Preparation of Moist Chamber Tree Bark Cultures: A Beginners Primer for Use at Home

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Many microscopic organisms spend their life cycles on live tree bark surfaces. Moist chamber cultures provide ideal conditions to observe the growth and development of these life forms on bark samples. Fruiting bodies of tiny fungi and myxomycetes are frequently observed by BRIT Researchers using these cultures. Some of these species are representative of new records for Tarrant County, the state of Texas, and even new to science. With this activity, you will enjoy the discovery of these organisms as well as myxobacteria, algae, mosses, liverworts, lichens, nematodes, insects, mites, and tardigrades, among others. The microenvironment of live tree bark is filled with understudied and diverse life forms. Join the Fungi, Myxomycetes, and Trees Research Team under the microscope and share your findings with us:

https://brit.org/urban-ecology-program/fungi-myxo-tree-program/

Email questions on species identification to haroldkeller@hotmail.com

For other questions, please email abordelon@brit.org

Tool Kit:

- Knife, flathead, or any other safe tool for tree bark removal
- Bag (plastic, paper, any bag!)
- Notebook & Pencil/Pen and Permanent Marker
- Pie tin (standard size is 9 in. diameter & 1-1/4 in. deep) or similar shallow container
- · Distilled Water
- · Clear plastic wrap
- Paper towels
- Hand lens or Microscope

This preparation is intended for live tree bark samples rather than fallen and dead parts of the tree. Before getting started, please make sure you have permission to be on the property of the tree you are sampling. Use caution when you remove the bark – we do not want to leave the tree injured or vulnerable to disease.

You are likely to have productive bark if sampling from Eastern Red Cedar (*Juniperus virginiana*) and American Elm (*Ulmus americana*), however you may not obtain results every time. Remember, this is science! Bark of many tree species is yet to be used in a moist chamber culture, so we encourage you to try new tree species or parts of the tree (base vs. canopy, trunk vs. limbs) to test for various results.

Procedure for Collecting the Bark Sample

- 1) Take your notebook and describe details about your collection
 - a) Where is this tree located? (ex. County, City, GPS Units)
 - b) Description of the tree you are sampling (ex. American elm tree, 38 ft. tall, 10 in. diameter)
- 2)Take photographs of the tree bark up close. You may not be able to visibly see anything with the naked eye, but these could be useful data in the future. You may also want to take a photo of the entire tree showing its habitat.
- 3)Carefully, use a safe tool of your choice to wedge a samples of bark off the tree. Be careful so as not to injure the tree; you only need the outermost trunk bark.
 - a) Strip enough bark to fill the bottom of a pie tin or two. Longer pieces, around 4 inches, are best but may not be feasible with certain tree species.
- 4)Place this bark in a paper or plastic bag with a written label with your name, and some addition unique identifier such as collection number, date, or tree species. This ensures your collection and data are linked!
- 5)It is best to put these bark samples in a moist chamber culture soon, within a day or two, but it will still work if you choose to wait longer.

Procedure for Preparing the Moist Chamber Culture

- 1) Take a pie tin and line the bottom with 1-2 sheets of paper towel.
- 2) Cover the bottom of the pie tin with your bark samples, giving them space without overlapping.
- 3) Measure around 50 mL of distilled water and pour around the outer edges of the pie tin. Try to avoid pouring directly onto the bark. The paper towels will start to soak up the water, followed by the bark.
 - i) If the bottom of your pie tin is not completely soaked, add small amounts of distilled water (15-20 mL at a time). You will know you have enough when the paper towel is soaking with no excess water. Use care if you need to pour out excess water, you do not want the bark samples disturbed too much.
- 4) Rip a piece of clear plastic wrap and write your initials, date of collection, tree species, and the date of adding water. This is called the "wetting" date.
- 5) Cover the pie tin with the plastic wrap.
- Place pie tin in an area with normal light conditions (no direct light) and average temperatures (around 68°-73° F).
- The following morning, remove any excess water from the pie tin or add more if it is needed.
- 8) You can remove the plastic wrap and start taking a closer look in the first few days! Use a hand lens or microscope and observe your bark samples.
 - a) Be mindful of the culture's exposure time to the air of your surroundings and possible contaminants when the plastic wrap is removed.
- 9) Record your observations.

10) Continue checking through the plastic wrap for any new growth over the next few weeks. Different species have different life cycles and will appear over the duration of your moist chamber culture.

Cultures can be kept up to thirty days. If you notice the paper towel and bark begin to dry out, add more distilled water and mark a second wetting date. This rewetting may stimulate the development of new species not found in the first round!

The BRIT Philecology Herbarium has both Mycological and Myxomycological collections that are available to the public virtually through www.mycoportal.org

You can also make an appointment to view specimens in person by emailing <u>herbarium@brit.org</u>

If you have found an interesting species that you would like to deposit as a specimen in the BRIT Herbarium, follow the steps below to create a good scientific collection:

- 1) Data! We cannot accept specimens with no data.
 - a. Examples of good data:
 - i. Scientific name (If you know it! We can assist with identification as well.)
 - ii. Date of collection, Date you added distilled water, and Date you observed & collected this species.
 - iii. Description of species color, habit, etc.
- 2) You will not be depositing your entire pie tin, only the pieces of bark with the species you are interested in. There may end up being more than one species on the bark pieces, you can indicate this in your data as well. These organisms are so tiny this is very common. Carefully remove the piece(s) of bark from the pie tin.
- 3) Optional * you may choose to use a pin to mark the location of the species on the bark.
- 4) Set the bark in a safe place to dry.
- 5) Once the bark piece is dry, you can place it inside a small box or container with your data and deliver it to the BRIT Herbarium! Contact <u>herbarium@brit.org</u> before arrival.