Discipline Courses-I Semester-II Paper III: Mycology and Phytopathology Unit-V Lesson: Morphology and life cycle of Puccinia and the symptoms of Smuts Lesson Developer: Anupama Shukla College/Department: Acharya Narender Dev College, University of Delhi

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#### SYSTEMATIC POSITION:

- **Phylum : Basidiomycota**
- Class : Urediniomycetes
- Order : Uredinales
- Family : Pucciniaceae

Class Urediniomycetes includes all those fungi which have the following characteristic features:

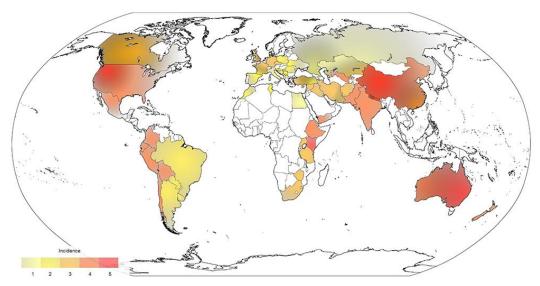
- 1. Presence of well-developed, branched and septate mycelium having simple septum.
- 2. Basidiocarp is absent; clamp connections are also absent.
- 3. The site of karyogamy is the probasidium and meiosis occurs in the metabasidium.
- 4. The basidium is transversely septate, each cell producing basidiospore laterally.

The Class includes 3 orders of which Uredinales is the largest as it includes the rust fungi. The order Uredinales consists of two families: Pucciniaceae and Melampsoraceae.

**Family Pucciniaceae** includes all those genera which form teliospores that are **stalked** and one- to many-celled. There are about 140-150 genera, with 7000 sps. They are all obligate pathogens attacking a wide range of plants like ferns, gymnosperms, and angiosperms. The name rust comes from the rust/reddish-brown colored lesions produced on their hosts.



**Figure:** Symptoms of wheat rust on common wheat. Source: <u>http://www.forestryimages.org/browse/detail.cfm?imgnum=5410641</u> They occur all over the world in a broad climatic range.



**Figure:** Incidence of wheat stripe rust in cereal producing countries Source: <u>http://striperust.wsu.edu/generalInformation/puccinia-striiformis-distributions.html</u>

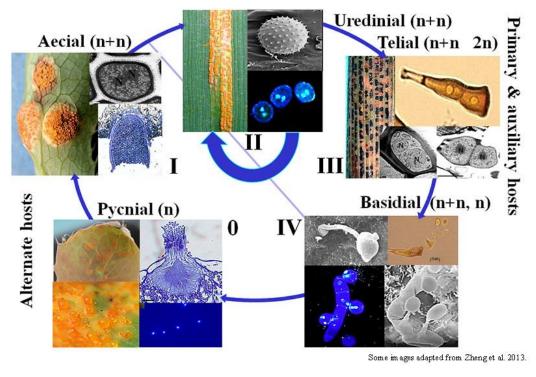
### The Rust Life Cycle:

The rusts typically produce **five distinct spore stages** in their life cycle in a regular sequence as follows:

**Stage O** = pycnidia or spermogonia bearing **spermatia** and **receptive hyphae**.

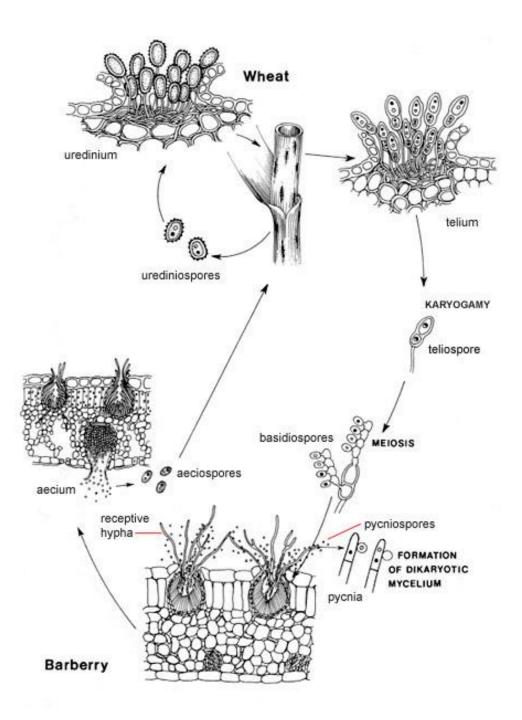
- Stage I = aecia bearing aeciospores.
- **Stage II** = uredia bearing **urediospores.**
- **Stage III** = telia bearing **teliospores.**
- **Stage IV** = promycelia bearing **basidiospores.**







**Figure:** Outline of the disease cycle of *Puccinia graminis*, which alternates between phases of growth on wheat and barberry. In summer, uredia form on grass from infection by aeciospores or urediospores, and there are repeating asexual cycles on the grass. In the fall, airborne urediospores (n+n) are released and telia form on grass. In winter, teliospores (n+n) form on straw, which is followed by karyogamy (2n). This is followed by meiosis, and teliospores (2n) germinate on straw, with a basidium and basidiospores (n) forming. In the spring, airborne basidiospores (n) are released and infect barberry; pycnia form on the barberry leaves. Pycniospores (n) are then transported by insects to receptive hyphae (n) of the pycnium of a different mating type, where plasmogamy occurs. Aecia (n-n) then form on barberry leaves (dikaryotization) and airborne aeciospores (n+n) are released, which begins the cycle anew.



#### Figure: Life cycle of Puccinia

Source: http://www.botany.hawaii.edu/faculty/wong/BOT135/Lect08.htm

The different rust species have different life cycles depending on the number of spores present. There are mainly three types of life cycles:

**Macrocyclic** – here all the spore stages are present in the life cycle.

**Demicyclic** – here 1/2 spores are absent, usually the Uredinia but in some the aecidia stage is absent.

**Microcyclic** – here only Telial and sometimes the Spermogonial stage is present.

The rusts can also be divided into two types depending on their host requirement. The **heteroecious** type of fungi require two hosts to complete their life cycle e.g. *Puccinia graminis tritici, Uromyces pisi*. The hosts on which the uredinial and telial stages occur are called the **Primary host** while the hosts on which the aecidial and spermogonial stages occur are called the **Alternate host**. The segregation of the stages occurs after the aecidial stage.

The **autoecious** types of forms are the second type. They complete their life cycle on the same host e.g. *Phragmidium* spp. on rose, *Puccinia asparagi* on *Asparagus*.

# **Physiological Races**

The **genus** *Puccinia* is the largest in the Order, having about 4000 species. The species is composed of several varieties and numerous physiologic races.

When, within a species, there are different individuals who are same morphologically but differ physiologically from others depending on their pathogenicity towards different hosts, they are called **physiological races**. The physiological races of *Puccinia gramini-tritici* have been identified using eight hosts (varieties of *Triticum aestivum*).*Puccinia gramini-tritici* has over 300 physiologic races. Races are designated by roman numbers (Race1, Race2, Race3...& so on). Physiologic races arise through hybridization and/or mutation.

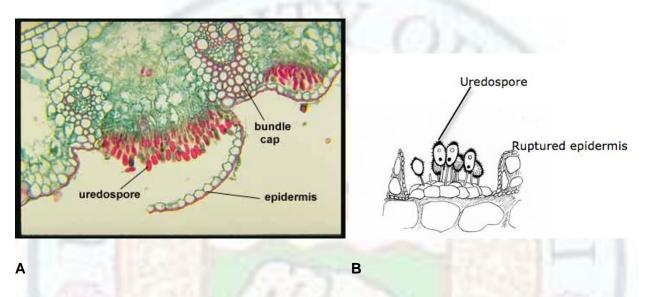
The infection reactions of a physiologic race are fixed after testing on different cultivars of species of wheat. The spores of a race are inoculated on the entire range of wheat cultivars. The hosts show different responses like: Immune; Extremely resistant; Moderately resistant; Moderately susceptible; Very susceptible; and, Mesothetic. Thus some show resistance, some susceptibility, others intermediate reaction. Spores from other race may show different reactions.

The genus *Puccinia* is an obligate parasitic fungus. It is a heteroecious form; its primary hosts are the grasses (cereals) while the alternate hosts are members of Family Berberidaceae.

# Life cycle of Black Stem Rust of Wheat (*Puccinia graministritici*)

Due to its cyclical nature, there is no true 'start point' for the life cycle. We can begin with the stages on Wheat, as it is the economically important host plant.

The infection begins in the leaf as the fungus enters through stomata/ injury. The mycelium develops in the host intercellular spaces taking nutrition from host cells through haustoria. Soon (1–2 weeks after infection) they start collecting below the epidermis in clusters called uredosori. Short, erect hyphae called uredinia are produced by the fungal mycelia. The uredinia function as conidiophores and form **Urediniospores** /uredospores from their tips. The urediniospores are dikaryotic, oval, stalked; wall is thick spiny/echinulate and brick-red/rust in color.



**Figure:** Stages in the life cycle of black stem rust of wheat caused by *Puccinia graminis*. **A**,. Stained section of a wheat stem with a pustule of uredospores breaking though the plant epidermis. **B**. Diagrammatic representation of the same.

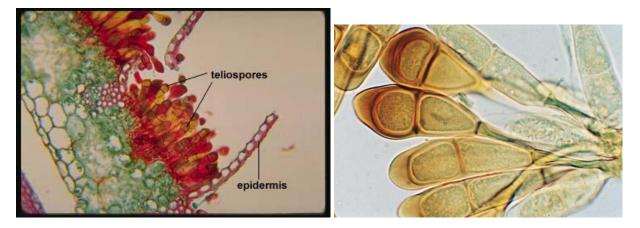
## Source: http://www2.puc.edu/Faculty/Gilbert Muth/botglosp.htm

When a large number of spores form, they exert pressure on the host epidermis and cause its rupture. This exposes the spores and facilitates their dispersal by wind. This appears as rust or brown colored pustules or lesions on the host. The infection first appears on the leaf then goes to the stem, glumes and awn.

Each urediniospore has two **germpores** (where the wall is thin). The urediniospores germinate by forming a **germ tube** when it comes in contact with a compatible host. The germ tube produces **appressoria** which in turn develop the infection peg. The infection pegs enter the host through stomata/injury and finally hyphal strands develop and hyphae spread intercellularly. When fully established, the uredosori are developed again. Urediniospores are the only type of spores which can re-infect the host.

Urediniospores spread from one wheat plant to another through wind, thus spreading the infection from plant to plant, and, field to field. This phase can rapidly spread the infection over a wide area.

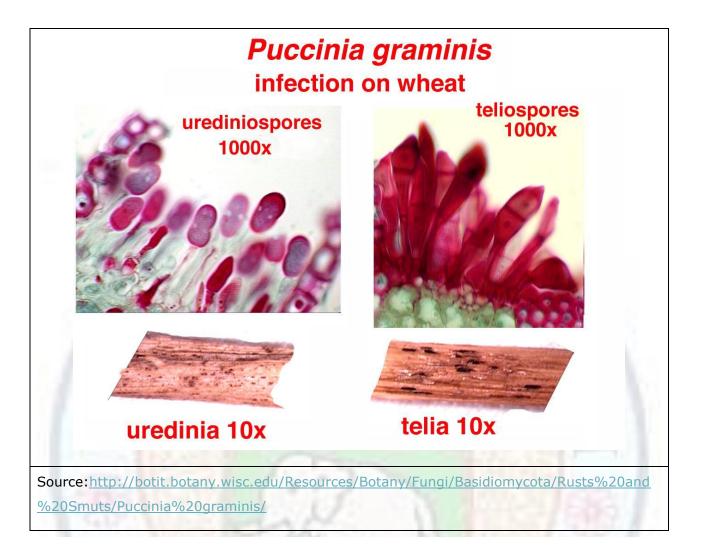
Towards the end of the cereal host's growing season, the mycelia produce structures called telia. Telia are produced in the same sorus as the uredinia. Telia produce a type of spore called **teliospore**. These are bicelled, black, thick, smooth walled spores.



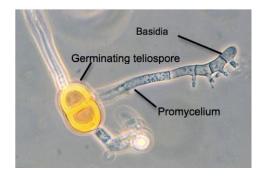
**Figure:** *Puccinia* – **A.** Section of wheat leaf showing teliospores **B.** Enlarged view of teliospores.

Source: <a href="http://www2.puc.edu/Faculty/Gilbert\_Muth/phot0094.jpg">http://www2.puc.edu/Faculty/Gilbert\_Muth/phot0094.jpg</a>, <a href="http://www.botany.hawaii.edu/faculty/wong/BOT135/Lect08.htm">http://www2.puc.edu/Faculty/Gilbert\_Muth/phot0094.jpg</a>, <a href="http://www.botany.hawaii.edu/faculty/wong/BOT135/Lect08.htm">http://www2.puc.edu/Faculty/Gilbert\_Muth/phot0094.jpg</a>, <a href="http://www.botany.hawaii.edu/faculty/wong/BOT135/Lect08.htm">http://www.botany.hawaii.edu/faculty/wong/BOT135/Lect08.htm</a>





They are the only form in which *Puccinia graminis* is able to overwinter independently of a host. They remain with the straw after harvesting where karyogamy occurs and the teliospores become diploid (2n). The teliospores germinate after a long resting period and exposure to freezing temperature.



#### Figure: Germinating teliospore showing promycelium and basidia

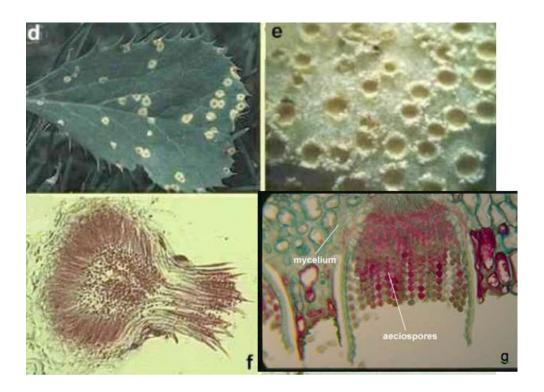
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The upper cell of the spore has an apical germpore, while the lower cell has two laterally apical germpores. A thin hypha comes out of the pore and is called the **promycelium**. The teliospore is the site of karyogamy and meiosis. Before germination the two nuclei fuse and the resultant diploid (2n) nucleus of the spore undergoes meiosis producing four haploid nuclei. These nuclei migrate into the promycelium, which then becomes septate. This four celled structure is the **basidium**. It is a septate, uninucleate **phragmobasidium** .Each cell produces a single haploid **basidiospore** on sterigmata. Basidiospores are thin-walled and colorless. They cannot infect the cereal host, but can infect the alternative host (Usually barberry). They are usually carried to the alternative host by wind.

Once basidiospores arrive on a leaf of the alternative host, they germinate to produce a haploid mycelium which directly penetrates the epidermis and colonizes the leaf. Once inside the leaf the mycelium produces specialized structures called pycnia/spermogonia. The pycnia are flask shaped structures. The pycnia look like small orange bumps on the leaf surface. They produce two types of haploid gametes, the pycniospore/spermogonia and the receptive hyphae. The spermogonia are produced at the tip of short, erect, unbranched hyphae which line the base of the spermogonium. They are formed in large numbers and released from the **ostiole** in a drop of sticky honeydew which attracts insects. The spermatia function as the male cells. In the neck of the pycnium, long, thin hyphae develop. They grow out of the pycnium through the ostiole and may branch a few times. These are called **receptive hyphae.** They function as the female gamete.

Insects carry spermatia from one leaf to another; splashing raindrops can also spread spermatia.

Spermatia can fertilise a receptive hypha of the opposite mating type, leading to the production of a dikaryotic mycelium. This is the sexual stage of the life cycle and cross-fertilization provides an important source of genetic recombination.



**Figure:** d) Lesions containing spermogonia on the upper surface of a barberry leaf. (e) Aecia erupting through the lower epidermis of a barberry leaf. (f) A spermogonium, showing the tiny spermatia and receptive hyphae. (g) Cross-section of an aecium.

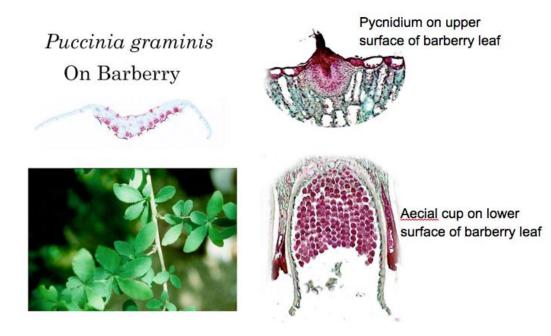
Source: <a href="http://archive.bio.ed.ac.uk/jdeacon/FungalBiology/rust.htm">http://archive.bio.ed.ac.uk/jdeacon/FungalBiology/rust.htm</a>, Courtesy of Jim Deacon, The University of Edinburgh. (g)</a><br/>
<a href="http://www2.puc.edu/Faculty/Gilbert\_Muth/phot0100.jpg">http://www2.puc.edu/Faculty/Gilbert\_Muth/phot0100.jpg</a>



#### Figure:

Source: <u>http://www2.puc.edu/Faculty/Gilbert\_Muth/botgloss.htm</u> (Displayed with permission)

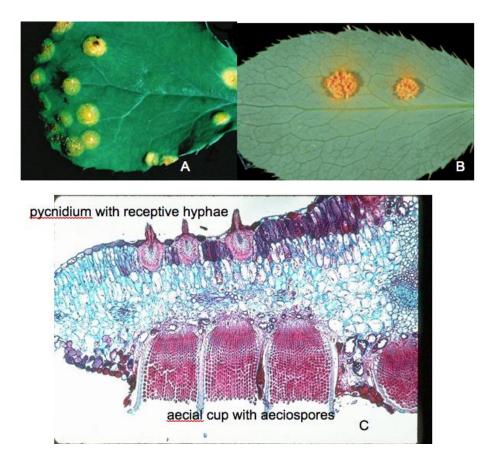
This dikaryotic mycelium then moves through the leaf tissue and reaches the lower surface, here they forms structures called **aecidio mother cells**, which produced a type of dikaryotic spores called **aecidiospores**. These spores have a warty appearance, hexagonal shape, and are formed in chains. The chains of aecidiospores are surrounded by a bell-like structure called **aecidial cup**. The aecidial cup is emergent i.e. half in and half out of the leaf and is made up of monokaryotic fungal cells. It looks like small orange colored cup like structure on the undersurface of the leaf.



Source: http://botit.botany.wisc.edu/Resources/Botany/Fungi/Basidiomycota/Rusts%20and %20Smuts/Puccinia%20graminis/On%20barberry.jpg.html



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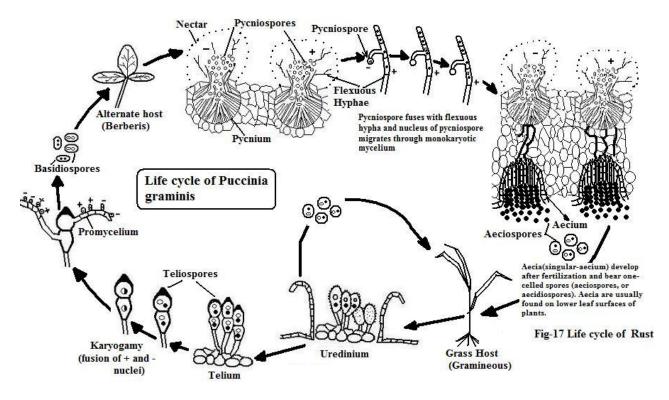


**Figure:** A. Pycniospores in honeydew (sticky nectar like drop carrying the pycniospores) on barberry plant (alternate host); **B.** Orange colored aecidial cups on the undersurface of barberry leaf. **C.** Section through barberry leaf showing pycnidium and aecidium. Note the hexagonal aeciospores in chains.

Source: <a href="http://www.apsnet.org/edcenter/intropp/HungryPlanet/Chapter11/Pages/ImageGallery.a">http://www.apsnet.org/edcenter/intropp/HungryPlanet/Chapter11/Pages/ImageGallery.a</a> spx, <a href="http://facultyweb.berry.edu/mcipollini/bio311/modules/page13.html">http://facultyweb.berry.edu/mcipollini/bio311/modules/page13.html</a>

The aecidiospores are able to germinate on the cereal host but not on the alternative host. They are carried by wind to the cereal host where they germinate and the germ tubes penetrate into the plant. The fungus grows inside the plant as a dikaryotic mycelium. Within 1–2 weeks the mycelium produces uredinia and the cycle is complete.

#### Morphology and life cycle of Puccinia and the Smuts



Source: http://www.peoi.org/Courses/Coursesen/bot/frame14.html

#### **Class Ustilaginomycetes**

This is one of the four main classes of the Basidiomycota. The name refers to the black, burnt and sooty appearance of the infected plant parts (the word ustus in latin means burnt or scorched). It includes over 1,500 species belonging to 77 genera. These are commonly called the "**smuts**," which are important plant pathogens on cereal crops and vegetables (around 4000 species of hosts). Among the Heterobasidiomycetes only the smuts and rusts do not produce basidiocarp. Unlike the rusts, smut fungi can be cultivated on artificial media.

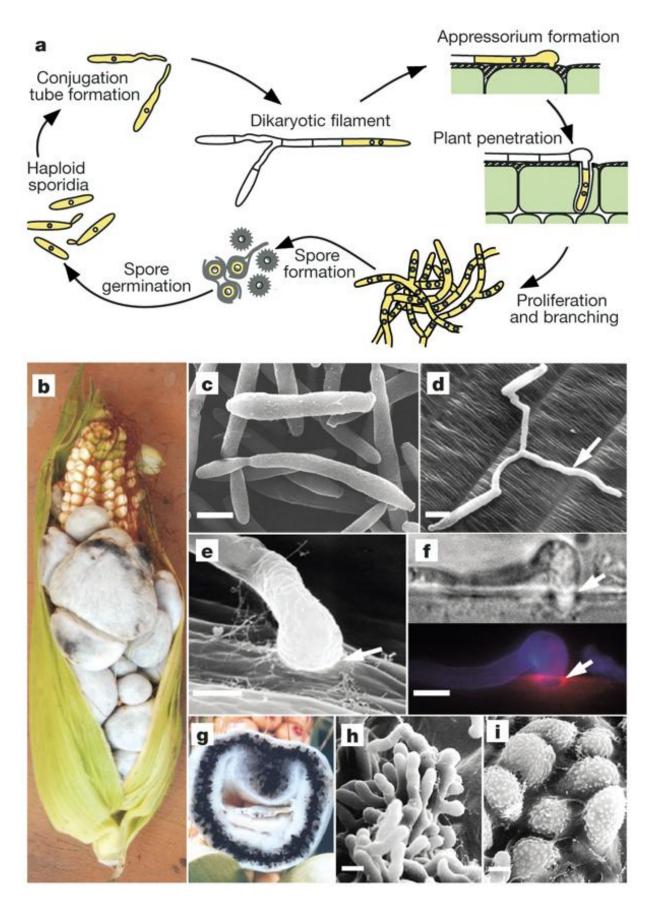
The smuts have a simpler life cycle than rusts forming only basidiospores(also called sporidia) and teliospores, the dikaryotic phase is terminated by the teliospore stage.

Teliospores in the Ustilaginales are conspicuous, dark masses (= pustules or spore masses). The spores are usually formed in the ovary of the host and produced in very large numbers. They often also induce hypertrophy in the host for e.g.in corn smut hypertrophy occurs throughout the plant. Some smuts sporulate only in the ovaries of the host which are thereby filled with the black mass of spores (this disease is called Bunt); but others form spores in other parts of the plant.

The teliospores or smut spores are usually spherical in shape, black in color and, held together in clusters, or spore balls, or, they may be free. Spores are retained in the host until the rupture of host tissue, and they have been given various names such as "winter spores", "ustospores" "smut spores", "brand spores", "teliospores" or "chlamydospores".

The teliospores germinate to form the promycelium where meiosis occurs. Each segment of this promycelium produces haploid basidiospores. The basidiospores cannot infect and germinate by budding to form yeast like cells. These cells soon fuse with compatible cells to form dikaryotic hyphae, which can infect hosts, wherein it forms mycelium. The mycelium forms teliospores, thereby completing the lifecycle.





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**a**, Developmental stages in the *U. maydis* life cycle. **b**, Tumour formation on maize. **c**, Scanning electron microscopy (SEM) image of haploid sporidia. **d**, SEM image of mated sporidia on plant epidermis; arrow denotes dikaryotic filament. **e**, SEM image of appressorium; arrow marks entry point. **f**, Top, differential interference contrast image of appressorium; bottom, epifluorescence image of fungal cell wall stained with calcofluor (blue) and endocytotic vesicles stained with FM4-64 (red). The bright ring indicates active secretion and endocytosis at the fungus-plant interface; arrows indicate the penetration point. **g**, Black teliospores visible in tumour section. **h**, SEM image of sporogenous hyphae and early stages of spore development. **i**, SEM image of ornamented teliospores.

Source: http://www.nature.com/nature/journal/v444/n7115/fig\_tab/nature05248\_F1.htm

#### The Smuts may be of two types: **Covered Smut** and **Loose Smut**

#### Symptoms of Covered Smut:

- **1.** The Teliospores are produced in the cereal grains by replacing all the internal tissues and thus remains covered by the outermost layer- the Pericarp.
- **2.** There is a deposition of fatty substances which keep the spores together in spore mass.
- **3.** The smut sori become visible clearly only when the ears emerge out and the spore mass is broken during threshing.
- **4.** At this time the spores get attached to the healthy grains, thereby contaminating them.
- **5.** The inoculum in this type of smut disease thus comes from these contaminated grains as the teliospores germinate with the seeds in the next season.
- **6.** This type of smut is thus externally seed-borne and spreads with the seedling.



http://www1.agric.gov.ab.ca/\$department/deptdocs.nsf/all/prm2431/\$FILE/c31.jp

### Symptoms of Loose Smut:

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- **1.** The teliospores are formed in the inflorescence by converting all parts of the floret into smut spores.
- **2.** The grains are not formed at all! The spikelet is completely converted into the black powdery mass of spores.
- **3.** Initially it is covered by a very thin membrane but this ruptures soon releasing the spores.



http://www1.agric.gov.ab.ca/\$department/deptdocs.nsf/all/prm2431/\$FILE/ cd13.jpg

- **4.** The spores are light and blow off easily and spread the infection to the adjacent plants.
- **5.** When they infect a flower, they reach the embryo and remain there. These seeds look healthy but carry the infection.
- **6.** This type of smut is thus internally seed-borne and the infection starts at germination.

# Summary

The Basidiomycota include two Classes of important plant pathogenic fungi – the Rusts (Class Urediniomycetes) and the Smuts (Class Ustilaginomycetes). They do not form Basidiocarps; instead the basidia are produced on the promycelium of germinating teliospores .The Rusts are heteroecious fungi forming up to 5 different spores in a lifecycle. The most well known species is *Puccinia graminis-tritici* causing the Black stem rust of wheat. Here we will study the life cycle of this fungus. The Smuts are important pathogens of many plants especially grasses (cereals). We will also see how the Loose and Covered Smuts differ from each other in the symptoms caused by them.

# Glossary

Aeciospores: A binucleate spore produced by the rust fungi in an aecidium.

Aecidium: A structure produced by the rust on alternate host .It consists of binucleate cells enclosed by a peridium half in and half out of the host tissue.

Autoecious: A pathogen which requires a single host to complete its lifecycle

Covered Smut: The smut disease where spore mass is covered by grain membrane.

Heteroecious: A pathogen which requires two hosts to complete its lifecycle

Loose Smut: The smut disease where the entire inflorescence is converted into the spore mass

Macrocyclic: A rust fungus which forms all five spore stages of the life cycle

Microcyclic: A rust fungus which forms only one binucleate spore stage in the life cycle

Primary Host: The host on which the teliospores of rusts are formed

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Secondary Host: The host on which the spermogonium of rusts are formed

Spermogonium: A flask shaped structure formed on the alternate host producing spermatia

Teliospore: Binucleate resting spores produced by the rusts and smuts

Urediniospore: Binucleate spore which spreads the infection

# Exercises

- Q1. What is the difference between autoecious and heteroecious rusts?
- Q2. How many types of spores are formed in a typical rust lifecycle?
- Q3. What is the macrocyclic type of life cycle?
- Q4. Give the difference between demicyclic and microcyclic type of life cycle?
- Q5. Why is wheat called the primary host of Rust fungus?
- Q6. What is an alternate host?
- Q7. Describe the differences between Loose and Covered smuts?

## Web Links

http://striperust.wsu.edu/generalInformation/puccinia-striiformis-symptoms-and-signs.html

http://www2.puc.edu/Faculty/Gilbert Muth/botglosp.htm