

ECOLOGICAL METHODS: FIELD SAMPLING TECHNIQUES

Lab Unit 4.1 - Fall: Leaf Herbivory and Fungal Occurrence on Tree Species

LEARNING OBJECTIVES

Students will....

- analyze leaves, stems, and trunks, of various tree species.
- evaluate the frequency of herbivory and fungus occurrence in various tree species by using basic leaf sampling techniques.
- characterize the tree as an ecosystem by observing patterns in and around various tree species.
- record, represent, and communicate findings (including photographing specimens).

BACKGROUND

Evidence of herbivory by invertebrates and fungal diseases on trees is a common occurrence and highlights the diverse interactions between many organisms and plants. Despite chemical and physical defenses employed by plants, there are many patterns of herbivory that can be observed on leaf surfaces that vary depending on herbivore foraging behaviors. For example, there can be chewing, scraping, mining, or piercing/sucking damage. There can also be damage by galls, commonly formed by oviposition (egg laying) and feeding behaviors of insects and spider mites. These cause abnormal growths, known as galls, of plant tissues which form an enlarged area that protects the developing organism. They can also be caused by bacteria, fungi, and nematodes (roundworms). Some insects can also damage the bark of trees by boring holes to feed or reproduce. Luckily for trees, many beneficial insects prey on the types of insects that feed and reproduce on trees, keeping their populations in check.

While herbivory can cause damage to the tree, by reducing leaf surface area and negatively affecting photosynthetic rates, transpiration, and overall health; fungal diseases add to this type of damage. The evidence of fungal disease on leaf surfaces is often manifested as brown spots and discolorations. It can also affect branches and tree bark. Fungal diseases as well as other types of viral and bacterial diseases of trees can cause bark weeping wounds, cracks, cankers, and cavities. Parasitic plants like mistletoe can infect trees by penetrating their branches and taking up water and nutrients. Although mistletoe plants carry out a small amount of photosynthesis, they rely on the host plant for much of its nutrition. If the amount of mistletoe is significant, it can cause the tree to die. Overall damage to a tree by herbivory or fungal infection can serve as an indicator of climate change since rates of damage can fluctuate with changing temperatures. Herbivory can also be looked at from the perspective of the “green world hypothesis” by Hairston, Smith and Slobodkin (1960), which states that percent herbivory is maintained at about 17%, primarily due to predation in combination with various density-dependent and density-independent factors.

In this lab, you will examine leaves to determine occurrence of herbivory and fungal diseases by assessing leaf surface area of a random sample of leaves from various trees on campus. In addition, you will record observations about any organism you see on or around the tree; including those that form non-harmful associations, such as lichens, mosses, and burls. In the following section, you will learn about each category of tree-to-species interaction you will be assessing.

NOTE: The following sections are accompanied by online materials found on the Lab Website for this unit. You will need to refer to the online materials to view images representing the type of herbivory, fungal infections, and other leaf damage.

OBSERVATIONS OF HERBIVORY

Chewing—Insects that chew on leaves will leave small holes in the center parts of a leaf or chewed areas along the edges. These insects can be caterpillars of moths or butterflies. Other common chewing insects are bagworms, beetles, and cankerworms.

Mining—Insects that “mine” through a leaf are considered substrate feeders because they form a tunnel through the leaf, living inside and eating the leaf between the lower and upper epidermis. Most common miners are the larvae of sawflies, a type of wasp, flies, and caterpillars of moths and butterflies. Sometimes, beetle larvae fall into this category, too.

Piercing/sucking—Many insects pierce the soft tissues of a plant, sucking out the nutritious sap content. They can also attach to stems, twigs, and branches. The most common examples are scale insects and aphids.

Scraping—Characteristic scraping damage on leaves: larvae feed only from one side (upper or lower surface) of the leaf. The damage caused by these insects may look similar to leaf miners, but they leave one of the surfaces intact. Mites and thrips, tiny slender insects, are in this category.

Galls—Abnormal growths that can occur on any part of a tree caused by various species of insects in the larval stage.

OBSERVATIONS OF FUNGAL DISEASES

Fungal spores can be transmitted through air or soil. Fungal infections are a major cause of disease in trees, and they can affect any part of the tree. They cause spots and discoloration, and it is not always easy to determine because damage by bacteria, insects, or nutrient loss can cause the same effects. Sucking and mining insects can also carry fungal spores on trees. Some of the best-known diseases caused by fungi include sudden oak death, blister rust, canker, wetwood, chestnut blight, and root rot; but there are many more.

OBSERVATIONS OF NON-HARMFUL ASSOCIATIONS

Lichens—An organism that is comprised of a symbiotic relationship between an algae and fungal hyphae. Lichens come in three main forms: crustose (encrusting), foliose (leaf-like with no branching), and fruticose (bush-like with branching).

Mosses—Tiny non-vascular plants that grow in moist areas. There are many varieties of moss that live in association on or around trees.

Burls—These are abnormal growths on trees of unknown cause, yet highly valued by wood carvers and instrument makers. They can range in size, shape, and amount on tree trunks and branches.

Mistletoe—Mistletoe grows as a parasite on trees, absorbing water and nutrients from their host. It is an obligatory plant parasite, meaning that it relies on attaching and living on other plants for its survival.

Bark Borers—Birds belonging to the woodpecker group which drill holes in the trunk of a tree in straight lines or around the bark in a perfect circle. They bore one quarter inch diameter holes to feed on the sap or insects that come out of the holes. Unless extensive, these birds rarely cause detrimental damage to the trees, yet the sap is vital to the birds' survival, especially for migratory species. Some insects may be classified as bark borers too, making holes that range from superficial to deep within the tree trunk. They lay eggs and the larvae chew their way out. These holes are random and not arranged neatly in rows or encircling the bark as seen with the woodpeckers.

Methods

You will be conducting data collection in any area where you can find trees (around your home, neighborhood, a local park, greenway, NCSU campus, etc.). **NOTE:** We are conducting non-destructive sampling, so do not remove leaves from the trees or damage surrounding areas when completing data collection. Download and use the "Data Collection Handout" from the Lab Website. Use the Lab Website for Unit 4.1 alongside this document to find helpful resources.

1 Location:

Select an area where you can locate at least **two** different trees. Ideally, they will be different species of trees so that you can compare them with respect to herbivory and fungal incidence, or you can pick different areas (for example, a completely natural area, a landscaped area, an urban area) and then select the same species of tree in each area. It is up to you how you want to design your study.

2 Approach your selected tree(s):

- a. Walk around the tree to visually inspect it, noting any organisms, overall leaf damage, bark damage, lichens, mosses, etc.
- b. Take pictures of your tree and any associated organisms or interesting observations (make sure you have GPS tags enabled on the picture settings).
- c. Use a phone application to identify your tree species (see the Lab Website for examples of herbivory and fungal occurrence).
- d. Include the location of the tree and its surroundings.
- e. Note all your observations in the space provided in the Data Collection Handout (see the Lab Website to download it), including a description of your sampling site(s).

3 Find a location on the tree that has evidence of leaf damage and "select" a leaf to evaluate:

NOTE: Refer to images in the Lab Web site and more instructions on how to measure leaf surface area. Look for herbivory and fungal damage/fungal presence using visual analysis (use a magnifier application on your mobile device). Without removing the leaf, you will place

a 1 cm² grid/graph paper transparency over the leaf to calculate approximate leaf surface area. See below for calculations:

- Full squares or squares more than half full = 1 cm²
 - Half-filled square is = 1/2 cm²
 - Less than half = 0 cm²
- a. *Calculate and record overall leaf area* (all possible squares that the intact leaf would take up). NOTE: if a large part of the leaf is missing due to damage, assess the total area that the leaf would have filled.
 - b. *Calculate area damaged by herbivory*. Take leaf surface area with herbivory considered. Count areas that are missing due to herbivory.
 - c. *Calculate area damaged by fungus or area of fungus present on the leaf*. Take leaf surface area with fungal damage/fungal presence considered. If fungus is spread throughout a box on the grid, estimate the amount of the box that it covers. For example, if it is spread throughout the entire box like dots, count it as one box worth of damage = 1 cm².

This will give you an approximate average surface leaf area lost to herbivores and to fungal infection. Record your data in Table 4.1-1 of the Data Collection Handout.

Example: In the image below (Figure 4.1-1), the darkened squares represent the part of the leaf still present, and lighter squares represent damage. The total surface area = 14.5 cm²

Herbivory leaf surface area = 6.5 cm²

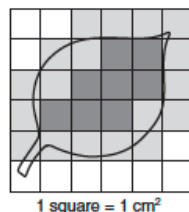


Figure 4.1-1. Estimation of Leaf Surface Area

- d. Repeat this protocol using simple random sampling for a total of ten different leaves and record your data in Table 4.1-1 (data collection Part II).
 - e. Using a piece of paper, cut it into four equal pieces and write, “Left,” “Right,” “Up,” or “Down.” You will randomly select which way you want to go by shaking up your paper pieces in a bag or other container and choosing one square each time. Return the used square to your bag/container. Repeat until done with all leaves. See below to learn how to generate a random number table. You will need the random number table before you start.
- NOTE:** If you get “Up” and you cannot go that high or cannot sample that leaf for any reason, go to the next random number until you find a leaf you can sample.
- f. Use random sampling technique as indicated on the Lab Website. (You will have to generate a table of random numbers using a random number generator:

<https://www.random.org/integers/>). You will start with one column of random numbers to move to your second leaf, take measurements, and move to the second random number. Repeat until done.

- g. Be sure to record your data in the Data Collection Handout, Table 4.1-1. Obtain SA totals for each category for your ten leaves.

WORKS CONSULTED

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