Time Flies When You Are Having Fungus

By: Alissa Jane Cotton

Jonesing for Fungi

As tree care professionals and arboriculture enthusiasts, there are many reasons to take an interest in fungi. For those of us who secretly identify as Indiana Jones, the fungal world holds extensive opportunity for exploration—discovering any number of the estimated 1-5 million unnamed fungi on earth may be enough to tempt any adventurous soul. For those more practical friends, simply getting a handle on what elements may be contributing to the decline of a single tree or stand of trees is a valuable practice.

Before you even ask—YES! It *is* possible to fulfill your appetite for adventure with arboricultural activities. Field-collecting fungal material from trees can be as swashbuckling as you make it (on par with the relic-seeking journeys of celebrity archaeologists). However, some might argue that the *real* thrill comes with the laboratory work-up of samples, which sheds light on the fungal friends and foes that exist in a tree. It is in the laboratory that we identify the fungal pathogens causing the tree diseases we love to hate: Dutch elm disease (caused by *Ophiostoma novo-ulmi*), oak wilt (caused by *Ceratocystis fagacearum*), and thousand cankers disease (caused by *Geosmithia morbida*), to name a few. For a glimpse into the elusive world of laboratory work involved with studying tree fungi, read on!

Keeping it Fresh

Procuring interesting fungi from around the world amounts to doodly-squat if you cannot get them back to the lab in a proper condition. A successful laboratory study on tree fungi begins with the material collection. The most important rule of thumb for fungal samples is to keep the material fresh, whether it be a fungal fruiting body or part of an affected tree. Taking live tissue is crucial; it is best to take several samples, keeping material cold until it can be processed in the lab, and stored in a way that minimizes water retention, which can lead to contamination.

Branch samples taken from Minnesota state capitol elm trees were packaged (Figure 1) and kept on ice before transfer into a -20C freezer (Figure 2) until processing:

Figure 1: Nice samples!

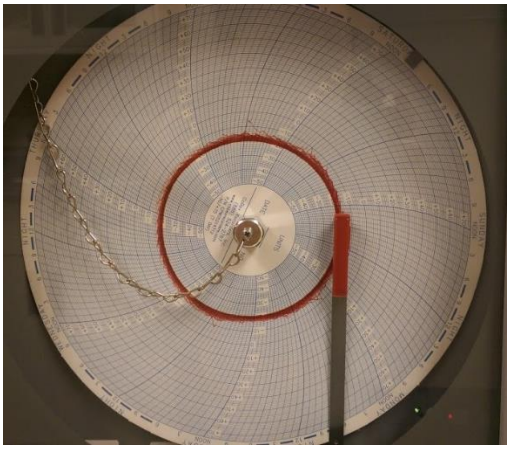

Figure 2 – Revolving monitor on -20C freezer records minute temperature fluctuations in red

<u>If You Feed It, It Will Come.</u>

The processing of fungal material is best performed in a flow hood which draws airborne contaminants away from the workspace. "But how do I get the fungus out of this wood sample in order to study it?" you ask. Answer: the same way you lure anything out of anywhere… offer it what it wants. In the case of a fungus, feed it and let it multiply! In laboratory lingo, this is called *culturing*. *Isolating* a tree fungus, or getting it *into culture,* involves selecting an appropriate media (fungus-food in a petri dish, or plate), using sterile technique to add bits of the affected tree material into the media, and allowing it to grow.

The basis for all fungal media production is water (water is life!), agar (when heated and cooled in water, creates a gelatinous base), and malt (food source). Additions such as streptomycin sulfate, cyclohexamide, and lindane, can create media that is specific for basidiomycetes (club fungi), stain fungi, and insect inhibition, respectively.

Initial plating of material often yields an array of fungi that must be individually transferred to separate media plates in order to analyze or identify each one. When a single fungus has been successfully isolated to a plate, it is called a *clean culture.*

These hands are gloved and ready to perform fungal isolations using the stacks of media in the hood (Figure 3):

Figure 3 – Ready to go!

Initial culturing of material yields many fungi (Figure 4), which must be separately plated onto fresh media and grown out individually (Figure 5):

Figure 4 – Multiple fungi growing on each media plate

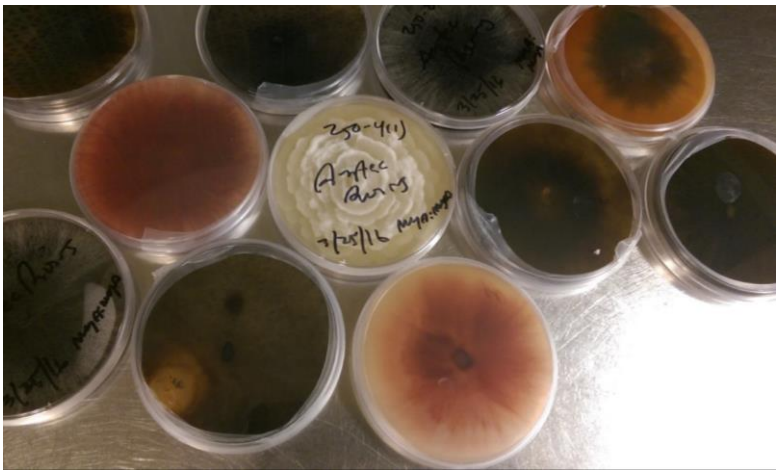

Figure 5 – An array of fungi in clean culture (yes, that says "Aztec Ruins"!)

What's on My Plate?

Examining the growth in culture can sometimes aid in the identification of a fungus. In situations where we are looking for a specific pathogen on a particular tree species (such as with Dutch elm disease on elm, or oak wilt on oak), identifying asexual structures grown on a specific media type can confirm the presence of the fungal pathogen. Figure 6 shows *Ophiostoma novo-ulmi* (Dutch elm disease pathogen) growing in culture. The structures, called synnemata, are made up of long, black stalks called conidiophores, with a bulbous sac on top of each, that is chock full of sticky conidia (asexual spores). Figure 7 shows the distinct, rectangular endoconidia (asexual spores) of oak wilt (*Ceratocystis fagacearum*) used to positively identify the presence of the pathogen taken from an oak sample.

Figure 6 – Ophiostoma novo-ulmi (Dutch elm disease pathogen) growing in culture, seen under dissecting scope

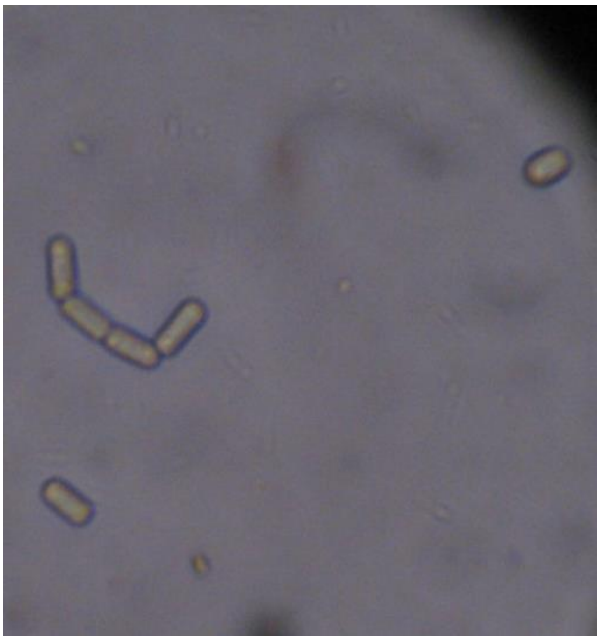

Figure 7 - The rectangular endoconidia of Ceratocystis fagacearum (oak wilt) are a unique identification piece for this pathogen, seen under microscopic magnification (Photo: Jennifer Flynn, University of Minnesota Plant Disease Clinic)

I'm Not So Sure About This…

Still not certain that what you have growing there is *Ophiostoma*? For the skeptics, the perfectionists, the tree-owners who simply do not believe a diagnosis until it is on paper, or anyone who has not quite developed their fungal-identification eyes yet, there is gene sequencing. To be entirely, officially, and scientifically certain of the identity of a fungus, all you need is love. Scratch that. All you need is a bit of its DNA. After extracting DNA from a fungal sample (just use what you have growing over there in that culture plate), a portion of its

gene sequence can be amplified, or copied about a billion times, using PCR (polymerase chain reaction). Having a billion copies of a gene sequence makes it easier to visualize and identify the base pairs that it is made of, using sequencing software. With the sequence of base pairs in hand, tippity-tap your fingers over to the NCBI Nucleotide BLAST database (blast.ncbi.nlm.nih.gov/Blast.cgi) and match it against thousands of other fungal species sequences. This process confirms, with great certainty, the identity of a fungus.

The first step in sequencing a portion of DNA is extracting the DNA itself from the fungal material, in this case, the hyphal growth in culture (Figure 8). After amplifying the gene sequence, it can be compared to other gene sequences registered on the NCBI BLAST database (Figures 9 & 10).

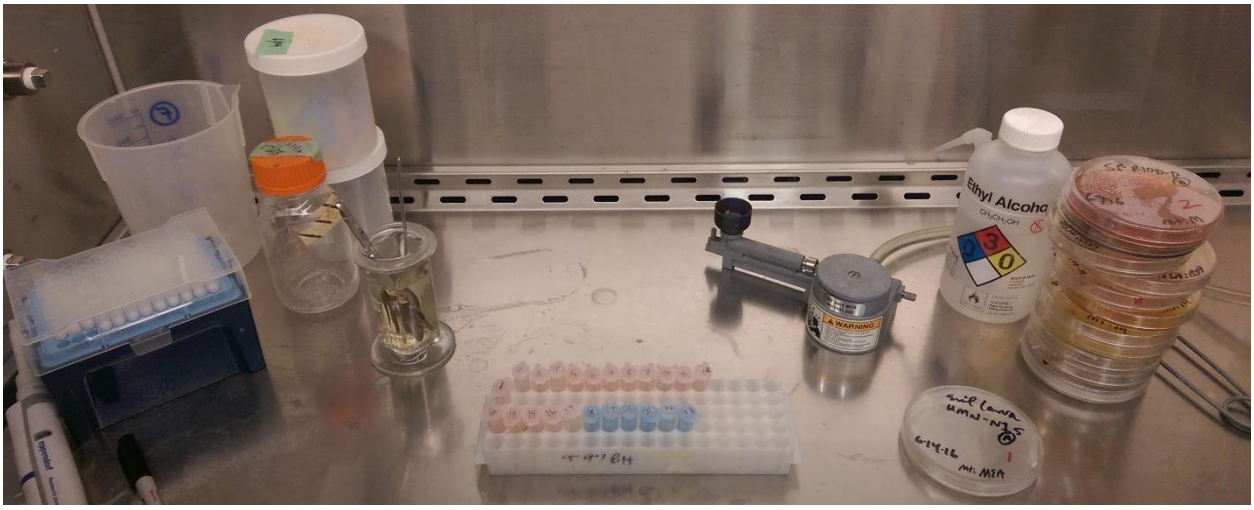

Figure 8 – First stage of a DNA extraction

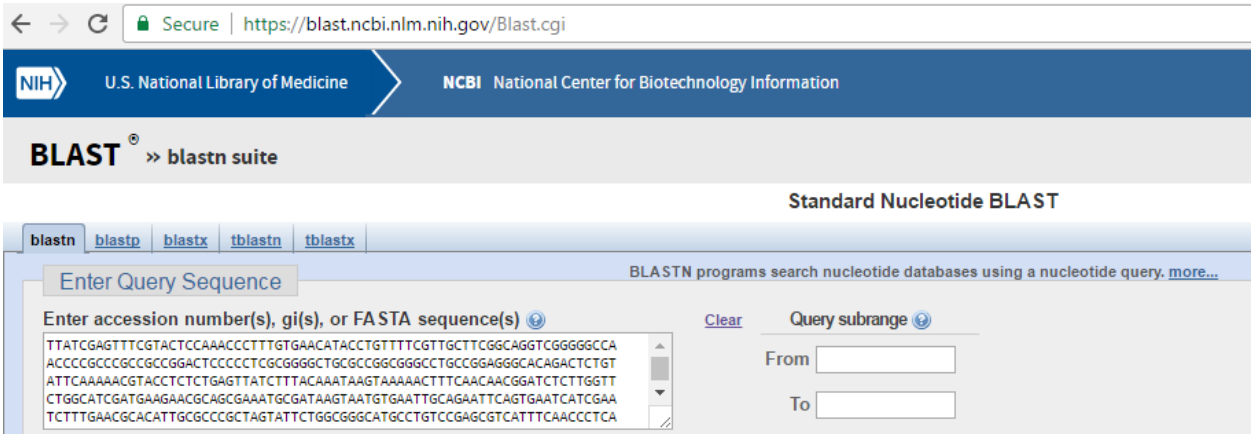

Figure 9 – The sequence of base pairs is entered into the BLAST database

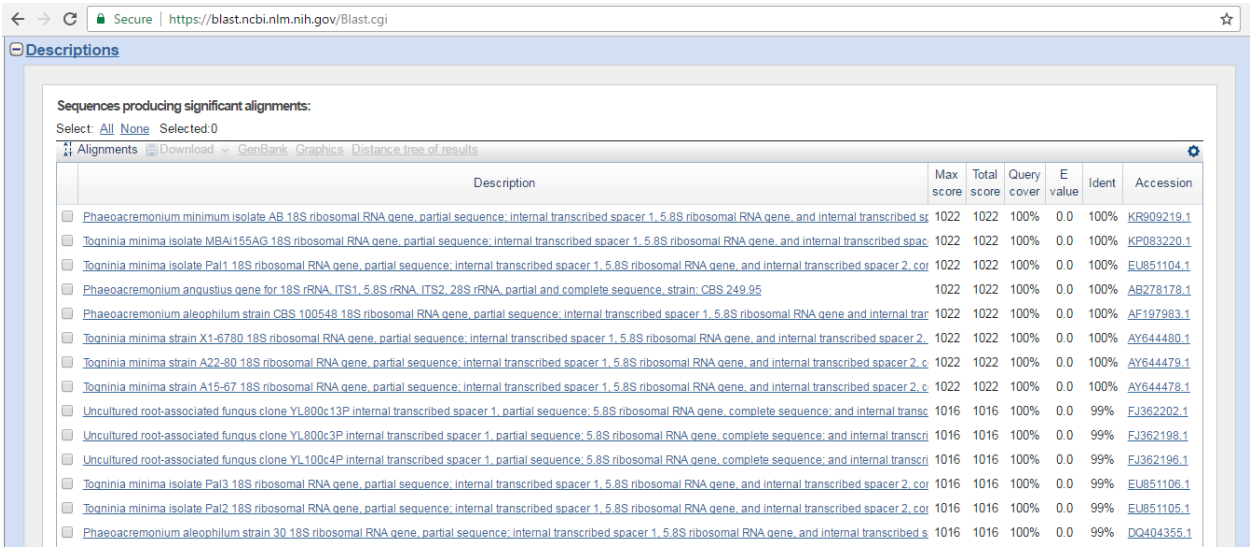

Figure 10 – The resulting species are listed by how well they match the sequence that was entered

Please, May I Have Some More?

Yes, yes you may! At almost any given moment, one of a handful of fungus-loving research laboratories at the University of Minnesota is diving into the fungal friends and foes of our forests. From the Dutch Elm Disease Resistance Program to the exploration of fungi surviving in extreme conditions where almost nothing else can— there is bound to be something that catches your fancy. Please check out the following University of Minnesota Forest Resources websites and investigate to your heart's content:

trees.umn.edu/

forestpathology.cfans.umn.edu/