Identification of Powdery Mildew Fungi anno 2006
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**Heffer, V., M.L. Powelson, K.B. Johnson**, and **N. Shishkoff**. Oregon State University and USDA-ARS

## OBJECTIVES:

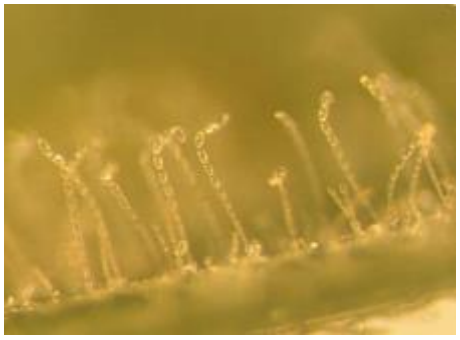
1. To observe powdery mildew diseases.
2. To use characteristics of asexual and sexual fruiting structures on fresh material to identify pathogen to genus.

## INTRODUCTION:

Powdery mildew fungi are obligate, biotrophic parasites of the phylum Ascomycota of Kingdom Fungi. The diseases they cause are common, widespread, and easily recognizable. Infection by the fungus is favored by high humidity but not by free water. Individual species of powdery mildew fungi typically have a very narrow host range.

Unlike most fungal pathogens, powdery mildew fungi tend to grow superficially, or **epiphytically**, on plant surfaces. During the growing season, hyphae are produced on both upper and lower leaf surfaces, although some species are restricted to one leaf surface only. Infections can also occur on stems, flowers, or fruit. Specialized absorption cells, termed **haustoria**, extend into the plant epidermal cells to obtain nutrition. While most powdery mildew fungi produce epiphytic mycelium, a few genera produce hyphae that are within the leaf tissue; this is known as **endophytic** growth.
**Conidia** (asexual spores) are also produced on plant surfaces during the growing season. They develop either singly or in chains on specialized hyphae called **conidiophores**. Conidiophores arise from the epiphytic hyphae, or in the case of endophytic hyphae, the conidiophores emerge through leaf stomata.

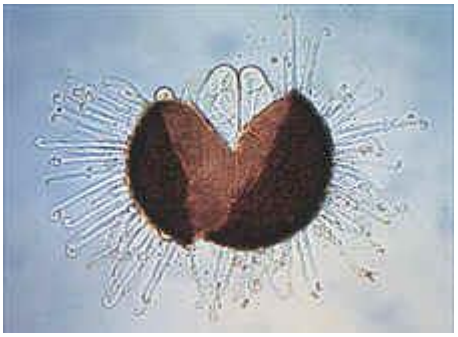
Conidia of powdery mildew in pseudochains, shown in profile. (Courtesy W. Gärtel)



Powdery mildew of a grape leaf. (Courtesy J. Schlesselman)

Hyphae and conidia of powdery mildew on the surface of a grape leaf. (Courtesy J. Schlesselman)

At the end of the growing season, powdery mildew fungi produce sexual spores, known as **ascospores**, in a sac-like **ascus** (pl. **asci**) enclosed in a fruiting body called a **chasmothecium** (pl. chasmothecia) (**cleistothecium** is a former term for this structure that is still widely used). The chasmothecium is generally spherical with no natural opening; asci with ascospores are released when a crack develops in the wall of the fruiting body. This type of fruiting body is unique among the Ascomycota. A variety of appendages may occur on the surface of the chasmothecia. These appendages are thought to act like the hooks of Velcro fastener, attaching the fruiting bodies to the host, particularly to the bark of woody plants, where they overwinter.



Ruptured chasmothecium showing several asci containing ascospores.
*Erysiphe* (Section *Uncinula* sp.)
(Courtesy B. Kendrick)

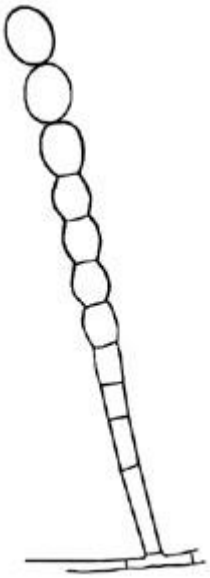
The taxonomy of powdery mildew fungi (order Erysiphales) recently underwent extensive revision based on DNA sequence data. Previously, identification was based largely on the teleomorph (sexual stage) and the morphology of the chasmothecium and its appendages, but the morphology of structure is not as conserved as originally assumed. With the new taxonomy, identification of powdery mildews now also requires attributes of the anamorph (asexual stage), so that it incorporates characteristics of the whole fungus (anamorph plus teleomorph, i.e., the holomorph). Powdery mildew genera are now grouped into five tribes, and some genera have been added or merged. The chart below shows the tribes and some representative genera of each; the previous teleomorphic names (and less commonly used anamorphic names) are given to aid in reference to the older literature.

| Tribe | New holomorphic genus | Anamorphic genus |
|---|---|---|
| Phyllactineae | *Phyllactinia* | *Ovulariopsis* |
| | *Leveillula* | *Oidiopsis* |
| Erysipheae | *Erysiphe* section *Erysiphe* | *Oidium* |
| | *Erysiphe* section *Microsphaera* | *Oidium* |
| | *Erysiphe* section *Uncinula* | *Oidium* |
| Blumeriae | *Blumeria* | *Oidium* |
| Golovinomyceteae | *Golovinomyces* | *Oidium* |
| Cystotheceae | *Podosphaera* section *Podosphaera* | *Oidium* |
| | *Podosphaera* section *Sphaerotheca* | *Oidium* |

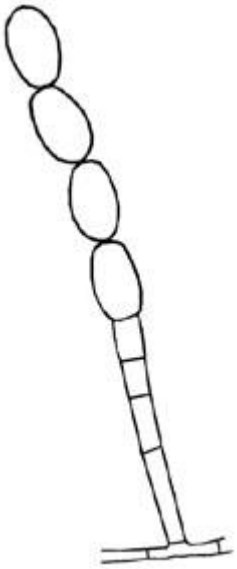
A major distinction for identification is whether conidia are produced in chains or singly. However, this distinction can be difficult to observe, and in some genera, particularly in the

Erysipheae, conidia that are produced singly can “stick together” to form **pseudochains**, which are not true chains.

conidiophore producing single conidium.



conidiophore producing conidia in true chain.



conidiophore and pseudochain.

Other characteristics that aid in classification are the location of mycelium (epiphytic or endophytic) and host specificity. In addition, the presence of one or several asci in each chasmothecium can also be useful for identification. From a practical perspective, the morphology of chasmothecium appendages remains important as a connection with the older descriptions and references concerned with powdery mildew diseases.

Powdery mildews are polycyclic diseases that can impair photosynthesis, stunt growth, and increase the rate of senescence of host tissue. The diseases they cause may be slight or, in some situations, if left untreated, they may result in severe economic losses on crops such as apples, grapes, cucurbits, and cereals.

## MATERIALS

- Fresh and dried plant materials with powdery mildew signs
- Clear scotch tape
- Dissecting needles/ single-edge razor blades
- Microscope slides
- Coverslips

## Laboratory Exercise Instructor Notes

### Identification of Powdery Mildews

Collect leaves that show signs of powdery mildew. Recently infected leaves are best for observing hyphae and conidia. Conidia can be more easily observed by folding the leaf and looking for the conidia in profile with a dissecting microscope.

Collect leaves with mature (black) chasmothecia on plants with powdery mildew in late summer and autumn. The chasmothecia should be visible with your eyes, although a hand lens may help. Leaves with abundant chasmothecia may be pressed and dried for classroom use for many years. They can be stored in large envelopes or cardboard boxes for use throughout the year. Dried leaves tend to crumble. It might be easiest to remove chasmothecia by gently scraping the surface with a moistened, single-edge razor blade. If this is done over a white sheet of paper, it is easier to see if chasmothecia have been successfully transferred to the water drop on the slide. Dried leaves also can be softened by steaming for about 10 minutes on a screen over boiling water. Students may find it easier to remove chasmothecia from fresh leaves and can be encouraged to bring their own samples to fall classes.

To properly use the identification key in the lesson, it will be necessary to observe both conidia and chasmothecia on the same plant host, so fresh plant material should be obtained for each dried specimen if at all possible. Also, as an introductory exercise, it can be difficult to discern between 'Conidia in chains' (Blumeria, Golovinomyces and Podosphaera) and 'Conidia in pseudochains' (common for Erysiphe). When uncertain, it is suggested that students work the key in both directions. Mycologists frequently require additional information to discern among genera, including DNA sequence, host range databases, and morphology of the appressorium (infection cushion) and the conidia.

Moreover, the distinction between "partially endophytic" mycelium, found in Leveillula and Phyllactinia, and "epiphytic" mycelium, found in the Erysipheae and other powdery mildews, can be difficult to determine without the preparation of thin sections of infected leaf tissue. The presence of conidiophores emerging through leaf stomata is a common occurrence in Leveillula and indicates the presence of endophytic hyphae in that genus. In Phyllactinia, however, some epiphytic mycelium may be present (typically on lower leaf surfaces only), with conidiophores generally arising from the epiphytic rather than the endophytic hyphae.

Genera that may be found are: Phyllactinia on hazelnut and dogwood; Erysiphe (sect. Erysiphe) on sweet pea and phlox; Erysiphe (sect. Microsphaera) on azalea, oak, lilac, dogwood, and viburnum; Erysiphe (sect. Uncinula) on grape; Podosphaera (sect. Podosphaera) on apple and cherry; Podosphaera (sect. Sphaerotheca) on rose, strawberry, and cucurbits; Blumeria on wheat and turf grasses; Golovinomyces on cucurbits, zinnia, sunflower, phlox, and chrysanthemum; and Leveillula on tomato and pepper.

Reference: Braun, U., R.T.A. Cook, A.J. Inman, and H.-D. Shin. 2002. The Taxonomy of the Powdery Mildew Fungi. Pages 13-55 in: The Powdery Mildews: a Comprehensive Treatise, Berlanger, R.R., W.R. Bushnell, A.J. Dik, and T.L.W Carver (eds.). American Phytopathological Society, St Paul.

## PROCEDURES

**Asexual stage**

With a dissecting microscope, examine the surface of a diseased leaf. Look for the presence

of mycelium on the leaf surface (epiphytic growth). Genera that are partially or completely endophytic will show reduced mycelium on the surface of the leaf.

Observe the leaf surface for conidia and conidiophores produced on the plant surface by epiphytic hyphae, or emerging through stomata from endophytic hyphae. Folding a section of the leaf may allow these structures to be more easily observed in profile along the crease.

Using a piece of clear tape (smaller than the microscope slide), hold one end of the tape and lightly smooth the rest of the tape (sticky side down) over the conidia and conidiophores. Place the tape, sticky side down, on a drop of water on the microscope slide. Observe with a compound microscope. Attempt to locate an intact conidiophore with conidia, and determine if the conidia are borne on the conidiophore singly or in chains.

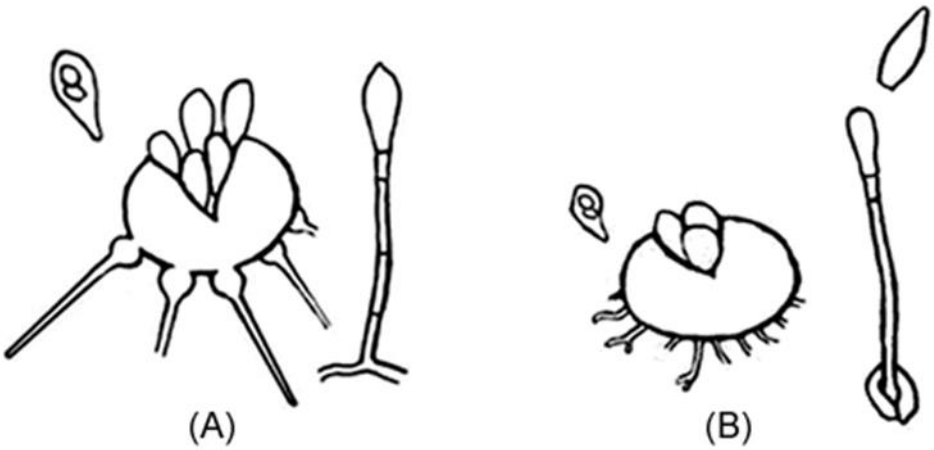
**Sexual stage**
Examine fresh or dried leaves using a dissecting microscope for small black spherical structures (chasmothecia). Remove several with scotch tape (as above) or with a moistened dissecting needle or razor blade, place them in a drop of water on a microscope slide, and add a coverslip if necessary. If the slide is placed over a white sheet of paper, it is possible to see if you have successfully moved some of the (black) chasmothecia to the drop of water on the slide.

Examine the chasmothecium with a compound microscope. Using the descriptions in the key below, determine the type of appendages present on the surface of the fruiting body. After observing the appendages, press gently on the coverslip or tape with the blunt end of a dissecting needle to break open the fruiting body and allow the asci to be released. Immediately examine microscopically to determine if one or several asci are contained in each chasmothecium.

Use the following key and diagrams of some of the common powdery mildew genera to identify the causal agent to genus. [Note: the section name is generally the genus name in literature published before 2003.]

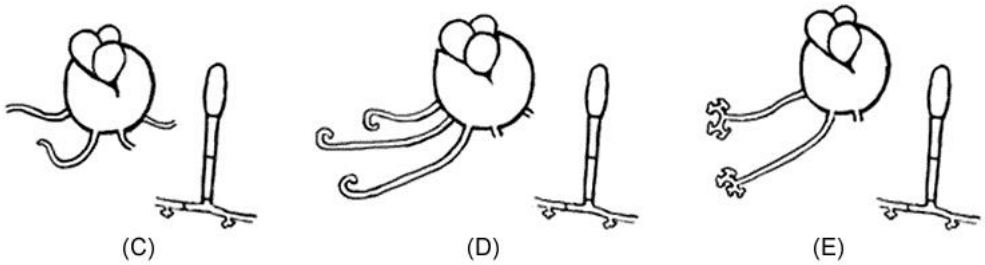
**Key to Genera of Powdery Mildew Fungi**

**Conidia formed singly**
Mycelium partially endophytic
Chasmothecium contains several asci
Appendages straight, bristle-like, with bulb-like base……..…..…..*Phyllactinia* (A)
Appendages simple, hypha-like……..…….……..…..…..….*Leveillula* (B)



**Conidia formed singly (or in pseudochains)**
Mycelium epiphytic
Chasmothecium contains several asci

Appendages simple, hypha-……..*Erysiphe* (section *Erysiphe*) (C)
Appendages coiled or hooked at tip.....……...…*Erysiphe* (section *Uncinula*) (D)
Appendages branched dichotomously at tip...……*Erysiphe* (section *Microsphaera*) (E)

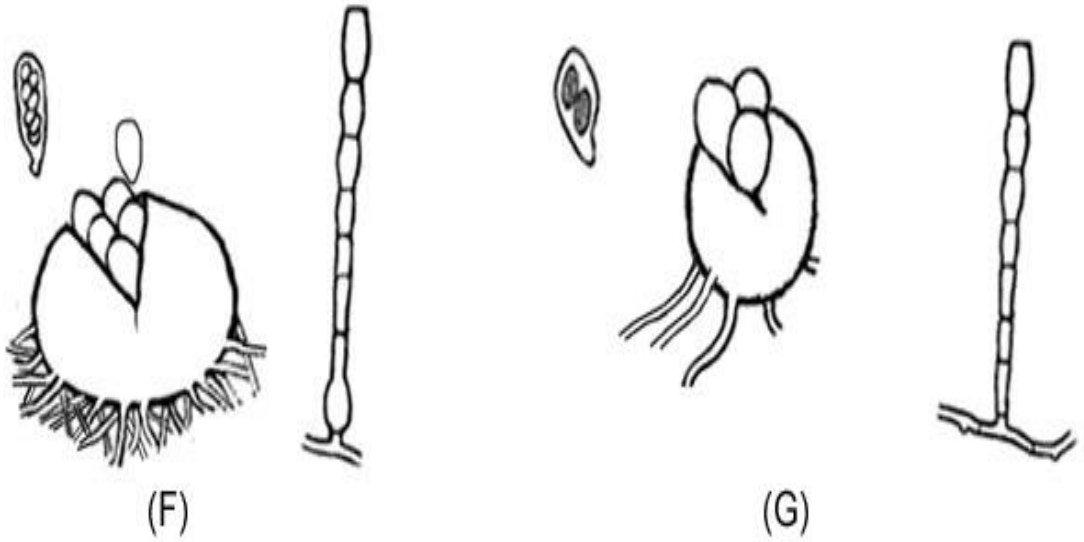



**Conidia formed in true chains**

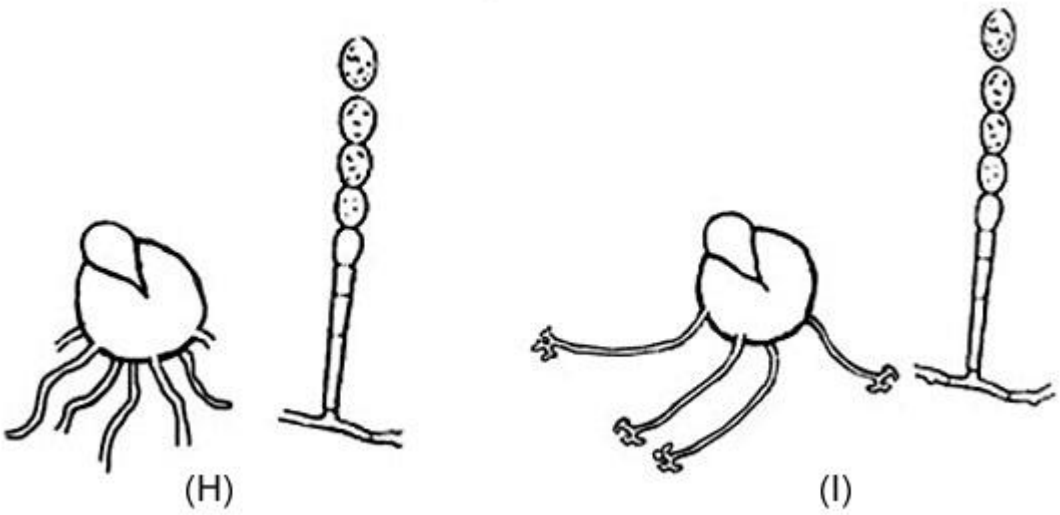
Mycelium epiphytic

Chasmothecium contains several asci
Appendages short, simple, hypha-like; infects grasses…..……...…*Blumeria* (F)
Appendages hypha-like, flexuous, simple or irregularly branched; infects dicots.

*Golovinomyces* (G)




**Conidia formed in true chains**
Mycelium epiphytic
Chasmothecium contains a single ascus:
Appendages simple, hypha-like……..…..*Podosphaera* (section *Sphaerotheca*) (H)
Appendages bristle-like, branched dichotomously at tip *Podosphaera* (section *Podosphaera*) (I)




LAB SHEETS TO BE PRINTED AND FILLED OUT BY STUDENTS)
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## OBSERVATIONS

Diagram the conidiophores and conidia seen on the diseased leaf.

In the table below, record the location of conidiophores, the type of conidia formation, the number of asci per chasmothecium, and a description of the appendages found on the chasmothecium. Using this information, determine the pathogen genus for each powdery mildew.

| Plant host | Appendage morphology | Pathogen genus |
|---|---|---|
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## CONCLUSIONS AND QUESTIONS

1. What physiological functions of the plant are impaired by powdery mildew fungi?
2. What is the function of the chasmothecium in the disease cycle?
3. What is the function of the conidia in the disease cycle?
4. Why are fungal pathogens frequently assigned to more than one genera? Why is the generic name based on the holomorph (whole fungus) concept important in the powdery mildews?
5. Speculate as to why free water on the surface of a plant might be unfavourable to a powdery mildew pathogen.