**Lichens in YOUR Local Landscape **

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**Pre-lab Reading**

The distribution and abundance of species are affected by the **abiotic** (non-living) and **biotic** (living) environment. Land-use modification and other anthropogenic drivers of environmental change (e.g., air pollution) affect landscape, local, and micro-site conditions. This, in turn, influences the ability of a given species to survive or thrive in an area. Lichens are an excellent group of organisms to explore how abiotic and biotic variables affect the presence and abundance of lichens - right outside your front door!

***What are Lichens?***

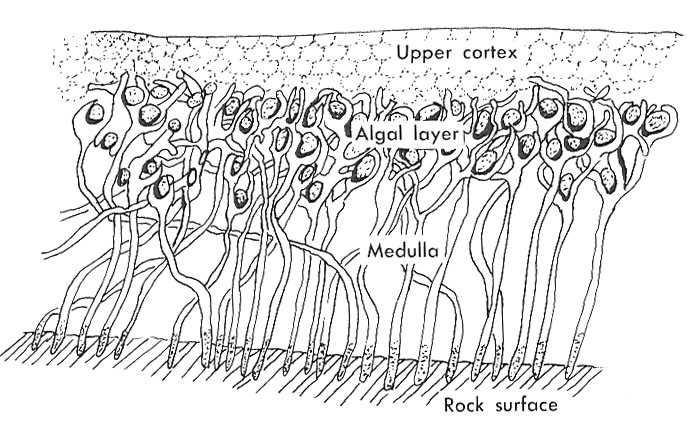
Lichens are **composite organisms**, which means that they are made up of two independent organisms living in symbiosis (close physical association). Figure 1 shows the generalized structure of a lichen. Lichens are **mutualistic symbionts**, meaning that both the fungus (the **mycobiont**) and algae or cyanobacteria (the **photobiont**) benefit from the close association. The algae or cyanobacteria are photosynthetic, so the fungus benefits from the carbon compounds (sugars) that the algae or cyanobacteria produce. In turn, the algae or cyanobacteria gain protection, nutrients, and moisture from the fungus. A win-win situation!

Figure 1. Generalized structure of a lichen thallus (non-reproductive tissues or vegetative parts): upper cortex (outer covering provided by the fungus), algal or cyanobacteria layer (photobiont), and medulla (stores nutrients produced by the photobiont) (<https://archive.bigelow.org/mitzi/spray_3.html>).

***Where are Lichens Found?***

Lichens are widespread and can be found in harsh climates, from hot deserts to cold alpine summits. However, within those ecosystems, they are found in specific **habitats** (natural environments in which they live). The key requirements for lichen habitat are: **water, air, nutrients, light, and substrate type** (USFS).

***Water*.** Lichens easily absorb water and water vapor through their **cortex** (outer layer of cells), but lack mechanisms to conserve water during drought periods. Lichens are **poikilohydrous** (water content determined by their surrounding environment); lichens lose water, dry up, and become dormant during dry periods, and rehydrate and become photosynthetically-active when moisture is available.

***Air*.** Not only do lichens absorb water from surrounding air, lichens also absorb nutrients and pollutants. They are sensitive to many air-borne pollutants, and the presence and abundance of some species of lichen are reduced in areas high in atmospheric pollution. For example, sulfur dioxide (SO2) combines with moisture in the atmosphere to form sulfurous acid (H2SO3) or sulfuric acid (H2SO4). High levels of SO2 pollution are associated with decreased lichen respiration and photosynthesis, deactivation of enzymes resulting in reduced metabolic activity, and reduced membrane integrity.

***Nutrients*.** Nutrients (e.g., nitrogen, carbon, oxygen) are needed for cellular processes that support lichen growth and survival. Lichens obtain their nutrients from air and water, and, to a lesser extent, their substrate.

***Light*.** Lichens produce their own energy through photosynthesis and, thus, they require light + carbon dioxide + water.

***Substrate*.** Lichens also require a substrate to attach to, and can be found on both natural (e.g., trees, rocks, soil) and artificial substrates (e.g., tombstones, buildings, abandoned equipment). Furthermore, characteristics of the substrate can influence how lichens interact with other aspects of their habitat. For example, substrate pH (e.g., limestone, tree bark with high (basic) pH) can counteract the acidity of SO2 pollution, so that in high pollution areas lichens may be found only on sites with high (basics) pH.

Although lichens are widespread, they need the right amounts of each of these key habitat requirements to survive and thrive!

***Lichens as Bioindicators***

Lichens are ubiquitous in urban and rural areas, and are also an important group of **bioindicators** - an organism whose presence, absence, and/or abundance in an area gives an indication of the degree of health of that ecosystem. For example, some organisms are very sensitive to pollution in the environment, so if pollutants are present, that organism may be absent, or may have different morphology or physiology, or may change its behavior. In contrast, other organisms are less sensitive to pollution in the environment. Documenting which of these bioindicator organisms are present, absent, and/or abundant in an area can be valuable in assessing ecosystem health and understanding whether or not an ecosystem may be impaired by pollution.

Some species of lichens are very sensitive to atmospheric pollution (e.g., nitrogen, sulfur, lead), whereas other species of lichens are tolerant to those pollutants. Lichens can be classified based on the structure of their **thallus** - their nonreproductive tissues or vegetative parts. Luckily, the degree of sensitivity of a lichen to air pollution varies roughly with their growth form. This makes lichens ideal to use as bioindicators.

There are three main types of lichens (Figure 2):

***Crustose*:**

* Crust-like
* Strongly adhere to the substrate; color varies

***Foliose*:**

* Leaf-like
* 2-dimensional (top and bottom “sides”)
* Flat and leafy like lettuce, or with ridges or bumpy

***Fruticose*:**

* Branched-upright or **pendulous** (hanging down loosely)
* 3-dimensional
* Hair-like or upright and shrubby

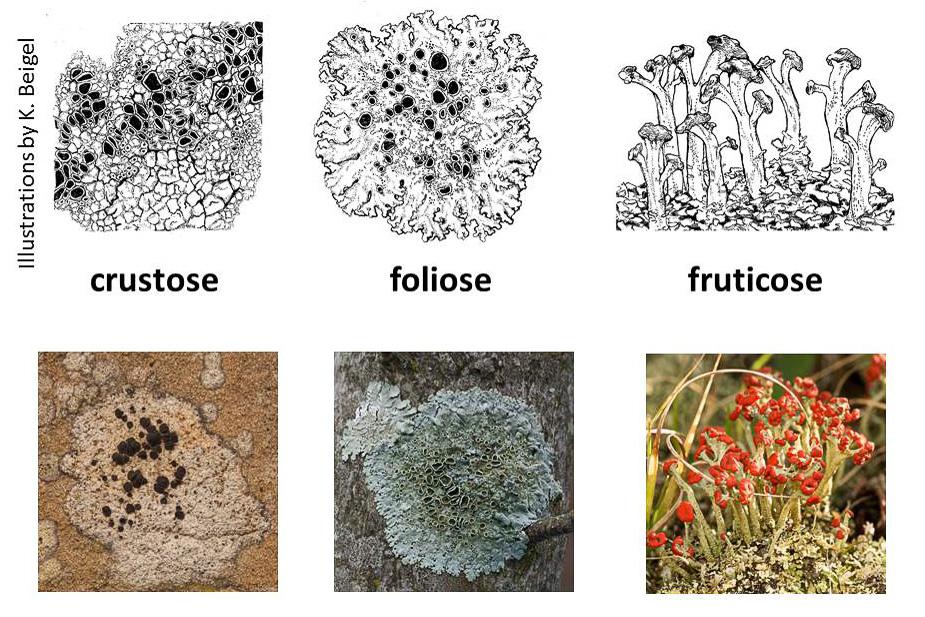


Figure 2. Three main types of lichens: crustose (thin, crust-like, very tightly attached to substrate), foliose (flat and leaf-like; 2-dimensional), and fruticose (upright, like tiny trees or shrubs, or hanging down like a miniature vine) (<https://ohioplants.org/lichen-biology/>).

For this lab, we will be using a modified version of the Hawksworth and Rose (1970) air quality index. This air quality index is based on the type of lichen(s) present, and ranges from 1 to 10, with 1 indicating the poorest air quality and 10 indicating the best air quality (Table 1). In general, lichens that are least sensitive to air quality are crustose, and lichens most sensitive to pollution are fruticose, with foliose lichens in between those two extremities. Two species of fruticose lichen, *Lobaria pulmonaria* and *Teloschistes exilis*, are extremely sensitive to air pollution and, thus, if either species are present, the air quality is considered very high.

*Lobaria pulmonaria Teloschistes exilis*

**Table 1. Lichen air quality index scoring.**

|  |  |  |
| --- | --- | --- |
| **Lichen Type** | **Air Quality** | **Air Quality Score** |
| No lichens present | very poor | 1 |
| Crustose only | poor | 3 |
| Foliose present, but no fruticose | moderate to good | 6 |
| Fruticose present | good | 9 |
| *Lobaria pulmonaria* or *Teloschistes exilis* present; fruticose lichens, very sensitive to pollution | very high | 10 |

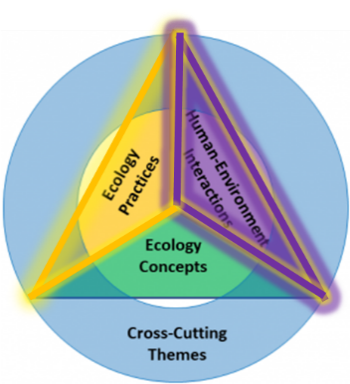
***iNaturalist Project EREN Lichen!***

Students will be contributing to a new iNaturalist project “EREN Lichen.” iNaturalist is a web-based and smartphone app platform that is used to document observations of species in a particular date and time. It is used and supported by an online community of naturalists, citizen scientists, and biologists, who help in crowdsourcing and confirming species identifications. Submitted observations are accessible to all members of the iNaturalist community, so they provide an excellent resource for exploring, teaching, and citizen science research opportunities. It is a great, user-friendly way to learn more about natural history.

The EREN Lichen project is new, so the number of observations in the iNaturalist project is limited. However, as classes from different locations adopt this module and contribute to the iNaturalist EREN Lichen project, these data can be used to explore hypotheses that address ecological questions across larger scales. The EREN Lichen project data can also be combined with other datasets (e.g., NEON, Living Atlas, NLCD) to broaden these types of analyses and fill in gaps.

We are looking forward to working with participants in the EREN Lichen project to explore these possibilities as the project grows. So get into the ground-level with this project, and let's see where it takes us!

In this learning module, students will use smartphones and simple equipment to collect field data on lichen presence, abundance, and growth forms on the tree surfaces. Students will join and upload their data into *i*Naturalist smartphone app’s project: EREN lichen. Students will collect measurements of abiotic and biotic variables, such as tree species, tree dbh (diameter at breast height), canopy cover, and bark pH (optional). For each tree sampled, students will document lichen presence and abundance at four locations, corresponding to the four cardinal directions: North, East, South, and West. Students will also have the option to take samples of tree bark to gather data on bark pH (although bark pH is optional because it requires access to deionized water and a pH meter or test strips). Students will develop hypotheses regarding the distribution, abundance, or lichen index of air quality and abiotic or biotic factors of interest. Students will analyze data gathered by their class or, if desired, data gathered from other classes that are contributing to the *i*Naturalist project: EREN lichens.

*** Student Learning Outcomes***

1. Gain experience in species identification, morpho-species classification, and how to make abiotic and biotic measurements in field settings.
2. Develop technical skills using modern technology (smartphone apps, spreadsheet programs, data analysis packages) and everyday materials (ruler, freezer baggie, sharpie permanent marker) to make scientific measurements and analyze data.
3. Examine how bioindicator organisms can be used to assess land-use change, air pollution, and other environmental impacts.

***Pre-Lab Activities***

***1. Developing Hypotheses***

***Forming Hypotheses to Test***

Overview: In this lab we will be exploring relationships between lichen presence and abundance and abiotic and biotic factors. Consider the key habitat requirements of lichens. Think about these questions: What abiotic and biotic variables may affect the presence and abundance of lichens, in general, or type of lichens present?

**Develop a potential hypothesis to test for this learning activity. Submit your hypotheses in the PreLab Assignment in Blackboard prior to your lab period.**

Response (Dependent) Variable:

Predictor (Independent) Variable:

What do you expect the relationship to be?

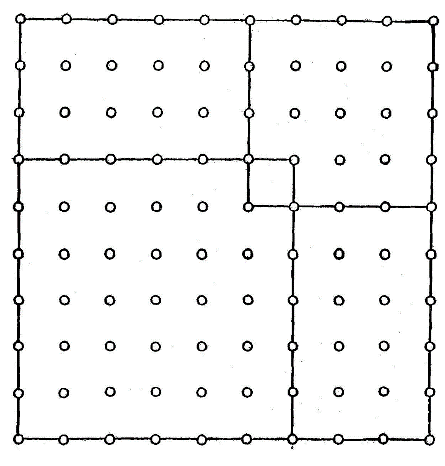
Rationale:

Write Out Your Complete Hypothesis:

***2. Download the following apps to your mobile device:***

* **For each of these apps, make sure you know how to use them before lab. If they need a username and password, do these important steps ahead of time!**
* *i*Naturalist. Make an account and join the EREN Lichen project. Note: It’s much easier to set up an account and join the EREN Lichen project via computer instead of smart phone. Once you have your account set up, log in on your smart phone so you’re ready for class
* Seek – great for identifying common organisms
* Canopeo – estimates vegetation cover
* A compass app

***3. If you are working from home, make a clear lichen sampling grid:***



* **Materials (provided in your lab packet)**
  + Quart or gallon baggie
  + Grid template. This print-out says “Appendix A. Lichen Sampling Grid Template” at the top and looks like the grid on the right
  + Sharpie
* **Make the sampling grid** following the instructions in “Appendix B. Making a clear plastic lichen sampling grid” found at the end of this document

***4. Watch the Field Sampling Demonstration video*** *showing field sampling in real time* [*(11 min)*](https://www.youtube.com/watch?v=zdJQ0YLqJpo&feature=youtu.be)*.*

* Also available in the lab component of the Blackboard module
* And at the project’s [Adobe Spark](https://spark.adobe.com/page/jFCQtlHNhMchS/) page

***5. Make a field data set***

Copy these two data table templates into your field notebook (wherever you keep your lab notes this term):

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | |  | |  | |  | |  | |  | |  |
| Title: Lichens in YOUR Local Landscape – Field Datasheet | | | | | | | | | | | | | | | | |
| Site: |  |  |  |  |  | |  | |  | |  | |  | |  | |
| Names: | |  |  |  |  | |  | |  | |  | |  | |  | |
| Date: | |  |  |  |  | |  | |  | |  | |  | |  | |
|  |  |  |  |  |  | |  | |  | |  | |  | |  | |
| Tree No. | Tree Species | % Canopy Cover | Tree diameter; dbh (cm) | %Lichen- North | %Lichen- East | | %Lichen- South | | %Lichen- West | | Air Quality Score | | Photos taken? (Y/N) | | Comments | |
| 1 |  |  |  |  |  | |  | |  | |  | |  | |  | |
| 2 |  |  |  |  |  | |  | |  | |  | |  | |  | |
| 3 |  |  |  |  |  | |  | |  | |  | |  | |  | |
| 4 |  |  |  |  |  | |  | |  | |  | |  | |  | |
| 5 |  |  |  |  |  | |  | |  | |  | |  | |  | |
| 6 |  |  |  |  |  | |  | |  | |  | |  | |  | |

Title: Lichens in YOUR Local Landscape – Photo Checklist:

|  |  |
| --- | --- |
| PHOTOS (take in this order) | Y/N? |
| 1: Dominant Lichen |  |
| 2: Canopy (N side) |  |
| 3: %Lichen - North |  |
| 4: %Lichen - East |  |
| 5: %Lichen - South |  |
| 6: %Lichen - West |  |
| 7+: tree ID photo(s) |  |

***6. Review the rest of this lab packet carefully.*** *Either print it out or have it accessible electronically for use during the lab.*

**Equipment list**

* **Smartphone and apps (**iNaturalist, Seek, Canopeo, a compass)
* **Flagging tape.** This is colorful and stretchy (provided, or sent in lab packet)
* **Thumb tacks**
* **Measuring tape**
  + These will be provided during lab
  + If you’re working from home, you will use the standard side of your DBH tape (carefully without bending the tape)
* **DBH tape** (cm) or string, thumbtack, and measuring tape. DBH tape is specialized measuring tape and was included in lab packets or will be provided during lab
  + One side of the measuring tape reports standard units (inches, cm, or both).
  + The other side reports diameter – it is marked to convert from circumference
  + Be sure to pay careful attention to which side you’re using and try not to bend them!
* **Clear lichen sampling grid**
* **Lichen air quality index scoring table** – provided at the beginning of this document
* **Field datasheet** to write down data before entering into *i*Naturalist
* **Sharpie**
* **Hand lenses.** For looking more closely at the lichen!

**Field Sampling Protocols**

Each student (pair or group of students) will collect data on several trees:

* If you’re working alone, collect data on 3 trees
* If you’re working in a pair, collect data on 5 trees
* If you’re working in a group of 3 or 4, collect data on 6 or more trees

Trees should be a minimum of approximately **30 cm (12 in) diameter**. A tree needs to have a large enough diameter to be able to take the four % lichen readings, without having any overlap. Be sure to bring your copy of the field data sheet (or notebook) to record measurements as you take them. At the end of sampling each tree, you will enter those data into the *i*Naturalist EREN Lichen project.

Remember that you can review the field sampling video [here](https://youtu.be/zdJQ0YLqJpo).

**Step 1.** **Identify the tree species.**

* Use field guides, identification keys, or smartphone app “Seek” to identify the tree to species level.
* Even if you initially record the common name, please use the scientific name (genus species) when you enter the name of tree species into the EREN Lichen project.
* ***Record the tree species on your datasheet.***
* If you cannot identify the tree to species level, then crowdsource tree species identification via *i*Naturalist:
  + pload an observation to iNaturalist.
  + Do not enter it directly into the EREN Lichen project - upload the observation to your general iNaturalist account.
  + Include one or more photos that show key details (e.g., leaves, fruits, bark). This will help with crowdsourcing species identification through iNaturalist.
  + When you enter your data into EREN Lichen, you can just skip that field for now - just enter “NA”.
  + Once you have the species, you can log into your *i*Naturalist account and update your observation.

**Step 2. Determine the north-facing aspect of the tree.**

* Use a compass or compass app on your smartphone to identify North.
* Then, move to the tree.
* Standing with the compass in your hand, your back to the tree, and facing away from the tree - move around to determine which part of the trunk faces North (00).

**Step 3. Measure percent canopy cover (if you do NOT have the Canopeo smartphone app, then skip this step).**

* Stand at the base of the tree, at the north side of the tree.
* Open up the Canopeo app.
* With your back to the tree, hold the phone overhead (making sure it is parallel to the ground), and take the photo.
* Canopeo will give you percent canopy cover.
  + Be sure that you are getting % canopy cover and NOT % open!
  + If CANOPEO is giving you % open, then subtract % open value from 100 - that will give you % canopy cover. (Equation: % canopy cover = 100 - % open.)
* ***Record the value on your datasheet or in your notebook.***

**Step 4.** **Measure tree diameter, in centimeters.**

* Tree diameter (diameter at breast height; dbh) is measured at 1.37 m (4.5 ft) above ground level.
* Measure with a dbh tape, which will measure the diameter directly.
* Find the side of the dbh tape that shows **“Diameter”** – the other side is standard units, which you do NOT want to confuse for diameter units.
* If you are working by yourself and the tree is too large, use the thumb tack to pin the loop at the end of the dbh tape to the tree, then wrap the dbh tape around the tree
* Measure the dbh by wrapping the tape around the tree and recording where the beginning meets the zero line (see image at right)
* Pay attention to what units you used, and record in your field notebook the **units** of your dbh tape (in or cm)
* If you do not have a dbh tape, you can use a string.
  + Affix a string to the tree (with a thumbtack) at 1.37 m (4.5 ft), and measure the circumference of the tree with the string.
  + After determining the circumference of the tree, convert it to diameter. (Equation: d = Circumference divided by pi.)
* ***Record the value on your datasheet or in your notebook. Be sure to upload dbh data in cm in your final data***
* **Wrap Flagging Tape:** Before you forget where 1.37 m (4.5 ft) is, wrap the colorful flagging tape around the tree at that height – this will help keep your sampling grid in place. Again, if the tree is too large, use a thumb tack to hold the flagging in place, wrap the flagging around the tree, and tie off in a knot. The flagging rips easily so you should not need scissors.

**Step 5.** **Record percent lichen at the north, east, south, and west faces of the tree.**

* Take the clear lichen sampling grid, and place the top of the grid so that it aligns with where you took the dbh reading (1.37 m; 4.5 ft above the ground; that’s why the flagging is there).
* Mark **“N”** on the flagging tape so you know where you started.
* The sampling grid has 10 rows of 10 circles. Count the number of circles that have lichens.
  + If you tally up the lichen circles systematically, it should be straightforward.
  + If you have a dry erase marker (and eraser), you find that helpful in keeping track of the count.
* ***Record the value on your datasheet or in your notebook.***
* **Repeat** for each cardinal direction: mark the direction with a Sharpie on the flagging tape, count the circles that have lichen, and record that value in your notebook.

**Step 6.** **Take photos and upload to the *i*Naturalist EREN Lichen project.**

* ***IMPORTANT***: Be sure that you take and upload photos in the specific order outlined below and indicated in the Photo Checklist you copied into your notebook. That way it will be standardized and we will be able to match up the grid photos with the corresponding tree aspect.
* Open up the iNaturalist app on your smartphone.
* Take the following photos IN THIS ORDER:
  + *Photo 1*: Look around the tree and find the **dominant lichen**. Find a good example of the dominant lichen, take a photo, and upload it as your first image. That lichen does not have to fall within your sampling grids.
  + *Photo 2*: Take an image of where you took the first lichen sampling grid reading, on the North face of the tree.
    - You will probably capture a bit more than the area you sampled with the grid - that is okay! This will give us a reference image so that if anyone wants to go in, and maybe zoom in to identify the lichens present, this will be a great resource.
  + *Photo 3*: Take an image of where you took the east-facing lichen grid sample.
  + *Photo 4*: Take an image of where you took the south-facing lichen grid sample.
  + *Photo 5*: Take an image of where you took the west-facing lichen grid sample.
  + *Photo 6*: Take an image of the canopy, from the north end of the tree. (If you took a canopy cover image with CANOPEO, you will take the image in the same manner.)
    - Move to the north end of the tree, hold your phone overhead, parallel to the ground, and take the image.
    - This will allow getting a measurement of canopy cover, regardless of whether you recorded percent canopy cover with CANOPEO.
  + *Photo 7 (or more)*: Take a photo (or photos) of the tree. Take whatever photo(s) that you think would be helpful in identifying the tree to species level.
    - These image(s) can be used as reference documentation, and may help getting some trees to species level.

**Step 7.** **Add this observation record to the *i*Naturalist EREN Lichen project.**

* In *i*Naturalist, click on Add to Project, and select EREN Lichen.
  + Please note that you had to have joined the EREN Lichen project in *i*Naturalist prior to going out in the field!
* Once you select the EREN Lichen project, there will be a number of fields to enter your data into:
  + EREN Lichen tree species (tree species that you are sampling for lichens; scientific name, if possible)
  + EREN Lichen percent canopy cover (taken at the base of the north side of the tree, taken with CANOPEO)
  + EREN LichenPercent NORTH (% lichen north-facing quadrat)
  + EREN LichenPercent EAST (% lichen east-facing quadrat)
  + EREN LichenPercent SOUTH (% lichen south-facing quadrat)
  + EREN LichenPercent WEST (% lichen west-facing quadrat)
  + EREN Lichen air quality index score for the tree (modified Hawksworth Rose index score)
    - No lichens present = 1
    - Crustose lichens only = 3
    - Foliose lichens present, but no fruticose = 6
    - Fruticose lichens present = 9
    - *Lobaria pulmonaria* or *Teleschistes exilis* present; fruticose lichens, very sensitive to pollution = 10
    - Note 1: Be sure to look over all areas of the tree (that are visible to you from the base of the tree) to determine the air quality index value. It is not restricted to where you measured with the lichen sampling grid.
    - Note 2: Only enter one value into this field. For example, if you ONLY have crustose lichen present, then it would be a score of 3. If you have BOTH crustose and foliose lichens present, the index score will be 6. If you have crustose, foliose, and fruticose lichens present, the index score will be 9.

**Step 9.** **SAVE or SHARE the *i*Naturalist record.** Exact wording may vary, depending on the type of phone or specific version of *i*Naturalist.

**Future Explorations with iNaturalist project EREN Lichen**

Once the *i*Naturalist project EREN Lichen gets contributions from multiple sites, there is excellent potential to expand spatial scale of analyses and breadth of research questions to address. Data can be explored from multiple EREN Lichen sites. These data can also be combined with other datasets (e.g., NEON. Living Atlas, NLCD) to broaden these types of research questions and analyses.

**References**

Hawksworth DL, Rose F. 1970. Qualitative scale for estimating sulphur dioxide air pollution in England and Wales using epiphytic lichens. Nature; London 227: 145–148.

Appendix B. Making a clear plastic lichen sampling grid

(if you’re working from home)

Items needed:

* Lichen sampling grid template (Appendix A.)
* Ziploc freezer baggie (or other brand; quart or gallon size)
* Tape (to hold the baggie and grid in place; optional)
* Sharpie permanent marker (or other brand; fine point - not too thick!)

Procedure:

1. Take the double-layer freezer baggie, and separate the two layers.
2. Place the sampling grid template into the freezer baggie. Make sure that any labels on the bag do not overlap the grid.
3. Secure the template and freezer baggie to a flat surface, so that they don’t move.
4. Using the Sharpie permanent marker, trace grid circles and lines (see photo). Try to avoid making any smudges. (Do not rush or you may cause the freezer baggie to slip.)
5. Let the ink dry.

