LESSON

SEEING FUNGAL DIVERSITY

DESCRIPTION:
Teachers and students collect leaves infected with powdery mildew fungi from different kinds of garden plants and examine them with dissecting and compound microscopes. This exercise can be used to explore the ecology of a diverse group of fungi and/or as a way for students to learn about how fungi are classified. This lesson introduces students to a common group of fungi: the powdery mildew fungi, which are obligate parasites of plants (or biotrophs, meaning that they cannot be cultured on nutrient media and require a living host to grow). This lesson can be done year-round after the initial collection of infected plant debris in late summer or autumn. Over time, your students can test which species tend to infect specific families of hosts.

BACKGROUND INFORMATION:
Powdery mildews are one of the most common, conspicuous, widespread, and easily recognizable plant diseases. As a group, powdery mildew fungi infect many species of plants, including many trees and shrubs, numerous ornamentals, vegetables, cereals, grasses, and even weeds. However, individual species of powdery mildew fungi are usually very host specific. For example, the species of fungus causing powdery mildew on pumpkin is different from that causing the disease on roses.

Extensive losses in plant growth and crop yield occur annually due to powdery mildew. The primary sign of powdery mildew is grayish white, powdery blotches on leaves. Usually, powdery fungal growth appears first on the upper leaf surface (Figure 1).

Figure 1. Hyphae and conidia of powdery mildew fungus on a grape leaf

Figure 2. The white, brown, and black spheres are cleistothecia at different stages of maturity.

Powdery mildew can cause distortion of leaves and stunting of plant growth. The type and extent of symptoms vary depending on the combination of powdery mildew fungal species.
and host plant species involved. Late in summer and fall, the sexual stage of many species of powdery mildew fungi, the cleistothecia, is visible as black or brown, pinhead-sized, spherical specks among the white to grayish mildew mycelium in the older infected areas on the leaves of many plants (Figure 2).

Collect these leaves that you find and press flat between newspaper for storage and use for this lesson.

The powdery mildew diseases of various crops and other plants are caused by many different species of fungi grouped into six main genera in the order Erysiphales, an order that includes a single family, the Erysiphaceae. The fungi causing powdery mildews are obligate parasites. These fungi reproduce by means of two spore types: asexual spores called conidia and sexual spores called ascospores.

Figure 3. Conidia (looks like water droplets), which grow from the base structures called conidiophores (the stick part).

Figure 4. Powdery mildew fungi absorb nutrients from the host plant through haustoria.

The conidia are usually barrel-shaped or oval and are usually formed in chains (Figure 3) at the ends of specialized hyphae called conidiophores produced from the mycelium growing on the surface of a plant's leaves, stems, flowers or buds.

The combination of the mycelium, conidia and conidiophores gives the leaf surface a powdery appearance from which the name powdery mildew is derived. The fungi are spread when the conidia are carried by air currents to new plant surfaces. Under favorable conditions, the fungal spores germinate. Fine, thread-like infection pegs penetrate the epidermal cells of the leaves and form haustoria (Figure 4).

Haustoria are specialized hyphae for nutrient absorption from the plant cells. The plant is damaged by the loss of nutrients to the fungus, disruption of photosynthesis, and premature death of leaves or other infected plant tissues.

When environmental conditions or nutrition become unfavorable for growth (usually later in the growing season), the fungus shifts to the sexual stage and produces cleistothecia (sing. cleistothecium). The cleistothecia are closed, thick-walled, tiny, black, spherical structures (white to tan when young) that house sacs called asci (sing. ascus) (Figure 5 and Figure 6).
Cleistothecia have ‘arm-like’ appendages that radiate out from their outer surface. Inside each cleistothecium is a single ascus or many asci. Currently, the powdery mildew fungi are classified to genus based on the number of asci contained in the cleistothecium and on the morphology (physical appearance) of the hyphal appendages (arms) growing out of the wall of the cleistothecium.

OBJECTIVES:
Students learn to use microscopes to find, identify and classify fungal plant pathogens from a school garden and beyond. Students become aware of the diversity of the fungal branch of life and witness host-parasite interactions on a macro and microscopic scale.

COMMON CORE STANDARDS:
CCSS:
12.RST 4: Determine the meaning of symbols and key terms as they are used in a technical context.
12.RST 7: Integrate quantitative or technical information expressed in words in a text with a version of that information expressed visually (e.g. graph, model, table).
12.SL 1: Engage effectively in a range of collaborative discussions with diverse partners.

NGSCS:
LS2.C: Complex set of interactions within ecosystems
LS2.C: Ecological disturbances (biological, physical, human-induced) may result in an ecosystem that reverts to a similar original state or become completely altered.
LS4.A: Phylogenies can be inferred by DNA, AA sequences, and morphological/embryological evidence
LS4.B: Natural Selection only can occur if there is trait variation
LS4.D: Biodiversity increases with more species and decreases with extinction
NGSCS Cross-Cutting Concepts:
1. Patterns: Different patterns may be observed at each of the scales at which a system is studied.
2. Patterns: Empirical evidence is needed to identify patterns.
3. Scale, Proportion, and Quantity: Patterns observable at one scale may not be observable or exist at other scales.
6. Structure and Function: The way an object is shaped or structured determines many of its properties and functions.

TIME: TWO 45 MINUTE DAYS. DAY ONE: PART A & STATION B; DAY TWO: STATION C & STATION D.

MATERIALS:
Materials for collecting and drying leaves (best done in late summer, autumn):
- Newspapers or old telephone book paper
- Plant press or heavy books
- Envelopes

Materials needed for exercise:
- Water in dropper bottles (1 per group)
- Microscope slides
- Dissecting Needle or Toothpicks
- Clear Tape
- Light Microscopes (at least 1 per group)
- Dissecting Microscopes (at least 1 per group)
- Printed Fungi Keys
- Razors
- Cutting boards
- Paper Towels
- Gardening gloves and scissors
- Sharpies
- Fungal Cards
- Printed worksheets and Station Titles
PREP:

- Collect infected plant leaves in late summer or autumn that show signs of powdery mildew and mature (black) cleistothecia. Alternatively, assign groups of students to make their own collections. Check asters, beebalm (Monarda spp.), dandelions, lilac, phlox, rose, zinnia, evening primrose (Oenothera spp., often called sundrops), lawn grasses, wheat, crepe myrtle, cherry, apple, hawthorn, oak trees (red), willow, viburnum and many other shrubs, trees, flowers, weeds and garden plants for infected leaves. Look for a pale, dusty-white coating on leaves. The black, minute cleistothecia are generally visible to the unaided eye, although a magnifying glass may help.

- Infected leaves should be pressed flat between pieces of newspaper and placed in a plant press, or placed beneath some type of heavy object, e.g. large heavy books. Use several layers of newspaper to absorb moisture from the leaves to prevent the leaves from becoming moldy while drying. You can also easily improvise a “plant press” by placing infected plant leaves between the pages of an old telephone book and then stacking a heavy book on top of the closed telephone book. Once dried, place dried leaves protected by newspaper/telephone book paper inside envelopes for storage. These can be re-examined over many years.

Prep for Station A

- Station A Title page, Station A worksheet, Dissecting microscope, Light microscope, small razor, tape, water dropper, cutting board, glass slides, coverslips

Prep for Station B

- Station B Title page, Station B Worksheets, Dissecting microscope, Light microscope, small razor, tape, water dropper, cutting board, glass slides, coverslips, Fungi Key for species identification

- Two infected plants (Plant A & Plant B) or two infected leaves infected by different powdery mildew species

Prep for Station C

- Station C Title, Station C Worksheet, Sharpies, Fungal Cards
ACTIVITY:
DAY ONE
1. Start with a Think-Pair-Share activity:
   - Have students individually think about the following question: What is the largest organism on Earth today? Do you think it is large in size or weight? (one minute).
   - Have students form groups of four to discuss (five minutes). Possible answers:
     - Mass: the Aspen Trees in Utah nicknamed “Pando” are actually all connected as one organism because their roots are connected. Each tree formed through root sprouts coming off an original parent tree. It covers 0.43 km² and weighs 6,600 tons.
     - Height: A California Redwood named “Hyperion” is 115m (379 ft) tall
     - Area: Honey Fungus (Armillaria ostoyae) in Oregon, spanning 8.9 km² and weighs 605 tons.
   - Discuss as a class.
2. Watch a video by MinuteEarth called “The Biggest Organism on Earth” (5 minutes). Tell students that they will be hunting for fungi, but looking for a specific type, called powdery mildew, which doesn’t have a mushroom top. With that hint, have the student-groups explore in the garden and see if they can find powdery mildew any plants (10 minutes).
3. Have the class examine the first mildew found and use that as a reference to look for more. Have each group collect only one live sample if possible. Teacher should collect one sample. Arrive back in the classroom.

Station A
1. Guide the classroom on how to properly dissect their samples to see powdery mildew. Also guide them through the tape-method. If necessary, review how to use the dissecting and light microscopes.
   - To see whether fungus is growing inside the leaf, need to make a cross-section microscope slide. To do this, use a dissecting microscope, a leaf-sized cutting board and a small razor to slice as thin a piece of infected leaf as you can. Once you have a thin sliver, place it on a glass slide with water and a coverslip. Check it under a light microscope.
   - To get a closer look at the fungus growing on the leaf’s surface, take a small piece of scotch tape and stick it to an infected area. Slowly peel away the conidia and stick to a glass slide. Use a water dropper to place some water underneath the piece of tape.
2. Have student-groups inspect their samples and the dried samples prepared by the instructor. They may need to be dampened before cutting or using the tape on them. Cover in wet paper towels to do this.
3. While they are working on this, ask some Exploration Questions found in the Appendix.
DAY TWO

4. Tell students about the diversity of fungi, how they can be very big and very small. There are even differences within the ones that are small. Introduce the students to the diversity of powdery mildew fungi using photos of microscope images to show the different number of asci and different appendages. (10 minutes)

5. Have the same groups regroup. Have half of the groups go to Station B and the other half go to Station C. Instructions should be at the stations for the students.

6. Station B – Problem solving – can you figure out which pathogen is on Plant A and Plant B? (15 minutes).

Station C – reading about Phylum, Kingdom classification and structures of fungi. Not all fungi look like mushrooms! Which group contains mushrooms? (15 minutes)

DISCUSSION:

4. Now that you see how powdery mildew fungi are constructed, how do you think they attack their hosts?

5. What are some possible organic solutions you can think of to combat an infestation of powdery mildew fungi?

6. Why is it important to know which organism is infecting a plant or garden before creating a management plan and selecting something to fight it?

TEACHER TIP:

- If you wish to minimize time and replace an activity – you can have students make spore-prints and make drawings out of them. For more information, see: http://howtosmile.org/record/9300

REFERENCES


**Fungi Key**

**Key to Genera of Powdery Mildew Fungi**

1. Appendages coiled or hooked at tip — *Uncinula*
   Appendages simple and straight with bulb-like base — *Phyllactinia*

2. Appendages branching dichotomously at tip
   - Cleistothecium contains a single ascus — *Podosphaera*
   - Cleistothecium contains several asci — *Microsphaera*

3. Appendages simple or irregularly branched, often entwined
   - Cleistothecium contains a single ascus — *Sphaerotheca*
   - Cleistothecium contains several asci — *Erysiphe*
STATION A
Exploration
1. Place a leaf with cleistothecia under a dissecting microscope.
2. While looking through the dissecting microscope, place a small drop of water on a group of cleistothecia.
3. Still looking through the dissecting microscope, use a dissecting needle or toothpick to gently tease away several cleistothecia from the leaf surface into the water droplet.
4. Using a piece of clear tape (smaller than the microscope slide), hold onto one end of the tape and smooth the rest of the tape (sticky side down) over the cleistothecia and water droplet. Slowly peel back the tape.
5. Place the tape (sticky side down) on the microscope slide. If necessary, more water may be added by squeezing the water dropper gently at the edge of tape on the slide.
6. Place the slide on the stage of the compound microscope and examine the cleistothecia, first under 100x magnification (10x objective).
7. Draw and identify the different parts below:
STATION B
Pathogen Identification
Can you figure out which pathogen is on Plant A and Plant B? Use what you learned yesterday to prepare your slides. If you dissected yesterday, then let your partners do it this time with some group guidance.

1. Once you find cleistothecia, look at their appendages.
2. Carefully examine both the base and tip ends of the appendages. Compare them to your Fungi Key.
3. Decide what kind of appendages they are and record your observations in your notes/table.
4. Determine how many asci are in the cleistothecia by gently pressing down on the clear tape with a dissecting needle or toothpick. It is helpful to look through the microscope at low power while doing this in order to see the asci pop out of the cleistothecia.
5. Once you break open a mature cleistothecium, how many asci do you see? One ascus or several asci? Record your observations on the data sheet.

DATA TABLE 1

<table>
<thead>
<tr>
<th>PLANT</th>
<th>APPENDAGE MORPHOLOGY</th>
<th>NUMBER OF ASCI/CLEISTOTHECIUM (ONE OR SEVERAL)</th>
<th>POWDERY MILDEW GENUS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
STATION C
Read and Create

Reading: Taxonomy is the field of biology dealing with the naming and classification of organisms. Every species has a unique two-part name situating it within a genus, and is further assigned to a series of higher-order taxonomic rankings. The basic taxonomic hierarchy or classification is generally agreed to consist of Domain, Kingdom, Phylum, Class, Order, Family, Genus, and Species. For example:

D: Eukarya
K: Plantae
P: Coniferophyta
C: Pinopsida
O: Pinales
F: Cupressaceae
G: *Sequoia* (genera and species are always italicized)
S: *S. sempervirens*. (each species name exists as a binomial, including the genus name and specific epithet)

Every named group is a taxon (e.g., Plantae, Pinales, *sempervirens*, etc.)

Taxonomy reflects the evolutionary relatedness of organisms and groups of organisms. The higher the taxonomic level, the more distantly related the organisms are within it, that is, the longer ago they shared a common ancestor. (Sometimes scientists find it meaningful to subdivide these levels further, into levels such as subphylum or subclass; these definitions are somewhat arbitrary and vary from field to field.) Groups are defined by possessing certain characters, or traits. Traditionally these have been observable or morphological characteristics, but with the invention of DNA sequencing, genes and molecular markers have also been able to be used as characters.

Another method to show the evolutionary relatedness among organisms is by looking at or creating a phylogenetic tree (also known as a cladogram). Traits of organisms can be plotted along the cladogram to show which organism has what characteristics. This is what you will be practicing using in this station.
Example 1: Imagine you have evidence that a bear and a dog are more closely related to each other than either a cat or a rabbit. In addition, you can say a rabbit and a cat are fairly close to each other based on your observations of the morphology. Then the tree should look something like this:

```
RABBIT    CAT    DOG    BEAR
```

```
HIBERNATES
GREAT SENSE OF SMELL
MEAT-EATING
FUR
```

Use the cladogram to come up with all the given characteristics of each animal and write them in on Data Table 2.

**DATA TABLE 2**

<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>CHARACTERISTICS</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEAR</td>
<td></td>
</tr>
<tr>
<td>DOG</td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td></td>
</tr>
<tr>
<td>RABBIT</td>
<td></td>
</tr>
</tbody>
</table>

Now obtain a set of fungal cards. Working as a group, try grouping the fungi on the cards into what you consider is the most likely evolutionary classification. When you come your decision, start filling in an evolutionary tree with the fungal cards. Take 10 minutes to make your tree.
<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Can Have 2 Nuclei Per Cell</th>
<th>Made of Chitin</th>
<th>Septate Cells</th>
<th>Has Ascospores</th>
<th>Has Flagella for Motility in Water</th>
<th>Have Mushrooms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ascomycetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Basidiomycetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chytridiomycetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Zygomycota</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>