

Dressed in Black:

FUNGI AND MELANINS

Hans Halbwachs

ABSTRACT

Melanins are ubiquitous in organisms across all kingdoms. In fungi, they serve a cornucopia of mostly defensive functions. They include protection against biotic and abiotic hazards, such as microbial attacks, thermal stress, radiation stress, and desiccation. Since the biosynthesis of melanins requires high energy input, the advantages obviously outweigh the cost. It may be expected that further research will bring additional beneficial properties to light.

Keywords: Pigments, extremophiles, environmental stress, Dark Septate Endophytes, radiotrophy

Melanins everywhere

Melanins are one of the most widespread organic substances in living beings across all kingdoms. Melanins are dark pigments that are common in many taxa (Fig. 1). It appears that melanins play a universal role and are physiologically highly relevant. Herrera (2018) even postulates that melanin is connected to the origin of life.

Back to more tangible issues: one may assume that melanins are mimicry-relevant (see Fig. 1 D, F) or have thermoregulatory functions because black absorbs heat radiation (see Fig. 1 H, J), but why should fungi, plants and bird feathers contain melanins? To answer such questions, the chemistry of melanins may give some clues.

Basic melanin chemistry

First, melanin is not a specific compound but an umbrella term that embraces a whole zoo of polymers that are synthesised using at least three

different chemical pathways (Bell and Wheeler, 1986) in different kingdoms. Fungal melanins are mainly produced in cell walls, e.g., conidia of molds (Fig. 2), using the so-called DHN melanin biosynthesis. This pentaketide pathway starts by converting organic acetic acid compounds during five steps into aromatic building blocks that finally aggregate to melanin, the basic component (monomer) of melanin polymers (Butler et al., 2001) (Fig. 3).

Melanin synthesis and polymerization are complex processes which require considerable energy input, implying significant benefits for a fungus (Halbwachs and Bässler, 2015). The higher the degree of polymerization, the less soluble and chemically inert are melanins. This is surprising because melanin contains highly reactive free radicals (unpaired electrons, <https://roempp.thieme.de/roempp4.0/do/data/RD-18-00055>), which are generally unstable. Since melanins feature unpaired electrons, they probably act as a “sponge” for other free radicals (mainly single oxygen atoms) that are generated, for instance, by radiation (Bell and Wheeler, 1986). Melanins also seem to have properties comparable with those of cation exchangers that are often used to purge solutions (White, 1958).

Fungal melanins are mostly brown to black and thus absorb visible light but also ultraviolet and, to a certain

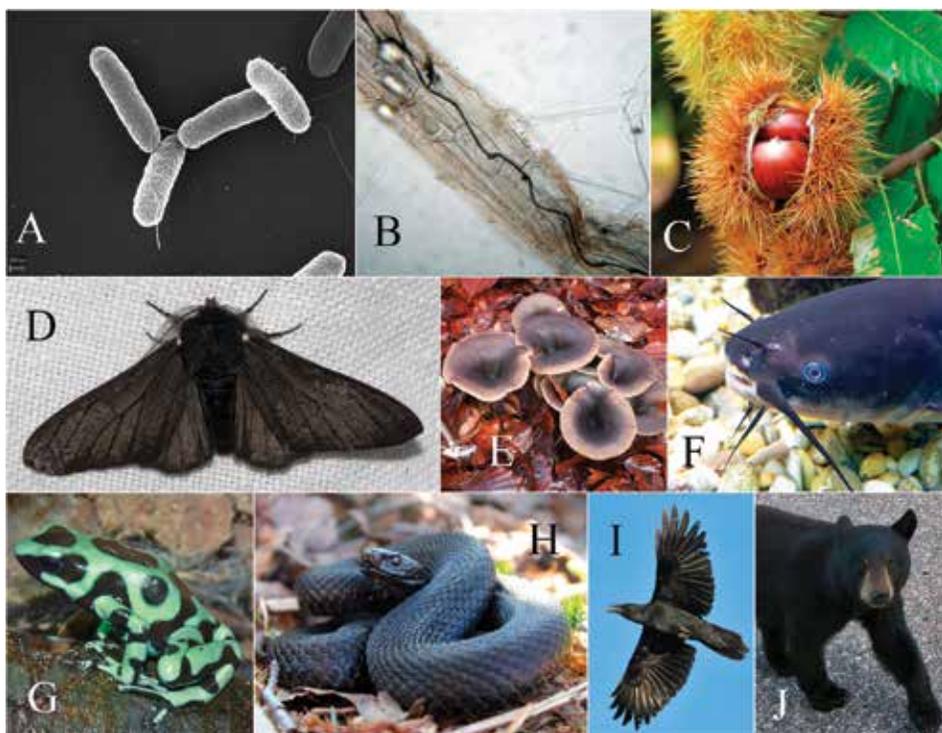


Figure 1. Melanized organisms:
A – *Azotobacter*, a nitrogen-fixing bacterium, image by alchetron.com/*Azotobacter*; **B** – Dark Septate Endophyte, a biotrophic microfungus that lives inside plants, image by the author; **C** – European chestnut with melanized fruit husks, image by Benjamin Gimmel (CC BY-SA 3.0); **D** – Melanized form of the peppered moth, image by Chiswick Chap (CC BY-SA 2.5); **E** – Goblet mushroom with melanized cutis, image by H. Krisp (CC BY-SA 3.0); **F** – Black bullhead catfish, image by Emőke Dénes (CC BY-SA 4.0); **G** – A poison dart frog with melanized ornaments, image by Adrian Pingstone (public domain); **H** – Black adder, image by Frogography (CC BY-SA 4.0); **I** – Common raven, image by USFWS Mountain-Prairie (public domain); **J** – Black bear, image by the author.

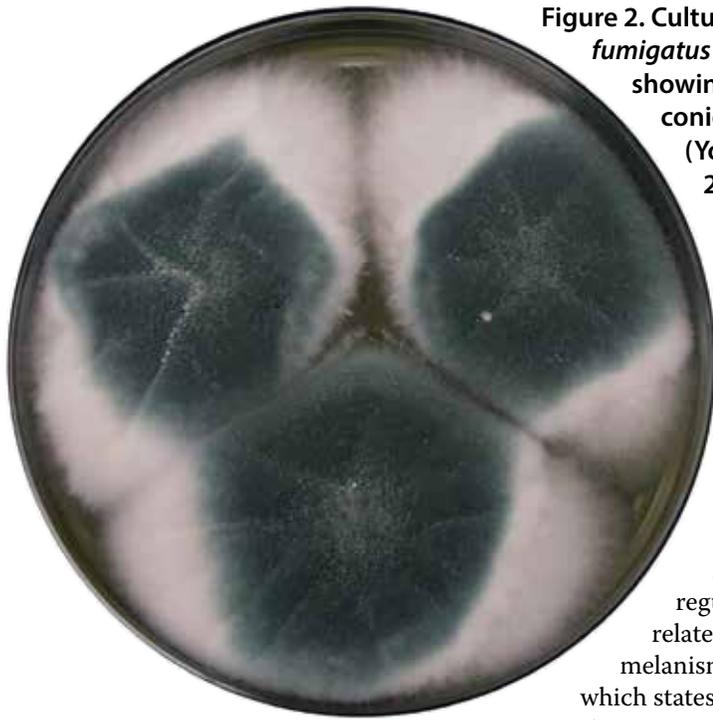


Figure 2. Culture of *Aspergillus fumigatus* in a petri dish showing melanized conidial aggregates (Youngchim et al., 2004), image by Dr David Midgley (CC BY-SA 2.5).

extent, infrared (Bell and Wheeler, 1986). Radiation energy is then converted into heat (Butler and Day, 1998). A more exotic property of melanins is their bistable conductivity (semiconductivity) triggered by environmental humidity (Mostert et al., 2012) and possibly by ionizing radiation (Dadachova and Casadevall, 2008). The biological implications of this rather strange phenomenon still wait for an explanation. But it is well known that melanins possess a wide range of protective properties against abiotic and biotic stress. Fungal examples of melanization are shown in Fig. 4.

Thermal stress

Melanins are involved in alleviating both heat and cold stress (Cordero and Casadevall, 2017). An example of heat protection is *Monilinia fructicola*, an ascomycete that causes brown rot of stone fruits. Rehnstrom and Free (1996) could show that melanin-deficient mutants were easier killed by high temperatures.

Many microfungi and lichens are melanized, especially those that inhabit extreme substrates such as rocks in cold environments (“black fungi”) (Gostinčar et al., 2012). Moreover, many fungal taxa associated with *Dryas* found in alpine or (sub-)arctic biomes have melanized hyphae (Bjorbaekmo et al., 2010). Soil fungi with dark septate hyphae dominate arctic and alpine soils (Smith and Read, 2008).

Another thermo-regulative function relates to the thermal melanism hypothesis, which states that ectothermic organisms are at an advantage under cold conditions when pigmented (Clusella-Trullas et al., 2007). It could, indeed, be shown that mushroom assemblages at higher latitudes feature darker fruit bodies than those in warmer areas (Krah et al., 2019).

Physical stress

Melanins show high tensile strength and thus reinforce cell wall structures such as spore walls (Cooke and Whipps, 1993). In this way, melanized

fungal cell walls better resist osmotic stress and turgor forces (Cordero and Casadevall, 2017). Spores are frequently melanised (e.g., ca. 50% of European larger basidiomycetes, Halbwachs et al., 2015) which protects them against desiccation (Fernandez and Koide, 2013) and hazardous UV-radiation (Cordero and Casadevall, 2017). Melanins even resist ionizing radiation (Bell and Wheeler, 1986). Remarkably, radioactive radiation can cause enhanced growth in melanised fungi, as it was observed with *Penicillium* in the vicinity of the Chernobyl reactor ruins (Dadachova et al., 2007). These fungi seem to harvest energy from ionizing radiation, making them autotrophic, analogous to photosynthesis (Rangel et al., 2018).

Chemical and biological stress

Overall, melanins have properties that bind noxious substances (Larsson, 1993). They contribute to the protection against chemical degradation, such as extreme pH, salt stress, and toxic heavy metals (Onofri et al., 2007; Cordero and Casadevall, 2017; Rangel et al., 2018). Melanins serve as antioxidants (Gessler et al., 2014) and resist lysis by enzymes as well as microbial attacks (Kuo and Alexander, 1967). The latter properties seem to enable many plant-pathogenic fungi to overcome host defenses and thus contributes to the virulence of the

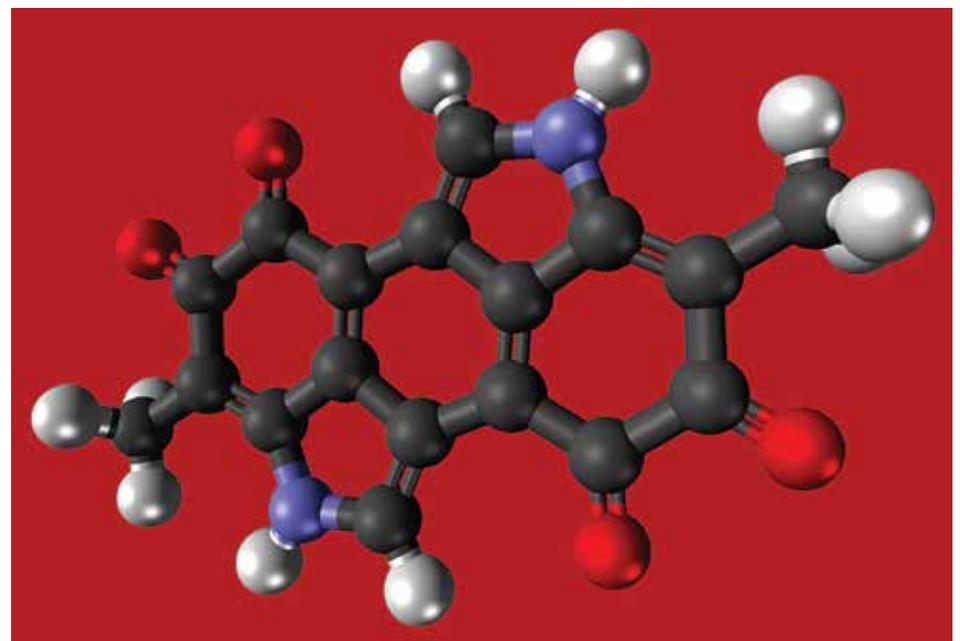


Figure 3. Ball-and-stick model of the basic melanin molecule (black – carbon, white – hydrogen, blue – nitrogen, red – oxygen), image by Mhotep (CC BY-SA 4.0).

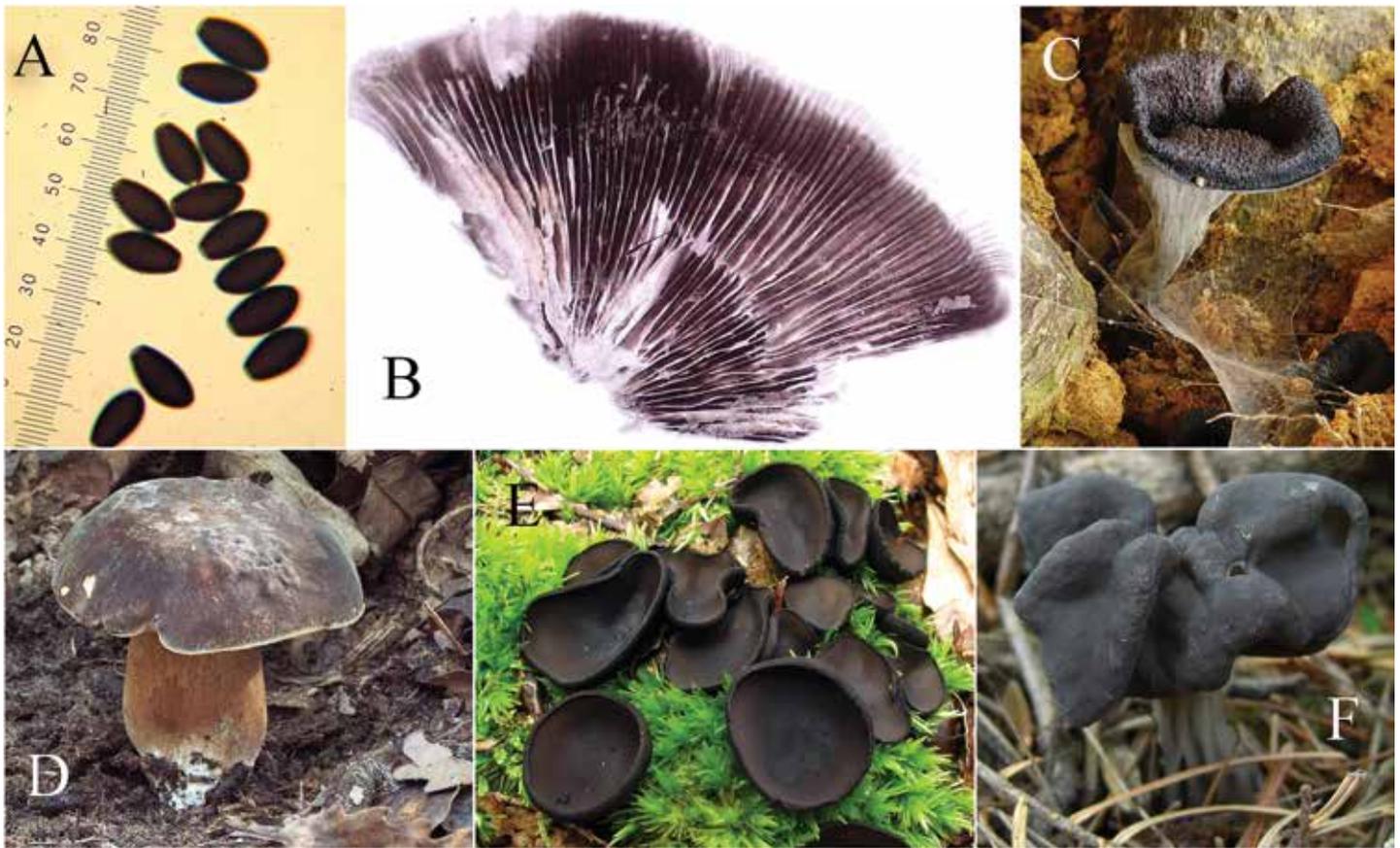


Figure 4. Examples of melanization in fungi: A – Spores of *Coprinopsis lagopus*, image by Ron Pastorino (CC BY-SA 3.0); B – Spore print of *Psathyrella candolleana*, image by Adam Singer (CC BY-SA 3.0); C – *Craterellus cornucopioides*, image by Lucarelli (CC BY-SA 3.0); D – *Boletus aereus*, image by Roberto Petruzzo (public domain); E – *Pseudoplectania nigrella*, image by Dan Molter (CC BY-SA 3.0); F – *Helvella lacunosa*, image by Jason Hollinger (CC BY-SA 2.0).

pathogen (Butler and Day, 1998). Finally, melanins in Dark Septate Endophytes (see Fig. 1 B) are toxic to herbivores (Mandyam and Jumpponen, 2005).

The bottom line

So far, documented and circumstantial evidence has exposed an impressive range of functional properties of fungal melanins.

- Structural enforcement (cell walls) against osmotic and turgor forces
- Desiccation protection
- Thermal stress protection
- Protection against ionizing radiation (UV, ionizing radiation, etc.)
- Salt and pH stress protection
- Mopping up heavy toxic metals
- Antioxidant
- Protection against lytic enzymes
- Fending off microbial attacks
- Enhancing virulence of plant pathogens
- Converting radioactive and other radiation to energy for metabolic processes

I would not be surprised if further research found more functional properties. For example, considering the molecule exchange capacity of melanins, one may wonder whether melanins also possess catalytic qualities.

BLACK HOLES: WHAT WE DON'T KNOW YET

Fungal melanins in deep time

Melanins seem to have played their role in deep time. The current oldest melanin-containing fossil has been reported from Coniferous cyclostomes (jawless fish, an ancestor of vertebrates, ca. 300 MYA) (Gabbott et al., 2016). Generally, fossilized melanin particles (melanosomes) seem to be very durable (Lindgren et al., 2015), which suggests that this applies to melanised fungi, too. Actually, most fungal fossils are dark

colored (Taylor et al., 2014; Halbwegs, 2019). But whether this is due to melanins still needs to be clarified. Should this be the case, the fossil record of fungi would be heavily biased.

The case of DSE (Dark Septate Endophytes)

Melanins in Dark Septate Endophytes (see Fig. 1 B) are assumed to take a symbiotic relationship with their plant hosts (Hidayat, 2019). DSE are claimed to protect host plants against abiotic and biotic stress (Berthelot et al., 2019; Naik, 2019), which is suggestive of the melanins in DSE being responsible. However, clear mechanistic explanations of how the fungal melanins could transfer their properties to the host plant is still missing, in particular, when considering possible mutualistic interactions with bacteria.

Conclusions

It becomes clear that melanins are one of the most versatile functional compounds synthesized by a plethora of

species across all fungal taxa. It suggests that the advantages offered by melanins outweigh the considerable production costs. It remains to be seen whether further studies will reveal even more functions of this miraculous substance.

References Cited

- Bell, A.A., and M.H. Wheeler. 1986. Biosynthesis and functions of fungal melanins. *Annual Review of Phytopathology* 24(1): 411–451.
- Berthelot, C., M. Chalot, C. Leyval, and D. Blaudez. 2019. From darkness to light: emergence of the mysterious dark septate endophytes in plant growth promotion and stress alleviation. In: *Endophytes for a Growing World*, T.R. Hodkinson, F.M. Doohan, M.J. Saunders, and B.R. Murphy, Eds. Cambridge University Press; pp. 143–164.
- Bjorbækmo, M., T. Carlsen, A. Brysting, T. Vrålstad, K. Høiland, K. Ugland, J. Geml, T. Schumacher, and H. Kauserud. 2010. High diversity of root associated fungi in both alpine and arctic *Dryas octopetala*. *BMC Plant Biology* 10(244): 2–12
- Butler, M., and A. Day. 1998. Fungal melanins: a review. *Canadian Journal of Microbiology* 44(12): 1115–1136.
- Butler, M.J., A.W. Day, J.M. Henson, and N.P. Money. 2001. Pathogenic properties of fungal melanins. *Mycologia* 93(1): 1–8.
- Clusella-Trullas, S., J.H. van Wyk, and J.R. Spotila. 2007. Thermal melanism in ectotherms. *Journal of Thermal Biology* 32(5): 235–245.
- Cooke, R.C., and J.M. Whipps. 1993. *Ecophysiology of Fungi*. Blackwell Scientific Publications.
- Cordero, R.J.B., and A. Casadevall. 2017. Functions of fungal melanin beyond virulence. *Fungal Biology Reviews* 31(2): 99–112.
- Dadachova, E., R.A. Bryan, X. Huang, T. Moadel, A.D. Schweitzer, P. Aisen, J.D. Nosanchuk, and A. Casadevall. 2007. Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. *PLoS One* 2(5): e457.
- Dadachova, E., and A. Casadevall. 2008. Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. *Current Opinion in Microbiology* 11(6): 525–531.
- Fernandez, C.W., and R.T. Koide. 2013. The function of melanin in the ectomycorrhizal fungus *Cenococcum geophilum* under water stress. *Fungal Ecology* 6(6): 479–486.
- Gabbott, S.E., P.C. Donoghue, R.S. Sansom, J. Vinther, A. Dolocan, and M.A. Purnell. 2016. Pigmented anatomy in Carboniferous cyclostomes and the evolution of the vertebrate eye. *Proceedings of the Royal Society B: Biological Sciences* 283(1836): 20161151.
- Gessler, N., A. Egorova, and T. Belozerskaya. 2014. Melanin pigments of fungi under extreme environmental conditions. *Applied Biochemistry and Microbiology* 50(2): 105–113.
- Gostinčar, C., L. Muggia, and M. Grube. 2012. Polyextremotolerant black fungi: oligotrophism, adaptive potential, and a link to lichen symbioses. *Frontiers in Microbiology* 3(390): 1–6.
- Halbwachs, H. 2019. Fungi trapped in amber – a fossil legacy frozen in time. *Mycological Progress* 18: 879–893.
- Halbwachs, H., and C. Bässler. 2015. Gone with the wind – a review on basidiospores of lamellate agarics. *Mycosphere* 6: 78–112.
- Halbwachs, H., R. Brandl, and C. Bässler. 2015. Spore wall traits of ectomycorrhizal and saprotrophic agarics may mirror their distinct lifestyles. *Fungal Ecology* 17: 197–204.
- Herrera, A. 2018. The origin of life according to melanin. *MOJ Cell Science Reports* 5(1): 00105.
- Hidayat, I. 2019. Dark septate endophytes and their role in enhancing plant resistance to abiotic and biotic stresses. In: *Plant Growth Promoting Rhizobacteria for Sustainable Stress Management*, R.Z. Sayyed, N.K. Arora, and M.S. Reddy, Eds. Springer; pp. 35–63.
- Krah, F.-S., U. Büntgen, H. Schaefer, J. Müller, C. Andrew, L. Boddy, et al. 2019. European mushroom assemblages are darker in cold climates. *Nature Communications* 10(1): 2890.
- Kuo, M.J., and M. Alexander. 1967. Inhibition of the lysis of fungi by melanins. *Journal of Bacteriology* 94(3): 624–629.
- Larsson, B. 1993. Interaction between chemicals and melanin. *Pigment Cell Research* 6(3): 127–133.
- Lindgren, J., A. Moyer, M.H. Schweitzer, P. Sjövall, P. Uvdal, D.E. Nilsson, J. Heimdal, A. Engdahl, J.A. Gren, and B.P. Schultz. 2015. Interpreting melanin-based coloration through deep time: a critical review. *Proceedings of the Royal Society B: Biological Sciences* 282(1813): 20150614.
- Mandyam, K., and A. Jumpponen. 2005. Seeking the elusive function of the root-colonising dark septate endophytic fungi. *Studies in Mycology* 53: 173–189.
- Mostert, A.B., B.J. Powell, F.L. Pratt, G.R. Hanson, T. Sarna, I.R. Gentle, and P. Meredith. 2012. Role of semiconductivity and ion transport in the electrical conduction of melanin. *Proceedings of the National Academy of Sciences* 109(23): 8943–8947.
- Naik, B.S. 2019. Functional roles of fungal endophytes in host fitness during stress conditions. *Symbiosis*: doi 10.1007/s13199-019-00635-1.
- Onofri, S., L. Selbmann, G. De Hoog, M. Grube, D. Barreca, S. Ruisi, and L. Zucconi. 2007. Evolution and adaptation of fungi at boundaries of life. *Advances in Space Research* 40(11): 1657–1664.
- Rangel, D.E.N., R.D. Finlay, J.E. Hallsworth, E. Dadachova, and G.M. Gadd. 2018. Fungal strategies for dealing with environment- and agriculture-induced stresses. *Fungal Biology* 122(6): 602–612.
- Rehnstrom, A., and S. Free. 1996. The isolation and characterization of melanin-deficient mutants of *Monilinia fructicola*. *Physiological and Molecular Plant Pathology* 49(5): 321–330.
- Smith, S.E., and D.J. Read. 2008. *Mycorrhizal Symbiosis*. Academic Press.
- Taylor, T.N., M. Krings, and E.L. Taylor. 2014. *Fossil Fungi*. Elsevier Science, London.
- White, L.P. 1958. Melanin: a naturally occurring cation exchange material. *Nature* 182(4647): 1427–1428.
- Youngchim, S., R. Morris-Jones, R.J. Hay, and A.J. Hamilton. 2004. Production of melanin by *Aspergillus fumigatus*. *Journal of Medical Microbiology* 53(3): 175–181. 📌