

CRIME SCENE INVESTIGATION

with Powdery Mildew Fungi

PURPOSE:

This exercise can be used to stimulate the investigative nature of your students as they use forensic plant pathology techniques with powdery mildew cleistothecia (the sexual stage of powdery mildew fungi) to prove their innocence in a mock murder investigation.

In order for students to truly understand the nature of science and technology, they must model the process of scientific investigation through inquiries. This lesson is suitable for grades 7-12 and does not require the purchase or maintenance of special cultures, since cleistothecia produced by powdery mildew fungi are easy to find on plants in nature. Teachers will use leaves infected with powdery mildew fungi that they have previously collected.

CRIME SCENARIO:

An eyewitness thought he saw you and two others running from a wooded park where a murder took place. The police have brought you all in for questioning and during their investigation they notice that the leaf pieces stuck to all of your shoelaces have the same powdery-like appearance as the leaf pieces that were found on the murder victim's clothes. Although only circumstantial, the public is demanding the arrest of a suspect, and it appears that you are going to be arrested for murder unless you can prove them wrong. You must delve into the depths of forensic plant pathology and use diagnostic skills and tools to prove your innocence...that is, if you ARE innocent!



Figure 1



Figure 2



Figure 3

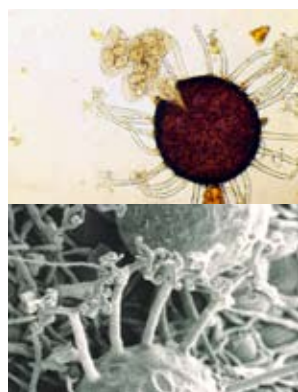


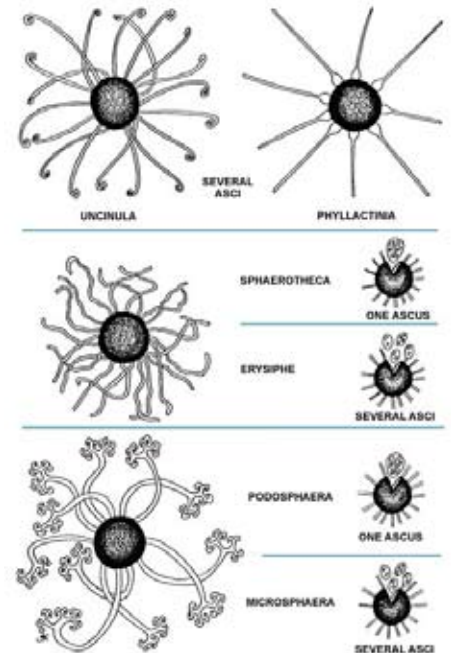
Figure 4

FORENSIC BACKGROUND INFORMATION:

Powdery mildew is a plant disease caused by a fungus that forms a whitish-gray coating of fungal hyphae (thread-like structures) and spores on the surface of leaves, stems, fruits, buds and flowers (Figure 1). Different genera of powdery mildew fungi can be identified most easily at

the end of the plant's growing season when they reproduce sexually to form pinhead sized, black, spherical structures called cleistothecia (Figure 2).

The distinctive 'arm-like' appendages that radiate from the surface of the cleistothecia and the presence of one or several spore-containing sacs called asci inside the cleistothecia help differentiate the genera (Figures 3, 4



Genera of Powdery Mildew Fungi
(Drawing courtesy of C.B. Kenaga, E.B. Williams, and R.J. Green)

Additional background information and color images are available in the K-12 Lab: Powdery Mildew Fungi: Classification and Ecology.

Disclaimer: Powdery mildew fungi have long been classified to genus based on the number of asci contained in the cleistothecium and on the morphology of the hyphal appendages growing out of the wall of the cleistothecium. Recently, new research has resulted in the reclassification of many of these genera according to the phylogeny (an estimate of evolutionary relationships) inferred from the DNA sequence of the ribosomal ITS region and a number of morphological characteristics. In particular, the morphology of the conidia (asexual spores) and conidiophores (the structures that produce the conidia) is important in the new classifications. Despite these changes, this lab can still be used to teach students about forensic evidence, the use of identification keys, and morphological variation among fungal species.

MATERIALS AND METHODS

Collection Methods:

In late summer or autumn collect leaves with mature (black) cleistothecia on plants that show signs (pale, dusty-white coating on leaves) of powdery mildew. The cleistothecia should be visible to your eye without the aid of a magnifying glass. Asters, azalea, cherry, filbert, grape, horse chestnut, lilac, oak, phlox, rose, zinnia, evening primrose (*Oenothera* spp., often called sundrops), viburnum, wheat, willow and many other shrubs, trees, flowers, weeds and garden plants are good sources of cleistothecia.

Leaves exhibiting abundant cleistothecia are good specimens for teaching purposes. Infected leaves should be pressed flat between pieces of newspaper. Pressed, dried, leaf specimens can be stored in envelopes for use over many years. Label the envelope with the pressed leaves giving the name of the plant, the powdery mildew genus (you will need to identify the genus with the forensic key and diagrams), and location of collection, for future reference. For your own purposes of cataloguing and ease of choosing teaching material for labs, you may want to number your envelopes and keep a separate list of the specific genera of powdery mildew found to be associated with each plant collected. You will find that several different genera of powdery mildew will infect the same host type. For example, *Microspora* and *Phyllactinia* may both be found on oak. This is why it is important to label your envelopes by powdery mildew genus and not host plant.

Lab Preparation:

Set out four empty envelopes and your envelopes of pressed, dried, leaf material infected with powdery mildew and covered with cleistothecia.

Take three empty envelopes and label each of them "Leaf Material from Suspect's Shoes." Label the three envelopes #1, #2, or #3. Note: for a large class you may want to make duplicate envelopes of each number. (This exercise works best when students work in groups of three or four.)

Remove several leaves from three of your storage envelopes that contain the pressed, dried, leaf samples of three different known genera of powdery mildew. Be careful to keep the leaves with each different powdery mildew genus separate from each other. Cut the leaves, one powdery mildew genus at a time, into smaller pieces. Clean the scissors before cutting the leaves of the next sample.

Place the cut leaf pieces of each different known powdery mildew genus into one of the numbered envelopes (#1, #2 or #3) labeled "Leaf Material from Suspect's Shoes." Note: the number of cut leaf pieces must equal the number of groups in the class so that each group gets one piece of "Leaf Material from Suspect's Shoes." On a separate piece of paper (your key) write down the envelope numbers and the name of the known powdery mildew genera in each numbered envelope.

Take one empty envelope and label it "Leaf Material from Murder Victim's Shoes." Choose one of the three known stor-

age envelopes from which you previously removed leaves (so the Murder Victim sample will match only one of the Suspect samples) and remove several more leaves from that one envelope. Cut the leaves from that sample into smaller pieces and place them in the envelope labeled "Leaf Material from Murder Victim's Shoes." Note: the number of total cut leaf pieces in this envelope must equal the number of groups in the class so that each group gets one piece of leaf material from the "Murder Victim." Record on your key the name of the known powdery mildew genus that you placed in the envelope labeled "Leaf Material from Murder Victim's Shoes."

Lab Set-up:

Place all of the labeled envelopes ("Leaf Material from Suspect's Shoes" and "Leaf Material from the Murder Victim's Shoes") on a designated table or lab bench.

Place several black permanent markers or wax pencils on the tables for students to label their petri dishes. For each lab group, set up a work station area consisting of :

dissecting microscope	compound microscope
clear tape	two petri dishes
forceps	several microscope slides
water in dropper bottles	

If supplies are limited, groups can easily share items.

Study questions:

Forensic Conclusions: Innocent or Guilty?

1. Compare the forensic plant pathology observations you have recorded for the two different samples. Are the appendages observed on the two different samples the same or are they different?
2. If the appendages you observed on the cleistothecia found on the plant material taken from your shoelaces (murder suspect) are different from appendages on the cleistothecia found on the plant material taken from the murder victim, what does this evidence suggest?
3. If the appendages on the cleistothecia found on the plant material taken from your shoelaces (murder suspect) are the same as the appendages on the cleistothecia found on the plant material taken from the murder victim what does this evidence suggest?
4. Do you need an alibi as to where you were when the murder took place? Explain.



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Full lesson plan with additional images, along with other great lab exercises, available in the APS Education Center at www.apsnet.org.

FORENSIC TOOLS:

You are a suspect in a murder investigation. You must attempt to prove your innocence and clear your name using dissecting and compound microscopes, clear tape, petri dishes, forceps, microscope slides, a water dropper and a forensic key with the special characteristics of powdery mildew cleistothecia appendages.

FORENSIC LAB PROCEDURES:

Collect and label forensic evidence.

First, locate the numbered envelopes labeled “Leaf Material from Suspect’s Shoes.”

Choose one of the numbered envelopes labeled “Leaf Material from Suspect’s Shoes” and using a forceps, remove a piece of the powdery looking plant material from the numbered envelope and place it in a petri dish you have labeled “Suspect.” Record the number of the envelope on the petri dish and on your data sheet.

Locate the envelope labeled “Leaf Material from the Murder Victim’s Shoes” and using a different or clean forceps remove a piece of the powdery looking plant material from the envelope. Place the plant material in a petri dish you have labeled “Murder Victim.” Note: Cleanliness is very important in this process of handling samples with forceps. There are several infamous cases of sloppy handling of forensic evidence that have led to false convictions.

Take the labeled petri dishes with your pieces of plant material to your work station.

Examine forensic evidence.

Put a small drop of water in the center of a microscope slide. Examine the leaf sample labeled “Suspect.” The black speck-like cleistothecia should be visible to your eye without the aid of magnification. Place the powdery looking leaf material peppered with black cleistothecia under a dissecting microscope, for increased visibility with magnification. Tear off a piece of clear tape that is approximately half the length of the microscope slide. While looking through the dissecting microscope, press the leaf piece firmly down with one hand. Using your other hand, hold onto one end of the tape and gently touch the rest of the clear tape on top of the black, speck-like cleistothecia. They should stick to the tape. Repeat this process several times on other areas on the leaf surface to gather more cleistothecia.

Place the tape (sticky side down) on top of the water droplet on the microscope slide. Gently push down on the tape and smooth the tape over the water droplet so that it sticks to the slide. If necessary, more water may be added by squeezing the water dropper gently at the edge of the tape on the slide. The clear tape is an excellent tool for gathering cleistothecia and other fungal structures from a leaf surface.

You can quickly check to see if you have successfully transferred cleistothecia to your microscope slide by placing it on a white sheet of paper. If you can see black pinhead-sized specks, then you have cleistothecia. If you do not see tiny black specks then remove the tape from the slide and repeat steps #4 and #5.

Now place the slide on the stage of the compound microscope and examine the cleistothecia, first under 100x magnification, followed by 200x and 400x (if available).

Identify forensic evidence.

As you examine the cleistothecia with a compound microscope, look at the appendages on the cleistothecia. Carefully examine both the base and tip ends of the appendages on at least fifteen different cleistothecia to be sure you have seen the correct appendage type. This is necessary since tips break off easily, leaving only stringy looking “arms” that might lead you to a false identification. Compare the appendages to the written descriptions and/or the drawings in the keys included with this exercise. Decide what kind of appendages they are, and record this description along with a drawing of the appendage type on the “Forensic Evidence Data Table” located on your Case Study Sheet.

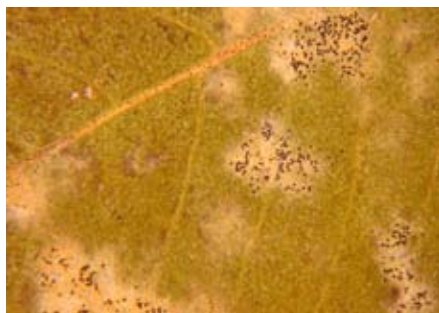
After observing the appendages, determine how many asci (spore-containing sacs) are in the cleistothecia by gently pressing down on the clear tape with the end of the forceps to break the cleistothecia open. It is helpful to look through the microscope at low power (100x) while doing this in order to see the asci pop out of the cleistothecia. Some cleistothecia may be immature and will not contain asci. Once you break open a mature cleistothecium, how many asci do you see? One ascus or several asci? Record your observations on the Case Study Sheet. This information may be important in your effort

to prove yourself innocent.

Repeat Sections B and C (Examination and Identification of Evidence) with the plant material in your petri dish labeled “Murder Victim” including the recording of results.

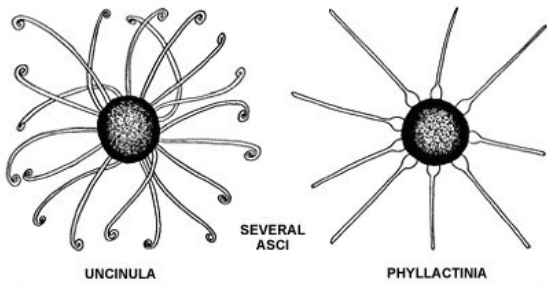
Record forensic evidence.

Locate the Forensic Evidence Data Table on the Case Study Sheet. Record and draw the type of appendages (arms) found on the surface of the cleistothecia (e.g. hooked tip; bulb-like base; antler-like dichotomous branching; or simple, string-like arms), the number of asci (one or several) contained within each cleistothecium, and the name of the powdery mildew genus.



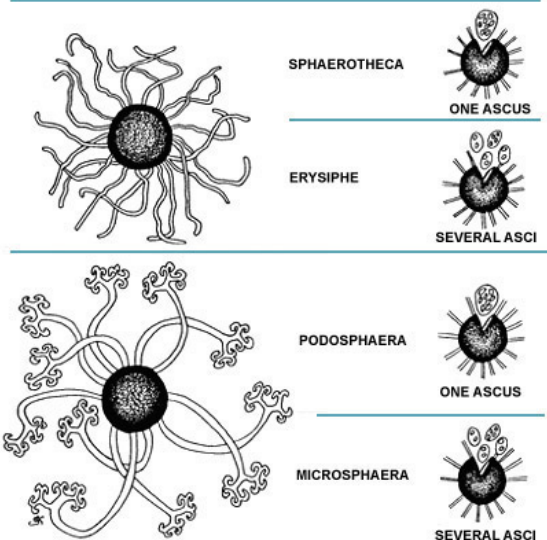
Forensic Evidence Data Table

	Characteristics of Cleistothecia		Powdery Mildew Genus
	Types of Appendages	Number of asci (sacs) per cleistothecium (one or several)	
Murder Suspect (YOU) # _____			
Murder Victim			



Forensic Key and Diagrams to the Genera of Powdery Mildew Fungi

- Appendages coiled or hooked at tip - Uncinula
- Appendages simple and straight with bulb-like base - Phyllactinia
- Appendages branching dichotomously (antler-like at tip)
 - Cleistothecium contains a single ascus - Podospaera
 - Cleistothecium contains several asci - Microsphaera
- Appendages simple or irregularly branched, often entangled
 - Cleistothecium contains a single ascus - Sphaerotheca
 - Cleistothecium contains several asci - Erysiphe



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