest of the spore. Many spores may show signs of collapse around these thinner areas, thereby changing the shape of the spore from globose to subglobose.

Stachybotrys - *Stachybotrys* is a soil fungus in the natural environment. It is commonly found indoors on wet materials containing cellulose, such as wallboard, jute, wicker, straw baskets, and paper materials. It is allergenic although little research has been done on the allergens. However, some isolates of *Stachybotrys* produce potent mycotoxins. There has been a great deal of controversy and publicity about the health effects caused by this fungus. Spores are oval with prominent warts warty. Spore color varies from dark brown to gray or black.



Stemphylium - *Stemphylium* occurs on decaying and living plants and in the soil. The genus is characterized by large, multicellular dark brown spores, which are divided by longitudinal and transverse septa. Spores are typically constricted in the middle and 30 to 40 µm long. A short beak may be present, and the walls are smooth or finely ornamented.

Torula – *Torula* is saprophyte found on soil, decaying vegetation, and wood. The spores are multicellular with only transverse septa and typically around 30 µm long. The spore has constrictions at each septum giving a spore the appearance of a short chain. Spores are dark brown and the surface has warts or spines. The end cell may collapse.