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Hello EMLab,

I hope you're doing well and will find the attached articles both interesting and useful. The first is about how heat effects fungi by Dr. Harriet Burge and the second is about the fungi *Bipolaris* by Dr. Michelle Seidl.

With best wishes,
Dave Gallup

How Does Heat Affect Fungi?

By Dr. Harriet Burge

The effects of heat on fungi depend on many factors, including the genus, species and strain of the fungus, the amount of available water, kinds of nutrients, and many other environmental factors. Of course, temperature is also a crucial factor. Most of the research on temperature relationships for the fungi has been done in the food industry where heat is commonly used to prevent fungal growth. Much of this research involves wet heat, which is more effective than dry heat.

Fungi can be divided into groups according to temperature requirements for optimal growth. Note that these requirements are dependent on water availability and nutrients, and are measured under carefully controlled conditions. These same terms are used for bacteria, but the temperature ranges differ.

The groups are as follows:

1. Psychrophiles: with optima less than 10°C
2. Mesophiles: with optima in the room temperature range (18-22°C)
3. Thermophiles: with optima at or above 37°C

There are also categories defining the ability of the fungi to withstand different temperature regimes. In this case, we have psychrotolerant and thermotolerant fungi, indicating that growth can occur at either low or high temperatures, but is not optimal. Thus, some mesophilic fungi may be able to grow or at least survive at either low or high temperatures, depending on the genetics of the strain and other environmental conditions. Another form of temperature tolerance lies in the spores, which can often withstand temperature extremes, and germinate when conditions return to normal. Finally, some fungi that are normally mesophilic have spores that require heat to stimulate germination. Temperature tolerance is strongly tied to the amount of water so that wet heat is much more effective at damaging spores than dry.

The effects of heat on fungi are related to the chemical reactions within the fungal cells. For optimum growth, temperatures must be in a range that allows the most efficient progression of the chemical reactions necessary for growth. As temperatures progress above the optimum temperature, the chemical reactions occur less efficiently, and growth slows. Eventually, the temperature can reach a point where growth stops, and cell components begin to be actually damaged by the heat. Enzymes are proteins that change structurally when heated to their limit of tolerance. Likewise, membranes, which contain lipids, change in structure, and their function of protecting and regulating the internal environment of the cell becomes compromised.

Most fungi are mesophilic, and have growth optima within the temperature range that people find comfortable. This is why so many fungi appear when moisture enters our homes, schools, and work environments. Because of air conditioning and heating, mesophilic fungi flourish in occupied environments in all climates. However, the fungal species that are abundant outdoors may vary considerably from one climate to another. In hot dry climates, fewer species of fungi are present, both because of the lack of water and the high temperatures. Thus, thermophilic and xerophilic (dry tolerant) fungi are likely to be more abundant than in cooler wetter environments. In tropical and subtropical places where both heat and moisture are present, thermophilic and thermotolerant fungi with mesophilic water requirements tend to be abundant. The incidence of fungal infections (including sinus infections) tends to be higher in these

areas in part because the fungi that can withstand human body temperatures are more abundant than in temperate climates. Finally, continental climates that tend to swing from hot humid to cold dry conditions have a few overwhelmingly dominant fungi (e.g., *Cladosporium* species) with other mostly mesophilic fungi filling in the gaps. Of course, this is an over-simplification, especially since, as mentioned many times, an array of factors are necessary for optimal growth of all kinds of fungi. Also, fungi live in microenvironments that may have very different temperature/water conditions than are represented by climate.

Table 1 lists some of the fungal temperature relationships that have been reported in the literature.

Table 1:

Activity	Temperature and Duration	Notes
Hot dry air sterilization	170 °C (340 °F), 1 hour 160 °C (320 °F), 2 hours 150 °C (300 °F), 2.5 hours 140 °C (285 °F), 3 hours	Kills virtually all spores
Wood heat treatment for rot resistance	200 °C (392 °F), 24 hours	Changes structure and color of the wood
Whole house treatment for termites and/or fungi	71 °C (160 °F), 4-6 hours	
<i>Penicillium</i> spore death in water	54.4 °C (130 °F), 30 minutes	
Ascospores activation in grape juice	70 °C (158 °F), 30 minutes	This is higher than the temperature which kills <i>Penicillium</i> in water
Germination of chlamydo spores in <i>Cladosporium</i> -related species stimulated by moist heat	75 °C (167 °F), 30 minutes	This is higher than the temperature which kills <i>Penicillium</i> in water

The question sometimes arises about heating an entire structure as part of remediation. In this process, an entire house (or part of a building) may be heated to a temperature that should kill most of the spores remaining following structural remediation and any invisible growth. Clearly, using heat will help the building dry faster because it reduces the relative humidity and increases the vapor pressure of the water in the building. Heat may also damage some plastics, some types of electrical equipment, and other items; so, naturally, heat sensitive items will need to be removed prior to heating the structure. There may also be spores that are particularly resistant to heat, microclimates that do not attain high enough temperatures, or spores that are stimulated to germinate. Also, spores that are left may still remain allergenic. As we do not have expertise in this method of remediation, and the science in this area is still in its infancy, we do not feel qualified to provide "the answer" for if or when heat remediation is good or bad. Also, like many aspects of fungal investigations it depends upon many situation specific criteria. Finally, it is important to remember that spores are continually entering the structure and the only permanent fix for fungal contamination is the maintenance of dry conditions throughout any building.

Fungus of the Month: *Bipolaris*

By Dr. Michelle Seidl

Bipolaris is relatively common with approximately 75 described species occurring worldwide. As described by Shoemaker (1959), *Bipolaris* is a filamentous, dematiaceous (darkly pigmented), anamorphic (asexual, conidial or mitosporic) fungus possessing phragmospores (multicellular spores). This anamorphic genus is well correlated with the teleomorphic (sexual or meiosporic) genus *Cochliobolus* in the Pleosporaceae family of Ascomycetes.

Bipolaris is considered cosmopolitan, associated primarily with grasses, but also occurs on a variety of plant materials, in soil, decaying food, and indoors on various substrates (Knudsen *et al.*, 1995). A few species, e.g. *B. australiensis*, *B. hawaiiensis* and *B. spicifera*, are well-documented human pathogens.

Spores from *Bipolaris*, *Dreschlera* and *Exserohilum* are not differentiated on spore trap and usually not on direct exam samples unless there is growth and the sporulating structures are recovered. Difficulty in differentiation is due to similarities in appearance of the spores and the critical features necessary to accurately identify the genera are usually lacking. Some laboratories also group *Helminthosporium* in with the previous

three, however, *Helminthosporium* can be distinguished by its obclavate (larger at the base) spore shape and straight conidiophores.

In the outside air, spores in the *Bipolaris/Drechslera* group are occasionally recovered in spore trap samples. They are recovered most frequently during the fall season. Recovery rates vary from about 10% in February to 23% in September. When recovered, the spore density is relatively low, with the 50th percentile value being about 13 spores per cubic meter, which is marginally above the detection limit for many samples.

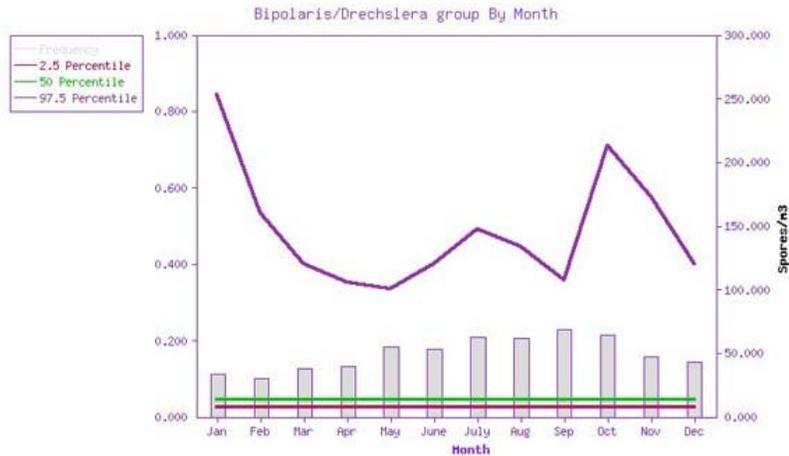


Figure 1: *Bipolaris/Drechslera* group frequency of detection and spore density by month. The gray bars represent the frequency of detection, from 0 to 1 (10%), graphed against the left axis. The red, green, and purple lines represent the 2.5, 50, and 97.5 percentile airborne spore densities, when recovered, graphed against the right hand axis. (Source: EMLab™ MoldRange data. Total sample size for this graph: 39,878.)

Recovery rates and spore levels are still low even in various weather conditions. In this dimension, recovery rates vary from about 6% in moderate rain to 18% in clear weather. When recovered, the spore density is again low, with the 50th percentile value being about 13 spores per cubic meter. Again, this is marginally above the detection limit for many samples.

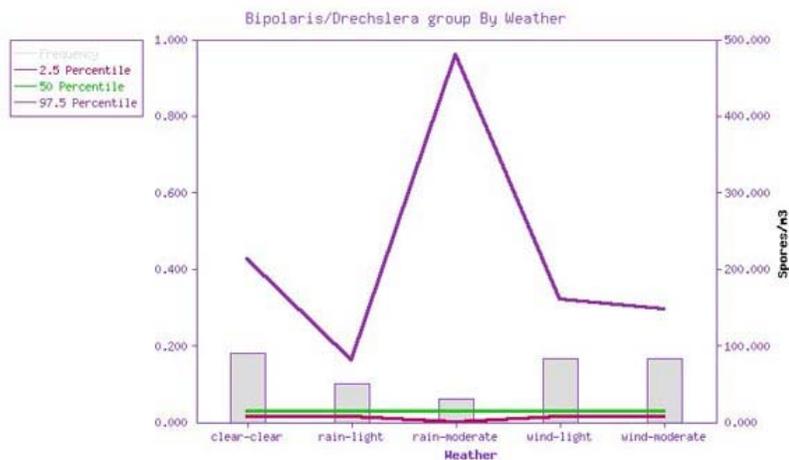


Figure 2: *Bipolaris/Drechslera* group frequency of detection and spore density vs. weather. The gray bars represent the frequency of detection, from 0 to 1 (10%), graphed against the left axis. The red, green, and purple lines represent the 2.5, 50, and 97.5 percentile airborne spore densities, when recovered, graphed against the right hand axis. (Source: EMLab™ MoldRange data. Total sample size for this graph: 20,191.)

With culturable samples, differentiation between these genera and some species level identification is possible. Dematiaceous fungi are conidial fungi that produce dark brown, black or green colonies. Colonies of *Bipolaris* are initially whitish (prior to spore maturation) becoming grey to dark olive to black on the surface. The colonies of *Bipolaris* are dark on the reverse, both initially and when mature. The growth rate is rapid and it has tolerance to the fungicide benomyl (Hoog et al., 2000). Colony texture is suede-like to hairy or downy. Microscopically, the conidia (asexual spores) are

brown, ellipsoidal to fusoid, straight or slightly curved, typically ranging in size from 4-17 microns wide and often up to 120 microns long, with one species up to 250 microns. They are multicellular, appearing thick-walled and with the central cells not much darker and broader than the end cells. Conidia are produced along a main axis which appears bent or as a zigzag.

The key feature of *Bipolaris* spores is the germination pattern in that they grow and develop only at the ends (bipolar). These spores are unique in that they are not made up of normal cells separated by septae (cross-walls). Instead the cells are compartmentalized by distosepta, meaning they are contained in sacs that have a wall distinct from the outer wall of the conidium. This feature cannot be accurately determined using the light microscope alone and requires higher magnification than is used for analyzing spore trap samples. After spore germination, the hyphae of *Bipolaris* are septate and brown.

In the indoor environment, *Bipolaris* that has been recovered from the air is generally from outdoor sources, however it has been occasionally recovered growing on indoor substrates such as wood, plant materials, and some food stuffs.

Historically, *Bipolaris* has been described under *Dreschlera*. Despite the similarities between genera, species of *Dreschlera* have straight conidia (spores) that germinate from any cell and develop perpendicular to the main axis. *Bipolaris* germinates only from the end cells and develops along the main axis. *Exserohilum* is distinct from the other two genera by possessing a protruding or prominent attachment point. Both *Bipolaris* and *Dreschlera* lack this feature. There are two additional genera that may be grouped here as well. *Curvularia* is somewhat similar but differs by a few features including having truly septate conidia that are usually slightly curved. As mentioned above, *Helminthosporium* spores are straight and larger at the base. Due to morphological similarities, confusion has resulted in the nomenclature between these five genera. For example, *Bipolaris* and *Exserohilum* were once grouped under the name *Helminthosporium*. According to St-Germain and Summerbell (1996), *Helminthosporium* is limited to plants. Clinical cases reported in the past as caused by *Helminthosporium* are now identified as *Bipolaris* or *Exserohilum*. As a plant pathogen, *Helminthosporium* causes various turf grass diseases such as leaf spot, root rot and crown rot. *Bipolaris* causes leaf spot and melting out of tall fescue grass. It favors high temperatures and normally develops in late summer. In addition, species of *Dreschlera*, *Curvularia* and *Exserohilum* all occur on grasses (Sivanesan 1987). *Dreschlera* leaf spot is similar in appearance but is more common in late spring to mid-summer, favoring long periods of leaf wetness and moderate temperatures as this disease is inhibited by hot weather (Maloy 2005).



Bipolaris is occasionally found in humans and other mammals. In older literature, it is a causative agent for the disease (primarily in cattle) referred to as "helminthosporiosis". In humans, *Bipolaris* mainly causes allergic sinusitis and is also linked to eye infections (keratitis), allergic bronchopulmonary disease, abdominal cavity inflammation (peritonitis), hay fever, asthma and other respiratory problems (Washburn *et al.*, 1988). These conditions are generally treatable.

Bipolaris is a causative agent of phaeohyphomycosis, which is a fungal infection characterized by dematiaceous septate hyphae, yeast-like growth, or a combination of both (McGinnis *et al.*, 1986). Colonization may become chronic or invasive in the case of impaired immunity. If the fungus becomes invasive, bone erosion may occur which can also lead to brain lesions (Killingsworth and Wetmore 1990). Adam *et al.* (1986) reported *Bipolaris* and *Exserohilum* infections, demonstrating the capability of both fungal genera to cause invasive as well as "allergic" disease. They note that possibly all cases of *Helminthosporium* and *Dreschlera* infections reported in the literature are

caused by *Bipolaris* or *Exserohilum*, indicating infections due to the latter two genera are more common than previously recognized.

In the immunocompromised individual, *Bipolaris* and *Exserohilum* are known to cause tissue death, central nervous system disease, and vascular invasion and osteomyelitis (inflammation of bone and bone marrow). Sinusitis, the most common form of disease caused by *Bipolaris* and *Exserohilum*, occurs in otherwise healthy patients as nasal polyp formation and allergic rhinitis (allergic or non-allergic inflammation and irritation of the nose). In summary, the diseases that have been documented specifically for *Bipolaris* include: allergic sinusitis, subcutaneous infections, endocarditis (inflammation of the heart membrane), pulmonary disease, cerebral disease and meningoencephalitis. The most common *Bipolaris* species indicated in human disease is *Bipolaris spicifera*.

In closing, this large fungal genus is primarily associated with plants and occasionally found in the indoor environment. Cases affecting humans do exist, becoming serious in individuals with impaired immune systems.

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