Identification of Dematiaceous Fungi and Their Role in Human Disease

A. W. Fothergill

From the Fungus Testing Laboratory, Department of Pathology, the University of Texas Health Science Center at San Antonio, San Antonio, Texas

Phaeohyphomycosis is the disease caused by the dematiaceous hyphomycetes or those fungi that are darkly pigmented because of the presence of melanin in their cell wall. Infections with these fungi may remain localized at the site of traumatic inoculation or within the sinuses or may become disseminated. As these fungi pose a significant problem to both the clinician who must treat the patient and the laboratorian faced with identification of the organism, a selection of representative organisms was presented at the meeting entitled "Focus on Fungus Infections 5." These fungi often infect patients who are not overtly immunocompromised and therefore may be encountered in a wide variety of cases.

The most common site of infection is the sinuses. Often a patient is subjected to repeated courses of antibiotic therapy without success before the physician determines that surgery is necessary. When a dematiaceous fungus is responsible for infection, the substance removed from the sinus cavity is a thick, black mass with the consistency of peanut butter. Routine staining of the substance may show hyphae that are not distinguishable from common fungal pathogens such as *Aspergillus* species. However, Fontana-Masson staining demonstrates the melanin in dematiaceous hyphae, thus confirming the presence of a dematiaceous mould. One would expect the culture to yield a black-to-brown-to-olivaceous colony. Table 1 summarizes data on the dematiaceous fungi.

Bipolaris Species

Bipolaris species are by far the most common fungi implicated in sinusitis. Macroscopically, the colonies are floccose and gray-to-black with a black reverse, and moderate growth with mature colonies occurs in 5 days. It is important to differentiate *Bipolaris* species from *Drechslera* species. To my knowledge, *Drechslera* species have never been documented in the literature as agents of human disease despite the common misidentification of *Bipolaris* species as *Drechslera* species in the laboratory. *Bipolaris* species conidia germinate from each end (pole) or at a 180° angle to the conidium (figure 1), while *Drechslera* species germinate from cells within the conidium, usually at a 90° angle to the conidium.

Germination can be determined via a simple germ tube test that is similar to the test used for *Candida albicans*. A mature colony on an agar slant is overlaid with sterile distilled water. Scraping a sterile applicator stick across the surface of the colony liberates the conidia to form a suspension. Ten drops of the suspension is then added to 10 mL of a nutrient broth. Studies have shown that most any broth available in the laboratory may be used, including yeast nitrogen base, RPMI 1640 medium, Sabouraud dextrose broth, and brain-heart infusion broth as well as others. Following inoculation, the broth is incubated at 35°C. Germination should be visible within 2–4 hours. It has been noted that germination can be detected in sterile distilled water without the use of nutrient broths following a 24-hour incubation.

Bipolaris spicifera is the most common *Bipolaris* species recovered. Microscopically, *B. spicifera* is characterized by ellipsoidal, pale brown, pseudoseptate conidia (figure 2). These conidia are formed through pores on sympodial conidiophores. A sympodial conidiophore has a zigzag appearance. The conidiophore continues to grow in length past the pore where the newest conidium is being produced, thus causing the conidiophore to curve or bend around the conidium as each new conidium is produced. These conidia consistently contain three septa and measure 6–13 × 16–39 μm.

Bipolaris hawaiiensis, another cause of sinusitis, is known to be much more aggressive than other dematiaceous fungi causing sinus infection. A 25-year-old man was referred to an infectious diseases specialist because of chronic sinusitis and decreased vision [1, 2]. His history was unremarkable except for marijuana use. CTs revealed erosion of the bone into the brain as well as optical nerve involvement that resulted in total blindness in both eyes (figure 3). The patient was initially treated with amphotericin B but relapsed following his course of therapy. Therapy was changed to itraconazole; at the last follow-up, his condition was stable, and no new focus of disease was defined. A history of marijuana use has been noted in several patients with *B. hawaiiensis* infections.

Macroscopically, *B. hawaiiensis* cannot be differentiated from *B. spicifera*. Microscopically, *B. hawaiiensis* is also characterized by ellipsoidal, pale brown, pseudoseptate poroconidia that are formed through pores on the sympodial conidiophore (figure 4). These conidia also exhibit bipolar germination and contain three to six septa, with four septa being predominant.

Reprints or correspondence: Dr. A. W. Fothergill, Fungus Testing Laboratory, Department of Pathology, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7750.

Clinical Infectious Diseases 1996;22(Suppl 2):S179–84

1058–4838/96/2202–0014$02.00

Table 1. Summary of data on dematiaceous fungi.

Isolate	Characteristics	Disease
Bipolaris spicifera	Three-celled conidia formed from geniculate conidiophores; bipolar germination	Sinusitis, subcutaneous nodules, lung mass
Bipolaris hawaiiensis	Three- to six-celled conidia formed from geniculate conidiophores; bipolar germination	Sinusitis often with aggressive bony erosion, lung and bone infections
Drechslera species	Three- to six-celled conidia formed from geniculate conidiophores; germination from center cells	None documented
Exophiala species	Both yeast and hyphal stages present; nitrate-positive	Mycetoma, subcutaneous abscesses, keratitis
Ochroconis gallopava	Brown colony with diffusing red pigment, two-celled clavate conidia attached via denticles; grows at elevated temperatures	Brain lesions
Scolecobasidium constrictum	Brown colony, two-celled clavate conidia; fails to grow at elevated temperatures	Subcutaneous nodules
Phialophora repens	Brown colony with sausage-shaped conidia clustering at tips of phialides with slightly flared collarettes	Chromoblastomycosis, subcutaneous nodules
Phialophora parasitica	Colony begins light and darkens with age; phialides have inconspicuous collarettes	Subcutaneous abscess, joint infection
Phialophora richardsiae	Flask-shaped phialides with extremely flared collarettes	Subcutaneous abscess
Wallemia sebi	Tiny brown colony; vegetative hyphae round up into roughened conidia	Isolated from human nails and marine mammal respiratory specimens
Xylohypha bantiana	Long chains of conidia that remain intact on slide culture and tease mounts; conidia lack distinct hilum; grows at elevated temperatures	Brain lesions
Xylohypha emmonsii	Long chains of rounded conidia lacking a distinct hilum; no growth at elevated temperatures	Subcutaneous abscess, joint and bone infections
Zygosporium species	Chains of integrated vesicles with a sterile apical knob	Isolated from sputum

The conidia measure 4–9 × 16–34 μm and generally appear smaller than those of *B. spicifera.*

Exophiala Species

Other fungi frequently causing phaeohyphomycosis are the *Exophiala* species; these fungi are most often recovered from subcutaneous nodules and skin lesions resulting from traumatic inoculation. *Exophiala* species are polymorphic, often beginning as a black yeastlike colony that becomes velvety with hyphal development. Macroscopically, colonies are slow-growing and dark olivaceous-to-brown-to-black with a black reverse. Microscopically, the yeast synanamorph and the hyphal synanamorph are visualized simultaneously. The yeast stage consists of yeast cells that multiply via annellides, as opposed to the blastoconidia associated with *Candida* species. Annellidic conidiation occurs when the conidiophore continues to grow in length; it becomes more and more narrow with the production of each conidium. Rings or annellations similar to those of the earthworm are formed as each conidium is released, and

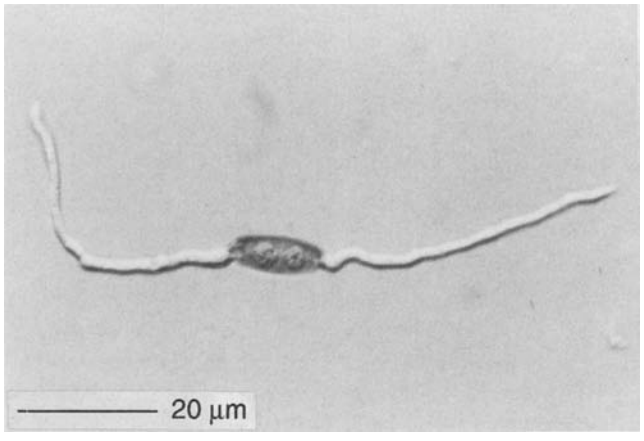

Figure 1. *Bipolaris* species germinating conidium. Note that germination is from each end cell at a 180° angle to the conidium (original magnification, × 1,225).

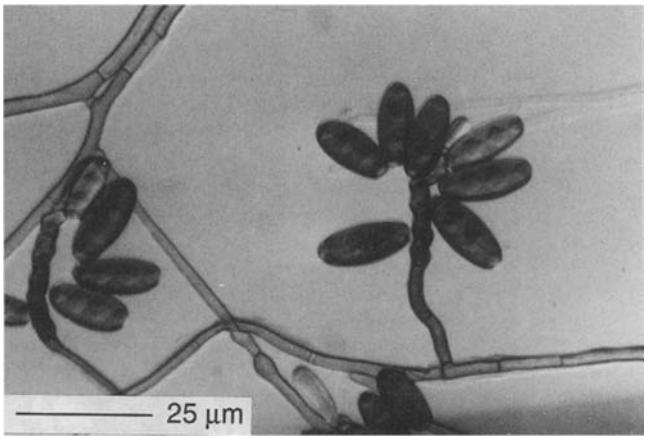

Figure 2. *Bipolaris spicifera.* Note the geniculate conidiophore and the three predominant pseudoseptate conidia (original magnification, × 920).

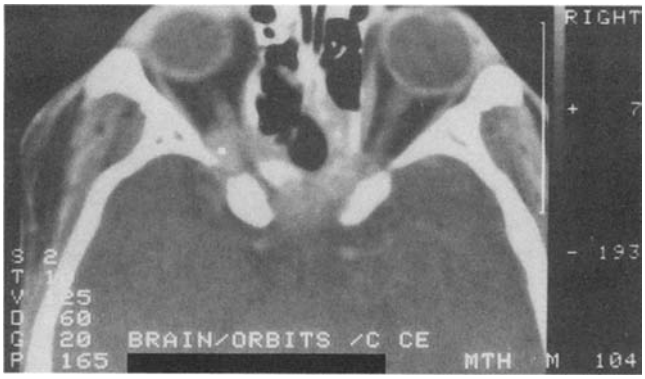

Figure 3. CT of a patient infected with *Bipolaris hawaiiensis.*

a portion of the annellophore is taken away as it is discharged. Although these annellations may be visualized in some organisms such as *Scopulariopsis* species or *Scedosporium* species, they will not be visible on slide cultures of *Exophiala* species.

Exophiala jeanselmei variety *jeanselmei* is the most common *Exophiala* species isolated. *E. jeanselmei* is a common cause of mycetoma. Microscopically, it is characterized by single-celled conidia formed from both medium-length annellophores and intercalary conidiogenous loci arising directly from the vegetative hyphae (figure 5). *Exophiala* species are known to assimilate nitrate, thus enabling their differentiation from *Wangiella* species (which are nitrate-negative). Colonies grow well at 37°C but poorly at 40°C, with diameters measuring >10 mm after 14 days of incubation. *E. jeanselmei* variety *leconi corni* resembles *E. jeanselmei* variety *jeanselmei* but lacks the medium-length annellophores that form conidia (their conidia are formed only from the loci within the vegetative hyphae).

An interesting case of phaeohyphomycosis due to *Exophiala castellanii* has been described [3]. A 61-year-old woman with a prosthetic heart valve presented with fever, chills, and right-leg pain. Auscultation of the chest revealed bilateral basal crep-

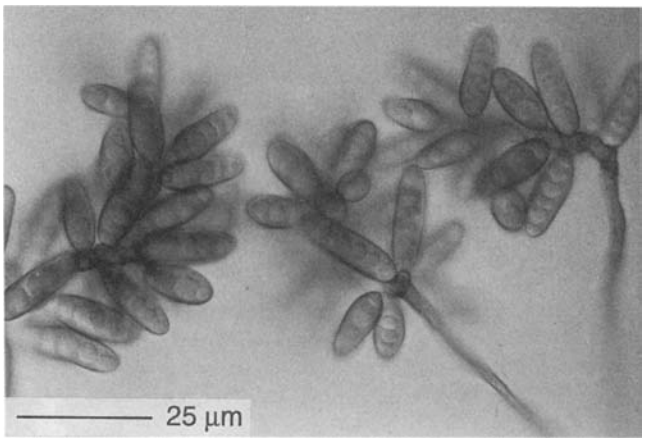

Figure 4. *Bipolaris hawaiiensis.* These conidia have more pseudosepta than do *Bipolaris spicifera* (original magnification, × 920).

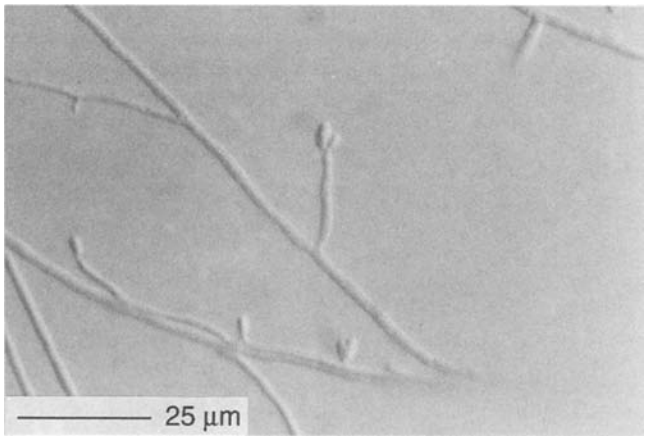

Figure 5. *Exophiala jeanselmei* variety *jeanselmei.* Annelloconidia are formed both from medium-length annellophores and from loci directly on the vegetative hyphae (original magnification, × 920).

itation and a grade 1/6 systolic ejection murmur along the left sternal border; no diastolic murmurs were heard. Blood cultures were negative. She was treated for 6 weeks with antibiotics for presumed culture-negative endocarditis. The patient became afebrile and was discharged. Three months later, the patient was readmitted because of fever. Six blood cultures yielded a black yeast later identified as *E. castellanii.* Treatment was changed to amphotericin B, and the mitral valve was replaced. The original prosthetic valve was noted to have a layer of black slime; culture of the black slime yielded *E. castellanii.* The patient was treated with amphotericin B and 5-fluorocytosine for 1 month and then with amphotericin B alone for 2.5 months. Within 5 months, the patient presented again with fever and pain in her left groin. Heart sounds were normal, but CTs demonstrated bilateral psoas and pelvic abscesses. Gram staining of the material obtained from surgical drainage was positive for a yeast consistent with *E. castellanii*; however, culture of the material was negative. The patient was discharged and treated with a 6-month course of itraconazole.

E. castellanii is characterized microscopically by the presence of yeast cells with very rare hyphae. Annellophores are not well differentiated from the vegetative hyphae, and conidia are formed from annellated loci otherwise known as conidiferous pegs. This organism is nitrate-positive, and it grows well at 37°C but fails to grow at 40°C. Colonies are wrinkled and brainlike and remain small, with a diameter of <6 mm after 14 days of incubation.

Exophiala moniliae, a common cause of fungal keratitis and mycetoma [4], is also a member of this group of nitrate-positive fungi. Colonies are hard and dry and are often depressed into the agar. Slide cultures reveal not only the yeast forms of the organism but also two types of annelloconidia (figure 6). Conidia are found as either broadly ellipsoidal or small and curved and are developed from swollen moniliform (beadlike) annellophores. Colonies grow well at 40°C, with diameters measuring >10 mm after 10 days of incubation.

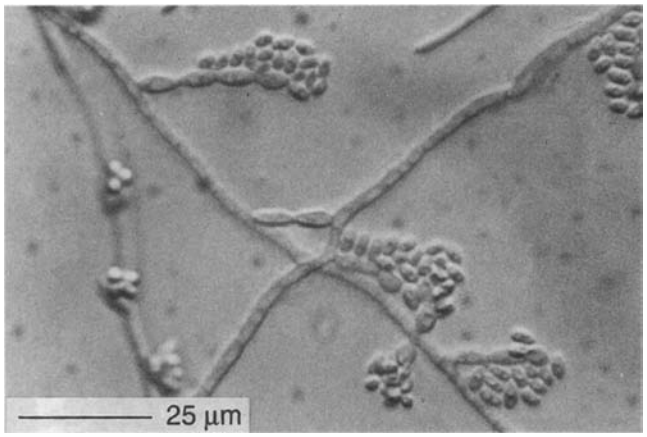

Figure 6. *Exophiala moniliae.* Note the beaded appearance of the annellophore and the two types of annelloconidia (original magnification, × 920).

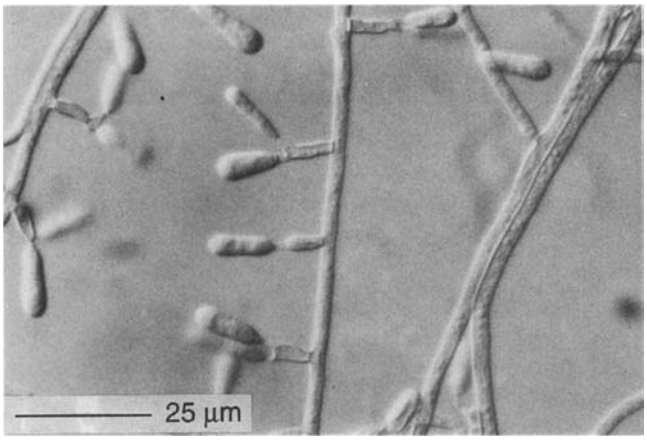

Figure 7. *Ochroconis gallopava.* Conidia are two-celled and are attached by denticles to the supporting structure (original magnification, × 920).

Exophiala pisciphiala is a fish pathogen but has also been implicated in human disease, most often subcutaneous nodules. Colonies have moderate to rapid growth, with diameters of >20 mm after 14 days of incubation. Microscopic examination reveals broadly ellipsoidal conidia with a conspicuous basal scar and prominent large black yeast cells. *E. pisciphiala* grows poorly at 37°C.

Exophiala spinifera is an organism whose name is derived from the long, narrow, spiny annellophores that are seen on slide cultures. Annellophores are darkly pigmented at the base and become lighter toward the tip; they are single or branched. Conidia are smooth and ellipsoidal and aggregate in clusters at the tip of the annellophore. Yeast cells are also prevalent.

Ochroconis gallopava

O. gallopava is a major cause of fait encephalitis in young turkey pullets but has also been isolated from brain lesions in humans. As with most neurotropic fungi, this organism grows at elevated temperatures (>55°C). An interesting case of brain abscess due to *O. gallopava* has been described [5]. A 60-year-old man presented with a 2-week history of confusion. CTs revealed multiple lesions requiring resection. Tissue stains showed many septate hyphal elements. Amphotericin B therapy was started, and 5-fluorocytosine was added to the therapeutic regimen after the presumptive diagnosis of aspergillosis by tissue stains. The therapy failed, and the patient died.

O. gallopava grows as a brown suedelike colony with a diffusing red pigment. Microscopic examination reveals two-celled clavate conidia attached to the vegetative hyphae via denticles or thin filaments of fungal material (figure 7). This organism can be differentiated from *Scolecobasidium constrictum,* an organism isolated chiefly from subcutaneous nodules, by its ability to grow at elevated temperatures, its susceptibility to cycloheximide, and its diffusing red pigment.

Phialophora Species

Phialophora species are common agents of chromoblastomycosis and phaeohyphomycosis. Chromoblastomycosis is usually characterized by draining sinus tracts and the presence of brown, round sclerotic bodies and hyphae in the tissue, whereas phaeohyphomycosis is confirmed via the presence of dark hyphae in the tissue by Fontana-Masson staining. These fungi often display hyphal fascicles or bundles and coils. *Phialophora repens* begins as a brown suedelike colony often possessing a diffusible red pigment similar to that of *O. gallopava.* It grows at 37°C but does not grow on Mycosel agar. Microscopically, however, *P. repens* is characterized by sausage-shaped conidia clustering in balls at the tip of phialides with slightly flared collarettes (fringes of material at the top of the phialide that may or may not be detectable) (figure 8). These phialides are often penicillate or in clusters similar to the ar-

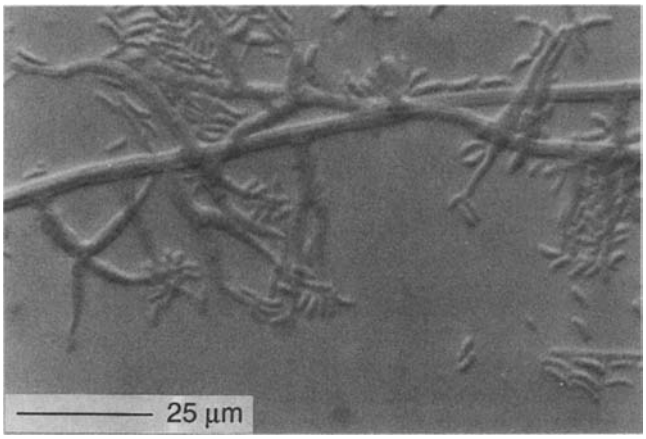

Figure 8. *Phialophora repens.* Note the penicillate arrangement of the phialides and the sausage-shaped conidia (original magnification, × 920).

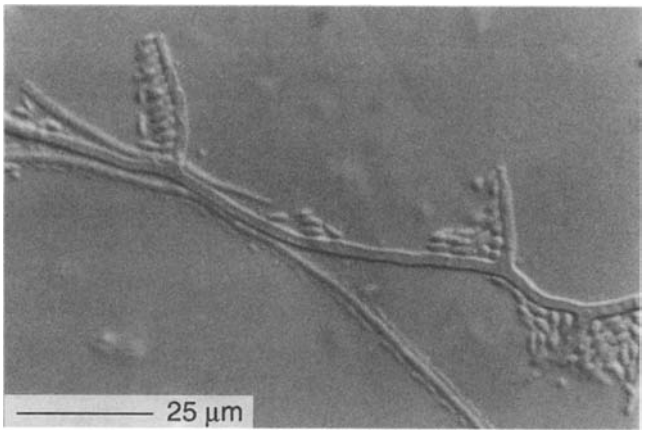

Figure 9. *Phialophora parasitica.* Phialides have inconspicuous collarettes and most closely resemble *Acremonium* species microscopically (original magnification, × 920).

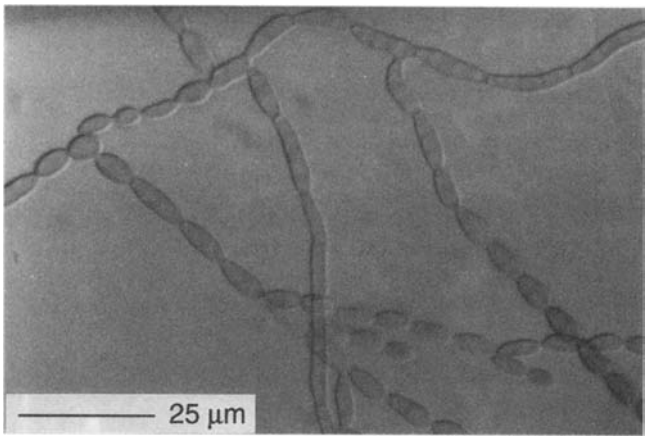

Figure 11. *Xylohypha bantiana.* This fungus is characterized by long nonfragile chains of conidia (original magnification, × 920).

rangement seen with *Penicillium* species. Phialides, another mode of conidiogenesis, do not change in length or width with the production of conidia. Although phialides are most often described as flask- or vase-shaped, they often resemble annellophores but lack the narrowing tip.

Phialophora parasitica was first reported to cause disease in a kidney transplant patient receiving maintenance therapy with immunosuppressants [6]. As in this first case, *P. parasitica* has frequently been isolated from subcutaneous abscesses. A case of chronic arthritis due to *P. parasitica* in a healthy patient has also been reported [7]. This isolate grows at 37°C and on Mycosel agar. It begins as a cream-to-pink colony and is most often misidentified as *Acremonium* species. The colony becomes dark, usually within 3–5 weeks. Phialides are single or branched and are slightly constricted at the neck with inconspicuous collarettes (figure 9).

Phialophora richardsiae has by far the most dramatic collarettes of the *Phialophora* species. The mode of acquisition is thought to be traumatic inoculation with plant material, and patients predominately present with subcutaneous granulomatous lesions [8]. *P. richardsiae* begins as a gray-to-brown suedelike colony with a mahogany reverse. Microscopic examination reveals flask-shaped phialides with very distinctly flared collarettes, which often measure up to 5 µm (figure 10). Two types of conidia are present: large round conidia and small sausage-shaped conidia.

Wallemia sebi

W. sebi has long been described as a pathogen of fish and as a problem in dried foods; recently, it has been recovered from the fingernails of humans and the respiratory tract of marine mammals. In dried peppers, *W. sebi* may reach numbers

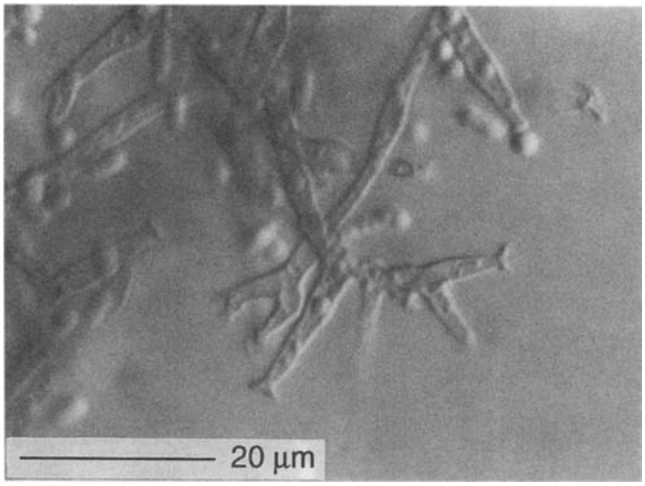

Figure 10. *Phialophora richardsiae.* Note the distinct collarettes and two types of conidia: large spherical conidia and small sausage-shaped conidia (original magnification, × 1,525).

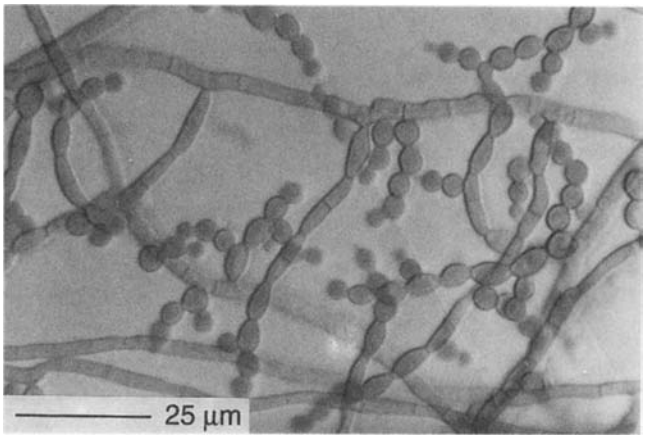

Figure 12. *Xylohypha emmonsii.* This fungus often has a sigmoid cell attaching the chains of conidia to the vegetative hyphae (original magnification, × 920).

as high as 10^9, and many people speculate that the high number may be responsible for the distinctive flavor of these peppers [9]. Macroscopically, the colony is brown with a deep brown reverse. The diameter of the colony is rarely >6 mm. Microscopic examination reveals vegetative hyphae that segment and round up into finely roughened conidia remaining in chains.

Xylohypha Species

X. bantiana, synonymous with *Cladosporium bantianum* and *Cladosporium trichoides,* is another of the neurotropic fungi often causing disease in the seemingly normal host. Recovery of this isolate from outside the CNS is unlikely, although not impossible with the ever-increasing incidence of unusual fungal infections. Since traumatic inoculation with this soil organism has not been reported to my knowledge, it has been speculated that the route of infection is via the respiratory tract despite the lack of lung infection or colonization in patients with this mycosis [10]. Colonies are olivaceous-to-gray-to-brown and floccose, and growth occurs at >42°C. Microscopically, the conidia are formed in long, sparsely branched chains consisting of as many as 35 or more blastoconidia, which lack the distinctive hila associated with *Cladosporium* species (figure 11). These chains arise from indistinct conidiophores and remain intact in contrast to those of *Cladosporium* species (which readily break apart with manipulation).

Xylohypha emmonsii, which also resembles *Cladosporium* species, has been recovered from subcutaneous lesions as well as from bone and joints. These colonies are olivaceous. When incubated at 25°C, the colonies measure 32 mm, but when incubated at 37°C, they measure only 3.5 mm; at 40°C, there is no growth. Microscopic examination reveals that conidia arise from undifferentiated hyphae, with the youngest conidium often being sigmoid or S-shaped (figure 12). Chains of up to 20 rounded conidia are formed; these conidia also lack a hilum.

Zygosporium species

The colonies of *Zygosporium* species are characterized by compact-to-effuse colonies that begin light but become gray-to-brown-to-black with age. Microscopically, the conidiophores are up to 100 μm long, with chains of up to six integrated vesicles giving a noded appearance (figure 13). The sterile apical region is hyaline and terminates in a small knob. Conidia are ellipsoidal, spherical, hyaline, and nonseptate. This organism has been isolated from the sputum of two patients from east Asia.

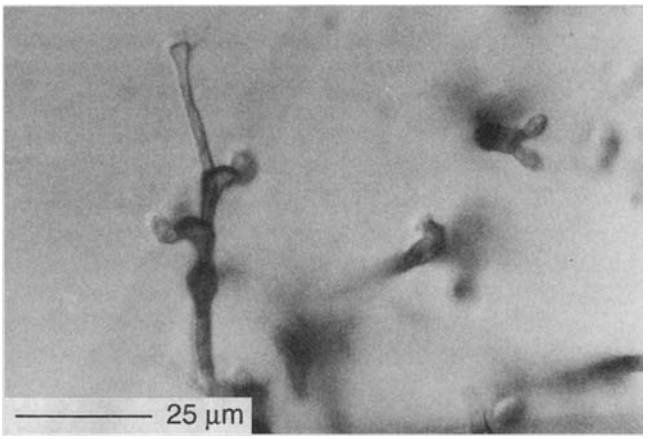

Figure 13. *Zygosporium* species. Chains of integrated vesicles give a noded appearance (original magnification, × 920).

Conclusion

It becomes evident that the dematiaceous fungi are an ever-increasing presence in human disease. As the population of immunocompromised patients continues to grow, the incidence of phaeohyphomycosis caused by a variety of never before reported human pathogens will most likely continue to increase. These organisms, well known by the plant pathologists, pose a unique challenge to the medical laboratorian and the clinician. Itraconazole is thought to be the drug of choice for the treatment of infections caused by the dematiaceous fungi, although little published data exist that aid in therapy decisions. Further studies are necessary to identify the best treatment options for phaeohyphomycosis.

References

1. Maskin SL, Fetchick RJ, Leone CR Jr, Sharkey PK, Rinaldi MG. *Bipolaris hawaiiensis*-caused phaeohyphomycotic orbitipathy. A devastating fungal sinusitis in an apparently immunocompetent host. Ophthalmology **1989**;96:175–9.
2. Sharkey PK, Graybill JR, Rinaldi MG, et al. Itraconazole treatment of phaeohyphomycosis. J Am Acad Dermatol **1990**;23:577–86.
3. Gold WL, Vellend H, Salit IE, et al. Successful treatment of systemic and local infections due to *Exophiala* species. Clin Infect Dis **1994**;19:339–41.
4. McGinnis MR, Sorrell DF, Miller RL, Kaminski GW. Subcutaneous phaeohyphomycosis caused by *Exophiala moniliae.* Mycopathologia **1981**;73:69–72.
5. Sides EH III, Benson JD, Padhye AA. Phaeohyphomycosis brain abscess due to *Ochroconis gallopavum* in a patient with malignant lymphoma of a large cell type. J Med Vet Mycol **1991**;29:317–22.
6. Ajello L, Georg LK. A case of phaeohyphomycosis caused by a new species of *Phialophora.* Mycologia **1974**;66:490–8.
7. Kaell AT, Weitzman I. Acute monoarticular arthritis due to *Phialophora parasitica.* Am J Med **1983**;74:519–22.
8. Pitrak DL, Koneman EW, Estupinan RC, Jackson J. *Phialophora richardsiae* infection in humans. Rev Infect Dis **1988**;10:1195–203.
9. Pitt J, Hocking AD. Fungi and food spoilage. Sydney; Orlando, Florida: Academic Press, **1985**.
10. Dixon DM, Merz WG, Elliott HL, Macleay S. Experimental central nervous system phaeohyphomycosis following intranasal inoculation of *Xylohypha bantiana* in cortisone-treated mice. Mycopathologia **1987**;100:145–53.