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Effects of Storage Temperature and Storage Time on Fungal Diversity

Pseudogymnoascus destructans is the fungus that causes white-nose syndrome, a disease that causes significant mortality to North American bat populations during hibernation. The fungus grows on the ears, muzzles, and wing membranes of hibernating bats, resulting in lesions that disturb the bats' torpor. This leads to abnormal winter behaviors, such as flying, which result in the premature consumption of fat reserves causing emaciation and death. *Pseudogymnoascus destructans* is present as inactive spores and can also grow in cave soils. Therefore, this fungus can spread from cave to cave by the transfer of cave soils by fomite (shoes, equipment, or other inanimate objects). Like many fungi, *P. destructans* can only flourish in a species-unique temperature range. The optimal temperature for the growth of *P. destructans* was found to be between 12.5˚C and 15.8˚C. However, factors outside of the cave environment can affect the biodiversity of fungi in cave soils. These factors can be anything from human influences (hiking, climbing, etc.) to the temperature of the external environment.

Figure 1 The subculturing of fungi isolates to a new PDA dish. Photo by Dan Raudabaugh, INHS.

Up to this point, investigations of *P. destructans* have not utilized the same soil storage protocol. Several research papers investigating cave fungal biodiversity showed differences in the times and temperatures in which the collected soil samples were stored. The purpose of this project was to investigate the impact of storage temperature and storage time on observed fungal biodiversity.

Approximately 10-g samples of soil collected from three infected bat roosting caves in Illinois were each divided into five 50-mL tubes and stored at 22˚C (room temperature), 14˚C, 7˚C, -20˚C, and -80˚C, respectively. These samples, at each temperature, were lawn plated after 48 hours, one week, one month, and six months at three separate dilu-

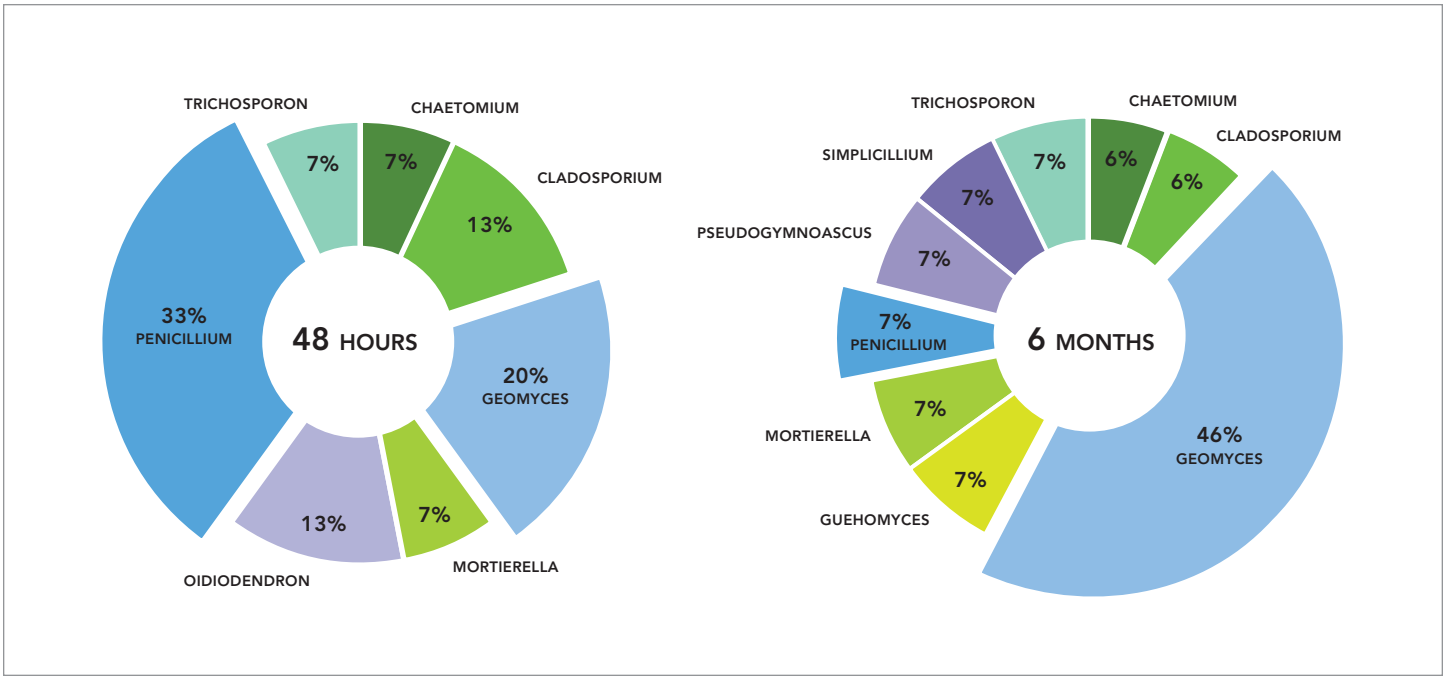

Figure 2 Changes in the presence and abundance from 48 hours of storage to 6 months of storage of fungal genera in cave soil samples when stored at a temperature of -20°C. The proportion of *Geomyces* increases with storage length and the proportion of *Penicillium* decreases as storage length increases.

tions. A 400-µL aliquot from each dilution was pipetted onto 90-mm Petri plates containing either Potato Dextrose Agar (PDA) or Sabouraud Dextrose Agar (both containing Penicillin G and Streptomycin) and spread evenly using a sterile glass spreader and rotator stand. The cultures were incubated at 14˚C for 48 hours to 1 week before being examined for fungal growth. Any visible fungal growth was transferred to a new individual PDA plate. Axenic fungal isolates were sub-cultured to an additional PDA dish and to a 1.5-mL centrifuge tube containing Potato Dextrose broth (Figure 1). DNA was extracted using a QIAGEN DNeasy Plant Mini Kit, a microwave method, or a NaOH method. A 1 to 3-µL aliquot (depending on amplification method) of the extracted DNA was amplified for identification through a PCR reaction using PuReTaq ready-to-go PCR beads (Promega) or GoTaq green master mix (Promega). Each sequence fragment from the PCR reaction underwent BLAST search to verify fungal identity. Identified fungi were then stratified by temperatures and storage lengths.

We found that the number of *Penicillium* organisms decreased drastically after six months of storage at temperatures of -20˚C and -80˚C. We also observed that the number of identified *Geomyces* organisms increased after six months of storage at -20˚C and -80˚C. *Guehomyces* was not detected at all until six months of storage (Figure 2). At 22˚C, 14˚C, and 7˚C, there was no significant decrease or increase in the number of species of any genera.

The majority of the observed changes in biodiversity occurred at lower temperatures and at longer storage lengths, indicating that for any study involving culture-based techniques, measuring the subject fungus's response to storage time and temperature is necessary. Further research is needed to elucidate how *P. destructans* responds to storage time and temperature before an exact protocol can be determined. Obtaining this information will help interpret past and future findings of research done on *Pseudogymnoascus destructans* and its effect on North American bats.

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Gretchen Anchor, Daniel Raudabaugh, Andrew Miller, and Nohra Mateus-Pinilla, INHS