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Paper 3: Mycology and phytopathology

Lesson: Oomycota

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Introduction

Oomycota include species which are commonly called as downy mildews and water molds. The word “**oomycota**” means “**egg fungi**”. This is because of the presence of a large oogonium i.e. the female gametes containing structure. Oomycetes show oogamous sexual reproduction in which large non-motile female gametes called eggs and male smaller gametes called sperms are produced. Fossil records are sparse in oomycota. Cretaceous amber is probably known to show an Oomycete. Phylogenetically, Oomycetes have been explained as a monophyletic group of the kingdom **Straminipila**.

Straminipila

Species of oomycetes because of their mycelia growth and absorbtive, heterotrophic nutrition resemble true fungi but they posses many characteristics which make them different from Eumycota. Oomycota is now classified under **non-photosynthetic chromista** having straminipilous flagellum called the kingdom **Straminipila.** The chromista includes several kinds of algae including phaeophyta or brown algae, Xanthophyta or yellow-green algae, chrysophyta or golden algae and Bascillariophyta or diatoms and other small groups The characters of Straminipila (also the oomycota) which differentiate them from Eumycota are as following:

Characters	**Eumycota / True Fungi**	**Straminipila**
Vegetative mycelium	Haploid or dikaryotic	Diploid
Cell wall composition	Chitin and proline	β-glucans, cell wall also contains amino acid hydroxyproline as well as some amount of cellulose.

Zoospore formation	Zoospores if produced have only one flagellum, whiplash type placed posteriorly (Chytridiomycota)	Presence of motile biflagellate zoospores. Two flagella are of different size and structure, condition placing them under Heterokonts. The larger anterior is straminipilous or tinsel flagellum. Its surface is having tripartite tubular hairs-the mastigonemes. The other flagellum is short, whiplash i.e. smooth and is placed posteriorly.
Sterol in Cell membrane	Ergosterol	Cell membrane in this sp. like plants has Fucosterol
Mitochondria	Inner memberane shows flattened or lamellate cristae.	Mitochondria with tubular cristae.
Golgi Bodies (dictyosomes)	Simple,single cisterna	Flattened, multiple cisternae
Synthesis of amino acid lysine	Synthesis by α-amino adipic acid (AAA) pathway	Synthesis takes place by α-diaminopimelic acid (DAP) pathway as in plants and prokaryotes.
Storage material	They have lipids, sugar, glycogen and trehalose as storage material.	The energy storing products are mycolaminarin similar to laminarin of red algae.

In addition Straminipila shows oogamous reproduction which takes place by gametangial contact resulting in the formation of a thick walled spore called **Oospore**.

The results of a number of studies such as molecular sequence data, combined with ultrastructural similarities, confirm common ancestory of oomycota with other members of heterokont algae or chromista. In addidition to oomycota, two smaller groups Hyphochytridiomycota and Labyrinthulomycota appear to be most closely related to chromista. Alexopoulos et. al. 1997 and 150 yr ago, Pringsheim (1858) suggested, in reference to the Saprolegniales (water molds), that oomycetes "were allied with certain algae".

The heterokont algae are distinctive among the algae in having similar kind of flagella as it occur in oomycota, tubular mitochondrial cristae and other ultrastructural similarities. The chlorophyll c and other pigments are charasteristics of photosynthetic members of chromista and are not found in any other groups. Thus although oomycetes are in the minority as heterotropic chromists, they definitely belong with these other chromist group. Some controversy still remain about exactly what to call this group of organisms. Most of the authors refer to the kingdom chromista, phylum heterokont while others place them in kingdom Straminipila – non-photosynthetic chromista (sometimes written as stramenopila, Patterson and Sogin, 1992).

The kingdom Straminipila has three phyla: **Oomycota**, **Hyphochytridiomycota** and **Labyrinthulomycota**. But we will be discussing Oomycota in details.

Oomycota as already mentioned are "egg fungi" which contain large round oogonium as the female gametes containg structure. They are also known as Peronosporomycota. The phylum contains more than 800 species. Species of this group are either saprobic or parasitic on other plants and animals which may be terrestrial or aquatic.

Ecology and Significance

Members of this class are common in almost all the habitats being aquatic as well as terrestrial. Most common condition is that they need moist habitat for their dispersal. They are filamentous protist absorbing their food from the surrounding soil or water. Many of them are parasitic, living on other plants and animals. Oomycota plays an important role in detritus cycle decomposing and recycling dead and decaying organic matter. They may be obligate aerobes inhabiting well aerated fresh water bodies or they may be facultative anaerobes. They also inhabit sea water, soil and can live on terrestrial and aquatic plants and animals as parasites. Parasitic species are major cause for the destruction of crops; they can also destroy fish (by growing on their scales) or amphibians, for e.g. *Aphanomyces astaci* is known for destroying European Crayfish from many rivers, *Saprolegnia* spp. which cause serious lesions on farmed fish especially *Salman* swimming upstreams to spawn. Aquatic invertebrates such as rotifers, nematodes, arthropods and diatoms are also parasitized by *Saprolegnia.*

Greatest impact of parasitic Oomycetes on human comes from their colonization on flowering plants which includes fungi causing root rots (*Phytophthora* sp.); blister rust, white rust (*Albugo* sp.), downy mildews (*Perenospora* and *Plasmopara*) and damping off disease (*Phytophthora* sp.).

History has been greatly influenced by two members of oomycota namely ***Phytophthora infestans*** causing late blight of potatoes and ***Plasmopara viticola*** which causes downey mildew of grapes. *Phytophthora infestans* caused great Irish potato famine of 1845-1848. The parasitic organism can cause death of the plant by growing into stem, leaf tissue etc and even infects the tubers. The disease almost entirely wiped out the crop in Ireland in one week during the summer season of 1846. Almost a million of people died because of this famine and approximately one and a half million emigrated to other countries.

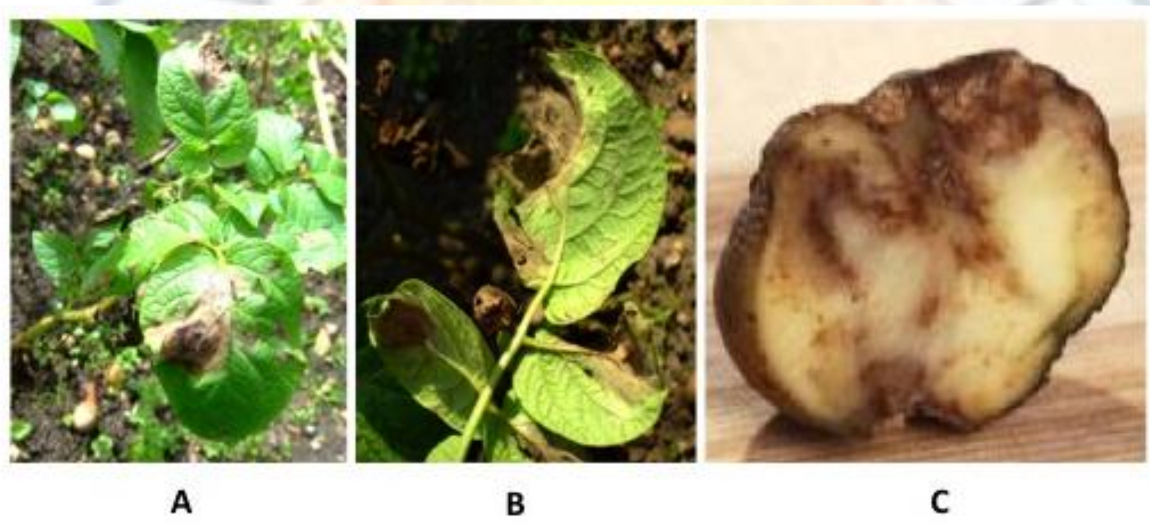


Figure: Late blight on potato caused by *Phytophthora infestans* (A) upper (adaxial) side, (B) lower (abaxial) side of leaf and (C) tuber

Source: http://www.staff.ncl.ac.uk/ethan.hack/disease-pictures/ (CC)

Figure: *Plasmopara viticola* causing downy mildew of grapes (A) abaxial surface of leaf (B) on fruits

Source: http://en.wikipedia.org/wiki/Plasmopara_viticola

Another oomycetous fungus i.e. *Plasmopara viticola (*causing downy mildew of grapes) is the native of North America. In late 1870s it was accidently introduced to Europe when French wine industrialists wanted to breed aphid resistant vine strains in their country because at that time massive aphid infestation was a major concern for French wine industry. American stocks brought with them downy mildew also because of which the entire French wine industry was wiped out. This breakdown caused serendipitous discovery of the first fungicide Bordeaux mixture (a mixture of lime and copper sulfate). A mixture of lime and copper sulfate was sprayed close to the road side so that the passerby could not pilfer the fruits. This caused the vines close to roadside to remain healthy. This led to discovery of Bordeaux mixture which is first known fungicide and still an effective fungicide to control *P. viticola* and other pathogens of oomycetes.

Characteristics of Oomycetes

- The organisms are either unicellular, holocarpic or they are eucarpic, filamentous species.
- The mycelium consists of profusely branched, non-septate, coenocytic hyphae.
- The vegetative state of oomycetes is diploid. Meiosis occurs in developing sex organs or gametangia of oomycetes.

- Septa are produced only when the thallus produce reproductive structure or when they are old.
- Oomycetes consists of many pathogens which are obligate parasites on important crop plants and are responsible for plant diseases.
- Hyphae of these pathogens grow intercellularly and they produce specialized hyphal branches termed as haustoria which enter the cell wall and invaginate the plasma membrane of the host cell. Haustoria are of different shapes depending on the species. They may be peglike, spherical or lobed.
- Hyphae show apical growth and enzymatic activity.
- Cell wall is made up of β-glucan in which β-1,4glucan provide the structural support and it is cross linked by β-1,3 and β-1,6 glucan. Cell wall of oomycetes is also characterized by the presence of amino acid hydroxyproline and some amount of cellulose.
- Cytoplasm contains typical cell organelles except mitochondria and golgi bodies as mitochondria contain tubular cristae as compared to lamellate cristae of Eumycota. Similarly Golgi bodies of oomycota are with flattened multiple cisternae while true fungi have simple cistern.
- The energy storing products are mycolaminarin (phosphorylated β-1,3glucan) which is present in **fingerprint vacuoles** or **dense body vacuoles**. (These vacuoles got their name because of presence of electron-dense lamellar array of paracrystalline material which look like finger print). These vacuoles are also involved in the formation of ooplast.
- Oomycetes reproduces both by asexual as well as sexual mode.

Asexual Reproduction

Oomycetes reproduce asexually by means of **sporangia** which are found on specialized hyphae called **sporangiophores**. Sporangia differ among various oomycetous pathogens with respect to their shape, mode of germination, their location with respect to host tissue and the structure of the sporangiophores.

- In *Aphanomyces*, sporangia are filamentous and resemble vegetative hyphae.

Figure: Sporangia of *Aphanomyces*

Source: http://www.plantmanagementnetwork.org/pub/php/diagnosticguide/aphano/

- In *Peronospora* and other genera causing downy mildews, sporangiophores are branched and tree like, with a single sporangium at the tip of each branch and often emerge out from the stomata.

Figure: Sporangia and sporangiophore of *Peronospora parasitica*, causal agent of downy mildew of cabbage

Source:https://www.apsnet.org/edcenter/intropp/LabExercises/Pages/Oomycetes.aspx

- An unbranched sporangiophore is produced in *Albugo*. This bears chain of sporangia.

Figure: Sporangia of *Albugo candida* are produced on short sporangiophores beneath the epidermis of a mustard leaf

Source:https://www.apsnet.org/edcenter/intropp/LabExercises/Pages/Oomycetes.aspx

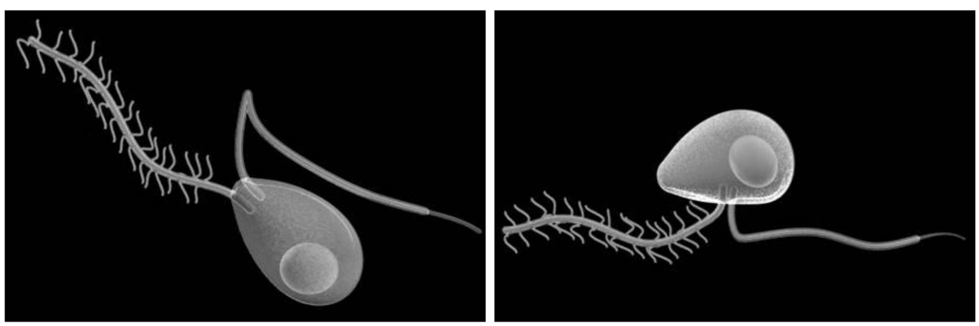
There are two mechanisms by which sporangium germinates in oomycetes. The germination mechanism is species specific. Some times depending on temperature or moisture conditions a species can show germination of the sporangium by either mechanism.

- Sporangium can germinate directly on the surface of a susceptible plant by forming a germ tube as in foliar pathogen (e.g. *Peronospora*). A sporangium which germinates in this way is called a **spore** or **conidium**.
- The sporangium may show germination indirectly by producing **zoospores**. In most of the genera which inhabit soil and water , indirect germination of sporangia takes place by producing zoospores. Zoospores are asexual spores and are motile.They lack cell wall. They bear two flagella which are of different kinds so called heterokontic. One of the flagellum is "tinsel" type characterized by the presence of fibers and is directed anteriorly while the other is posteriorly directed "whiplash" flagellum.

In some sp. like *Aphanomyces* and sp. of *Phytophthora*, zoospores are differentiated inside the sporangium while in many sp. of *Phythium*, the sporangium produces a short tube. A secondary structure called **vesicle** is connected to this tube. Zoospores are potentially differentiated in the sporangium and then they are transferred to the vesicle through the tube and final development and release of the zoospores take place in that vesicle.

Sporangia of some species, for example, *Phytophthora infestans* (cause of late blight) can germinate in either way. When the temperature is low, zoospores are formed while high temperature favours its direct germination. Soil moisture is also an important factor influencing sporangium germination, for e.g. in soil inhabiting sp*. Phytophthora cryptogea* very wet or saturated soil conditions favour zoospore formation where as drier conditions induce direct germination of the sporangium.

In some sp. of oomycetes two morphologically different types of biflagellate zoospores are produced **primary (or auxiliary)** and **secondary (or principal)** zoospores. The primary zoospore are pear shaped with flagella attached at their anterior end. Secondary zoospores are kidney or bean shaped with two flagella inserted laterally in a kinetosome which in turn is located within the lateral groove. Species producing both types of zoospores are called **dimorphic**, while sp. in which only one type of zoospore is produced are called **monomorphic.**



(A) (B)

Figure: (A) Primary zoospore, pear shaped with flagella attached at their anterior end; (B) Secondary zoospores are kidney or bean shaped with two flagella inserted laterally

Source: http://cronodon.com/BioTech/plant_diseases.html

Dispersion of the zoospores occur as they swim in water through soil pores or on the soil surface or they may swim in flowing water over long distances. Plant root exudates contain sugars and amino acids which attract zoospores. Depending upon the environmental conditions, the zoospores swim actively for a short or long period of several hours. After swimming period they become fatigue and ultimately retract their flagella,become spherical and secret a delicate wall around it to become encysted. The encysted zoospore is usually called a **cyst** or **cystospore.**Zoospores may have one **(monoplanetic)** or two **(diplanetic)** or many **(polyplanetic)** periods of motility called **swarm** or **swimming**

periods. Periods of motility are separated by period of encystment. Depending on weather mono, di or polyplanetic and environmental conditions, the zoospores encyst and will germinate by germ tube forming thallus or they may give rise to another zoospore which will undergo another swarming period followed by encystment and then germination and so on.

Sexual Reproduction

Sexual reproduction in oomycetes occur between two dissimilar gametangia, a large round oogonium containing one to several eggs, and a smaller antheridium that fertilizes the oogonium. Protoplast of the oogonium forms one or more **oospheres** which are uninucleate when mature. During the formation of the oosphere either the entire protoplast is utilized (Saprolegniales) or a part is left behind. The residual protoplast forms a peripheral layer termed **periplasm** around the oosphere. It is used up during oospore maturation.

Plasmogamy takes place by gametangial contact. Two types of arrangements of antheridium with respect to oogonium are known in oomycetes:-

- If the antheridium is located at the side of the oogonium, the arrangement is called as **Paragynous**.

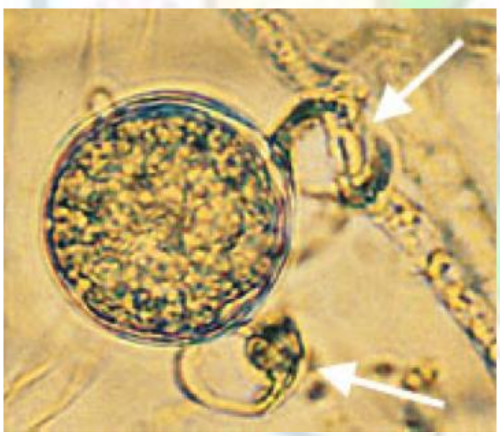


Figure: Paragynous arrangement of oogonium and antheridia in *Pythium.*

Source:https://www.apsnet.org/edcenter/intropp/LabExercises/Pages/Oomycetes.aspx

- It is **Amphigynous** type,when the oogonium grows through the antheridium and the antheridium will remain as a collar at is base.

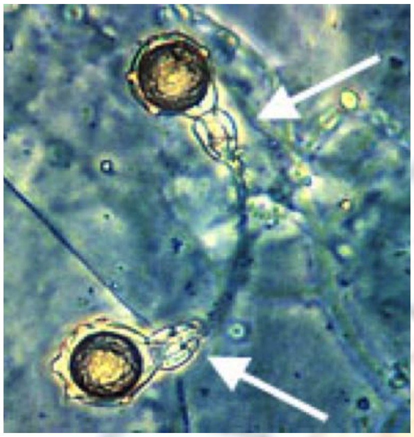


Figure: Amphigynous arrangement of oogonium and antheridium in *Phytophthora cambivora*

Source:https://www.apsnet.org/edcenter/intropp/LabExercises/Pages/Oomycetes.aspx

Oomycetes can be either **homothallic** (when fertilization occurs in a single strain) or **hetrothallic** (when two strains of opposite mating types are needed for fertilization). During fertilization a fertilization tube is involved which carries antheridial nuclei to oospore in oogonium. After fertilization a thick walled zygote called oospore is formed which functions as resting spores. These oospores are produced in infected host plants and after degradation of plant tissues they are released into the soil.

Classification

Martin (1961) and sparrow (1973) divided the class oomycetes into four orders on the basis of : (i) the nature of the thallus – weather Eucarpic or holocarpic. (ii) The nature and mode of zoospore formation (i.e. weather zoospores are formed inside the zoosporangium proper or in the vesicle). The four order and their characteristic are as following:

Order	Distinguishing features
Saprolegniales	Zoospores produced inside the sporangium, aplanetic, monoplanetic or diplanetic. Thallusholocarpic or eucarpic; in eucarpicthalli hyphae without constriction and arise from basal cell; several oospore present in oogonium. E.g.

	Saprolegnia and *Achlya*
Leptomitales	Zoospores produced inside the sporangium, zoospore aplanetic, monoplanetic or diplanetic. Thalluseucarpic; hyphae with constriction; Oogonium contain single oospore.
Lagenidiales	Zoospores formed in sporangium or vesicle; zoospores monoplanetic, reniform; thallus primarily holocarpic, mostly aquatic; parasitic on algae or water moulds.
Peronosporales	Zoospores formed in sporangium or vesicle; zoospores monoplanetic, reniform; thalluseucarpic, mostly terrestrial inhabiting soil or they may be parasitic on vascular plants.

The classification of oomycota is not yet completed. Eight orders have been given by Kirk et al. (2001) while according to others mycologist there are only six orders Dick (2001a,b)

Table. Summary of the most important groups of Oomycota and their characteristic features. Based on information provided by Dick (2001a,b) and Kirk et al. (2001).

Order	Number of species	Thallus and reproduction	Ecology
Myzocytiopsidales (incertaesedes) uncertain affinity	74	Holocarpic,later coralloid or breaking up into segments. Zoospores, oospores	Parasites of invertebrates or algae.
Olpidiopsidales (incertaesedes) uncertain affinity	21	Holocarpic,becoming converted into a sporangium.Zoospores, oospores.	Biotrophic parasites of Oomycota, Chytridiomycota and algae.
Rhipidiales	12	Eucarpicwith rhizoids. Zoospores, oospores.	Freshwater saprotrophs, facultatively orobligately anaerobic.
Leptomitales	25	Constricted hyphae	Freshwater

		producing sporangia. Zoospores, oospores..	saprotrophs or parasites of animals
Saprolegniales	132	Myceliumof wide stout hyphae.Zoospores, oospores.	Saprotrophs or necrotrophic pathogens of animals, plants and other organisms.
Pythiales	>200	Myceliumof relatively Narrow hyphae. Zoospores, oospores.	Saprotrophs or pathogens (often necrotrophic) of plants, fungi and animals.
Peronosporales	252	Intercellularmyceliumwith haustoria.Differentiated sporangiophores. Zoospores or‘conidia’, oospores.	Biotrophic plant pathogens, causing downymildews and other diseases.
Sclerosporaceae	22	Myceliumof very narrow hyphae.Differentiated sporangiophores. Zoospores or‘conidia’, oospores.	Biotrophic pathogens of grasses, causing downymildews.

Peronosporales is the most important order because it has many fungi which are parasitic on plants of economic importance.

Life cycle of *Albugo*

Systematic Position

Albugo is an important member of order **Peronosporales** and family **Albuginaceae**.

Characteristic features of Peronosporales are:

- In habit they may be aquatic, terrestrial or amphibians.
- Many species are highly evolved or specialized obligate parasites on plants, which produce lage amount of essential oils or alkaloids. A few may occur as parasites of thallophytes. The rest are saprophytes.
- The order is characterized by the fungi having well developed, branched, coenocytic and intercellular mycelium.
- The mycelium shows no distinction into rhizoidal and aerial hyphae.
- Septa may be formed to delimit the reproductive organs or in the older mycelium.
- The colourless hyphae have wall composed mainly of glucan-cellulose complex and protein containing hydroxyproline.
- In biotrophic sp., the mycelium is intercellular and possesses haustoria that vary in form from minute spherical or cylindrical form to a more lobed structure. In most of the Pythiaceae however, the hyphae are intracellular and the haustoria are rarely formed exception being few advanced species of *Phytophthora.*
- Asexual reproduction takes place by kidney shaped, biflagellate and monoplanetic zoospores. In few peronosporaceae, however, the sporangium functions as conidium germinating directly by producing germ tube.
- The sporangia are globose or oval or lemon shaped and are deciduous.
- They are developed on the tip of the ordinary somatic hyphae but in more advanced species the sporangia are borne at the tips of special reproductive hyphae known as sporangiophores.
- The sporangiophores are determinate and branched. The branching being characteristic of species. For eg.
    - Sporangiophore is long, stout hypha with many upright branches near the end. e.g. *Sclerospora*.
    - Sporangiophore branched.Branches irregularly spaced and occur at right angles e.g. *Plasmopara*.
    - Sporangiophore may be dichotomously branched. Branches are at acute angles and have curved, pointed tips e.g. *Peronospora* and *Pseudoperonospora*.
    - Sporangiophore dichotomously branched, tips of the branches expanded into cup shaped apophysis. Apophysis have four sterigmata along the margins of which sporangia are born.
- Sexual Reproduction is **oogamous** and meiosis occurs in the **gametangia** (oogonia and antheridia), resulting in formation of haploid oosphere and antheridial nuclei.

- The young oogonia in all species are multinucleate and differentiate into ooplasms and periplasm.
- Plasmogamy is by gametangial contact . The fertilization takes by fertilization tube. Fertilization may be **paragynous** or **amphigynous** type.
- The mature, usually single oospore lies free within the oogonial wall and may fill (**plerotic**) or may not fill (**aplerotic**) the oogonium.

The order is divided into three families on the basis of types of sporangiophore and sporangia. They are:

- **Pythiaceae**:Facultative parasites or saprophytes, sporangiophores undifferentiated from mycelium, branched, indeterminate,haustoria absent or branched.
- **Peronosporaceae**:Obligate parasites, well differentiated. Sporangiophores characteristically branched, determinate in growth, sporangia singly or in clusters,haustoria varies or branched.
- **Albuginaceae**:Obligate parasites, sporangiophores unbranched,clavate. Sporangia may behave like conidia, chain of conidiospores produced at the tip of conidiophores in basipetal succession; haustoria knob shaped.

Albugo candida (syn. *Cystopus candidus*)

The family, Albuginaceae has only one genus, *Albugo*. *Albugo* is represented by blisters or white rusts, but the most common amongst these is *A. candida*, causing white rust (not a true rust but named so because of its resemblance to aecial cups of rust fungi)on stems, leaves and fruits of cruciferous plants such as radish, mustard, chinese mustard, broccoli, cauliflower, cabbage etc. and several weed species.

The fungus is a biotrophic parasite of flowering plants and growing in pure culture is not possible. However growing callus culture of mustard plants having infection with *A. candida* has been possible recently which may help to understand the physiology and host pathogen relationships.

Symptoms

All the aerial parts of the plant like leaves, stem and flowers show the symptoms of the disease. The roots are not attacked by the fungus. The ventral surface of the leaf shows small white or cream-coloured shining, raised pustules in **concentric rings.** Pustules are smaller initially but later they coalesce to form large patches. The epidermis on the pustules remains intact for sometimes making the pustules appear shiny initially but as the fungus matures the epidermis on the pustule ruptures exposing the underlying sori and the powdery mass of spores making the pustules also powdery. The disease also causes deformation of flowers and fruits of the host. The distortion and hypertrophy of infected inflorescences is the most conspicuous symptoms of the disease. The leaves when severly infected become yellowish, shriveled and crinkled.

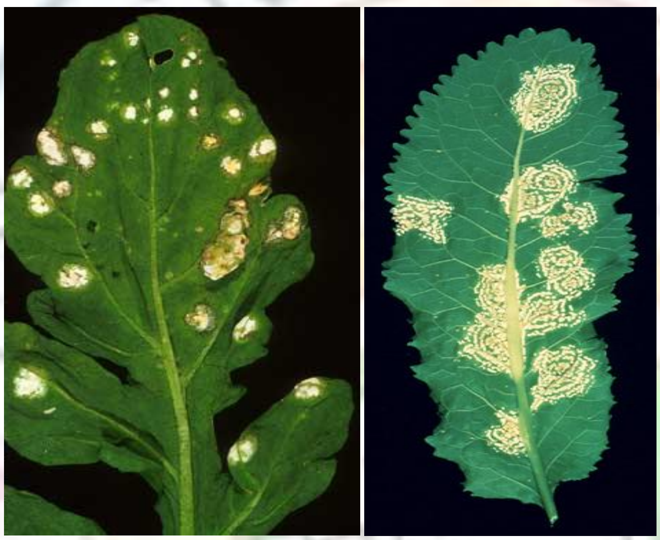


Figure: White rust on leaf of cruciferous plant, caused by *Albugo candida*
Source: http://www.apsnet.org/publications/imageresources/Pages/Lofng65.aspx

Developing stem and floral tissues when infected with spores show blisters and gross distortion of flowers, known as "**stagheads**" due to their spiny appearance. After the attack of young stems and inflorescence, the fungus spreads systemically in the whole plant causing the stem, inflorescence axis and the peduncles of the flowers to become thick and fleshy i.e. they become hypertrophied. They contain thick-walled oospore of the parasite. So extensive **hypertrophy** (increases in cell size), **hyperplasia** (increase in cell division) and chlorophyll and starch formation accompany fungal invasion. Other symptoms include deformation of petals which may become sepaloid, sepals and staments become thicker and leaf or carpel like. The pistil becomes swollen and grows beyond the limits of the petals and changes to large, conical, thick walled sac or changes into a sterile carpellary leaf.

Figure: Staghead Symptoms on Canola, Infected with Downy Mildew

Source:http://www.canolacouncil.org/canola-encyclopedia/diseases/white-rust/

Figure: *Albugo candida*: The hypertrophy and hyperplasia produced swelling and distortion in the floral tissues

Source: http://www.indiancropdiseases.com/mustard.aspx

Vegetative mycelium

The somatic mycelium consists of well developed, branched, aseptate and coenocytic hyphae. The hyphal wall is made up of cellulose and not chitin. The hyphae live and branch in the intercellular spaces. The protoplasm of the hyphae is granular and vacuolated in the older parts. Numerous nuclei, oil globules, glycogen and cell organelles like mitochondria, endoplasmic reticulum and ribosomes are present in these protoplast.

The intercellular hyphae absorb nutrition with the help of small globose or knob like haustoria. These haustoria arise as a lateral outgrowth which penetrate the host cells through minute perforations in the host cell walls. The slightly crescent-shaped bulge of the haustorial mother cell known as **haustorial initial** perforates the host cell wall at the **peneteration site** and protrudes into the lumen of the mesophyll cell to develop into a haustorium. It is bordered by the invaginated host plasma memberane.

Structure of Haustorium

The haustorium is a small, spherical structure consisting of two parts:

- The haustorial **stalk** or neck which connects the haustorium to the intercellular mycelium.It is 0.5μm in size. The protoplast of the haustorial neck has ribosomes but lacks other cell organelles.
- The terminal haustorial **head** or body :The cytoplasm of the haustorial body contains mitochondria, ribosomes and some times cisternae of RER and lipoidal inclusions but lacks nuclei and perinucleardictyosomes. The reason for this is that nuclei of *Albugo* are 2.5 μm in diameter and may not be able to penetrate through a narrow constriction (0.5 μm) which joins the haustorium to the intercellular hyphae. An electron dense, granular amorphous material forms the encapsulation on the outer face of the hyphal wall of the haustorial body. This sheath matrix, also known as the **extrahaustorial sheath** is absent in the neck region. The invaginated host plasma membrane which completely surrounds the haustorium and remains appressed to it is called the **extrahaustorial membrane.** It is flexible and thrown into a series of tubular projections extending into the host cytoplasm. Thus it delimits the extrahaustorial matrix from the host cytoplasm. From the plasma membrane of the hyphae develop a system of unit membranes and tubules towards the fungal cytoplasm. This system represents **lomasomes**.

A collar-like sheath surrounds the haustorial base which is an extension of the host cell wall, but this wall does not surround the haustorium. The pathogen induces secretory processes in the host which are not observable in the uninfected cell. There is an increase in the number of ribosomes, golgi complexes, various vesicular and tubular elements near the body of the haustorium in infected cells as compared to uninfected cells.

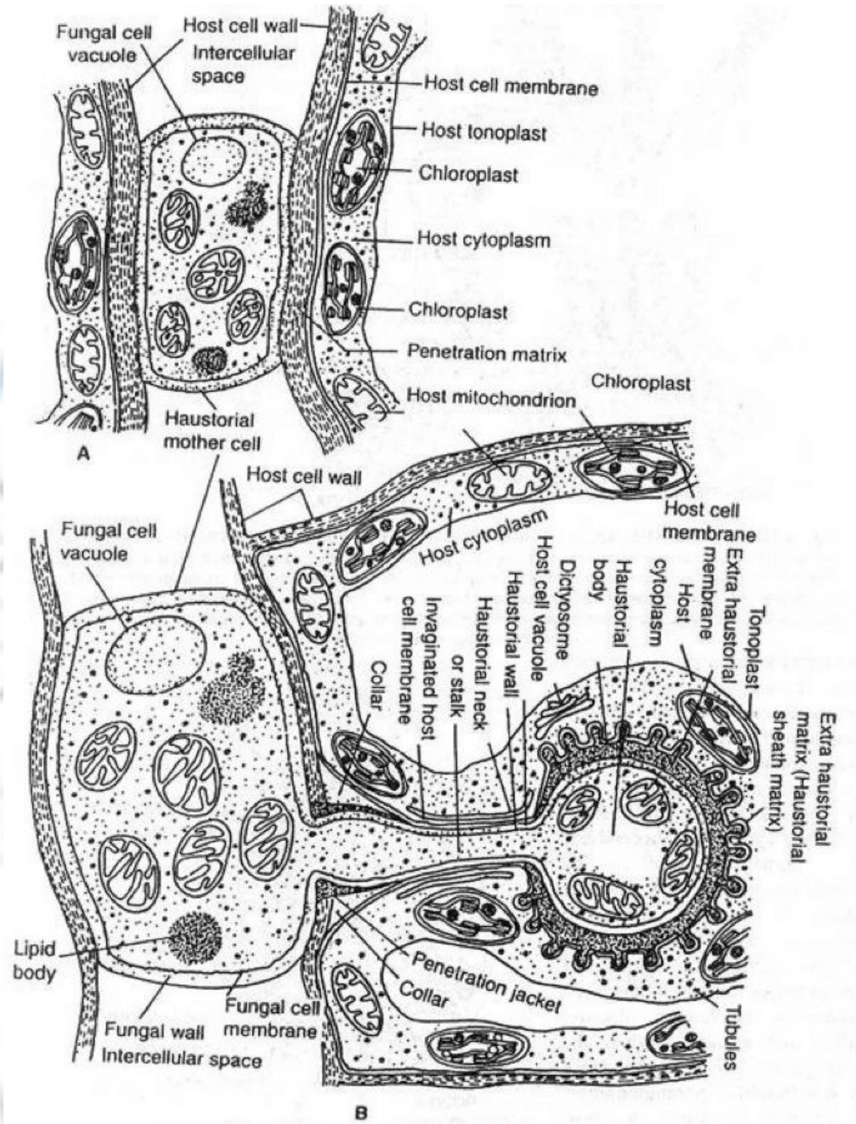


Figure: *Albugo candida* : (A) part of intercellular mycellium; (B) haustorium developed from intercellular mycellium in host cell.

Source:http://www.yourarticlelibrary.com/reproduction/2-methods-of-reproduction-in-genus-albugo-or-cystopus/3903/

Reproduction in *Albugo*

The two most important method of reproduction in genus ***Albugo*** or ***Cystopus*** are as following:

Asexual Reproduction

The asexual reproduction takes place by means of **biflagellate zoospores** formed inside the sporangia. In the beginning the intercellular hyphae accumulate just beneath the epidermis of the infected leaf and on maturation, these hyphae produce certain thick walled, clavate aerial sporangiophores forming a solid or palisade layer. There are about a dozen nuclei and sufficient cytoplasm in each sporangiophore. The terminal end of the sporangiophore becomes constricted and 5-8 nuclei and cytoplasm are included in each sporangium. Sporangia are produced in chain successively in **basipetal** succession i.e. the new sporangia are constricted at the base and the mature or oldest sporangium is at the top. The basipetal arrangements of sporangia in the chain serves two purposes.

- It permits ready dispersal of the oldest sporangia by air currents or rain water.
- It helps in proper nourishment of younger ones.

In between two sporangia a gelatinous pad or disc develops called as **disjunctor**. The disjunctor acts as separator of two successive sporangia. The sporangia is double walled, normally rounded and smooth but due to the pressure during its formation results in flattened sides so that some of them are cuboid or polyhedral in shape. In an infected area several fungal hyphae accumulate below the epidermis and give rise to innumerable sporangiophores which abstrict sporangia inabundance, due to which a pressure is built up and the epidermis bulge out. The host epidermis finally ruptures exposing sporangia on the surface of the host in the form of white creamy powder forming raised pustules or blisters. The distal sporangia by the time gets matured, the connectives or gelatinaous pads between them dry, shrink and finally disintegrate in moist air. The sporangia in chain thus separate and are disseminated from one place to another by various agencies such as wind, insects, water ect.

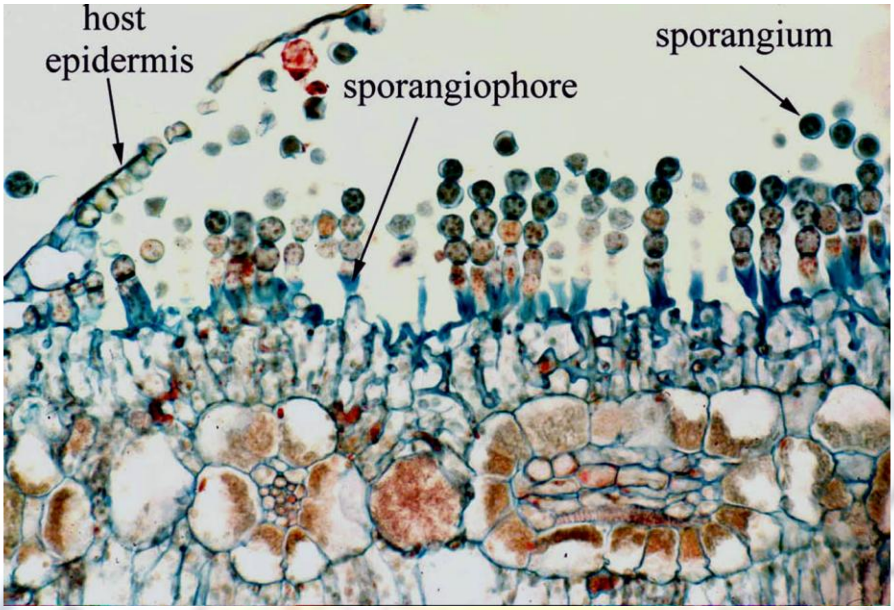


Figure: *Albugo candida*: T.S of leaf showing ruptured epidermis; sporangiophores arranged in palisade like manner bearing sporangia in basipetal fashion.

Source:https://atrium.lib.uoguelph.ca/xmlui/bitstream/handle/10214/5932/Albugo_candida_asexual_sporangia_from_host.jpg?sequence=1

Germination of Sporangia

On maturation of the sporangium, the protoplast is cleaved into uninucleate protoplasts. Each protoplast metamorphoses into naked, biflagellate, uninucleate, reniform and vacuolated zoospore. The sporangium bursts anteriorly and the zoospores are liberated in the film of water. Number of zoospores formed per sporangium is eight. After swimming for sometime the zoospores withdraw their flagellum and encyst. The encysted protoplast when lands on a suitable host germinates producing germ tube. The germ tube enters through stomata and branch profusely in intercellular spaces to form new mycelium.

Sometimes sporangia behave as **conidia** as they germinate directly by producing germ tubes. The behavior of the sporangium generally depends upon the temperature and moisture conditions of the environment.In presence of moisture and low temperature sporangium functions as zoosporangium. The optimum temperature for germination being 10°C.High temperature and comparatively drier condition induce the sporangium to behave like conidium.

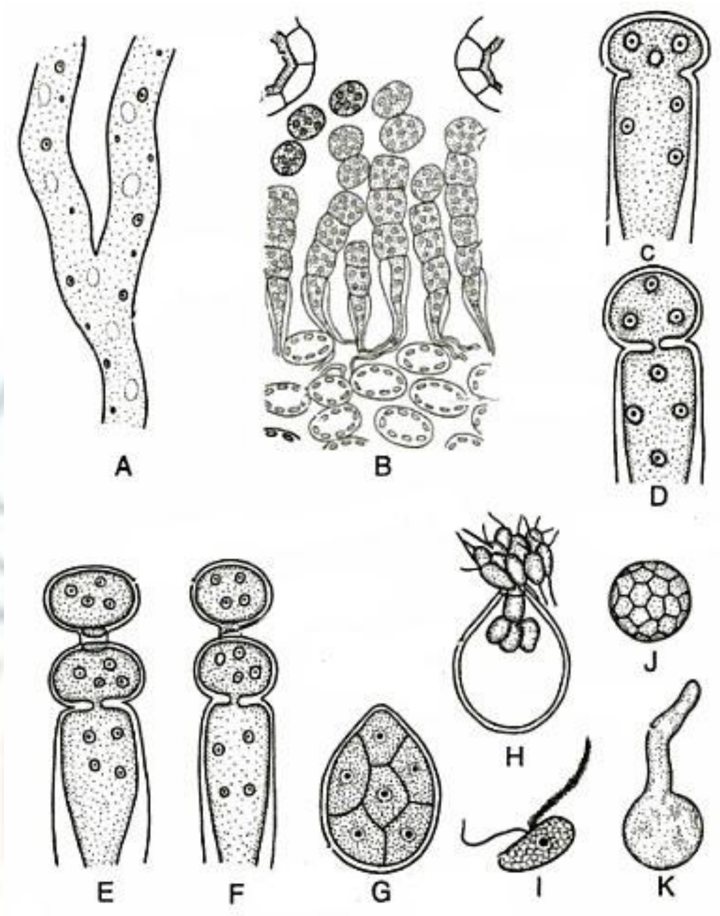


Figure: Asexual reproduction in *Albugo candida* (A) Somatic hyphae (B) sporangiophores bearing sporangia in basipetal fashion; (C-F) developmental stages of sporangia; (G-H) germination of sporangium and formation of zoospores; (I) biflagellate zoospores; (J) encysted zoospore; (K) germinating encysted zoospore.

Source :http://www.asturnatura.com/especie/albugo-candida.html

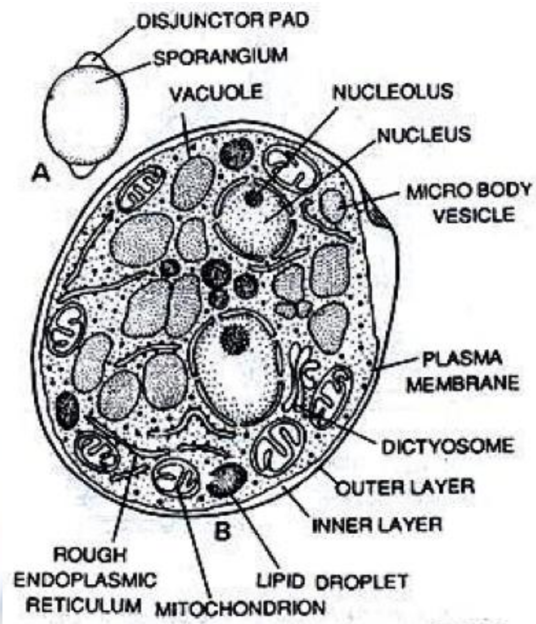


Figure: *Albugo candida*: (A) sporangium; (B) ultra structure of sporangium (diagrammatic).

Source: http://www.yourarticlelibrary.com/reproduction/2-methods-of-reproduction-in-genus-albugo-or-cystopus/3903/

Sexual Reproduction

Towards the end of the growing season of the host, the hyphae penetrate deeper into the tissues of stem and petiole and forms sex organs. Sexual reproduction is oogamous. The male sex organ is called an **antheridium** and the female sex organ as **oogonium**. They develop near each other in the intercellular spaces. The sex organs arise on separate hyphae called the male and female hyphae. Hypertrophy and distortion in shape are the external indications of the development of sex organs within the host tissue. The developing sex organs are separated from rest of the mycelium by septa.

Antheridium

Antheridium is elongated, club shaped, multinucleate and develops on terminal end of a hypha lying very close to the oogonium. The hyphal end swells and becomes multinucleate. A septum develops at the base of this swollen portion and cuts it off from the rest of the hyphae. This swollen, multinucleate, club shaped portion is called antheridium.

Oogonium

It arises as a globular enlargement of the tip of the female hypha, sometimes it can be intercalary in position. The swelling is multinucleate and is cut off from from the rest of the female hyphae by a cross wall at the base of the swelling. The cytoplasm, vacuoles and nuclei are uniformly distributed in the young oogonium. On maturation of the oogonium its protoplasm differentiates into two regions, the outer region is called as **periplasm** which has thin cytoplasm and is largely vacuolated. All the nuclei except one migrate to the peripheral periplasm. The central protoplasm is dense and has few vacuoles, this portion is called as **oosphere** or the **egg.** There is only one functional female nucleus in the dense cytoplasm of the oosphere. To begin with the development of the oogonium there are many nuclei, which later degenerate leaving one functional female nuclei. Prior to fertilization a spherical and granular cytoplasmic body appears in the centre of the oosphere called as **coenocentrum**. The single female nucleus is attracted to a point near it.

Fertilization

The antheridium soon arranges itself in **paragynous** arrangement i.e. contacting the oogonium at the side. At the point of contact between antheridium and oogonium a fertilization tube develops from the antheridium. The fertilization tube penetrates the oogonial wall and reaches the coenocentrum through the periplasm. This fertilization tube help in transfer of the functional male nucleus to the egg. The male nucleus fuses with the female nucleus and the rest of the nuclei of the antheridium degenerate. Thereafter the fertilization tube collapses but persists and the coentocentrum vanishes.

The two nuclei fuse to form a diploid zygote nucleus. The oosphere develops a warty wall and becomes the **oospore.** The diploid zygote nucleus divides mitotically many times and a multinucleate oospore is formed. The ridges of the oospore wall are characteristic of various species of *Albugo*.

Oospore

Mature oospore are globose (40-55µm)in diameter, have brown warty wall with much folded exospores. In this condition the oospore undergoes resting period of several months and after the resting period the oospore germinates. During germination outer wall of the exospore bursts and endospore comes out as a thin spherical vesicle, which may be sessile or formed at the end of a wide cylindrical tube. Within the vesicle 40-60 reniform biflagellate zoospores are differentiated. The vesicle breaks down after sometimes and the zoospores are liberated. After swimming for sometime the withdrawl of flagella followed by encystment of the zoospores take place .Soon the encysted zoospore germinates to form germtube which enters the host through stoma where it grows vigorously and forms the mycelium again. This completes the lifecycle of *Albugo*.

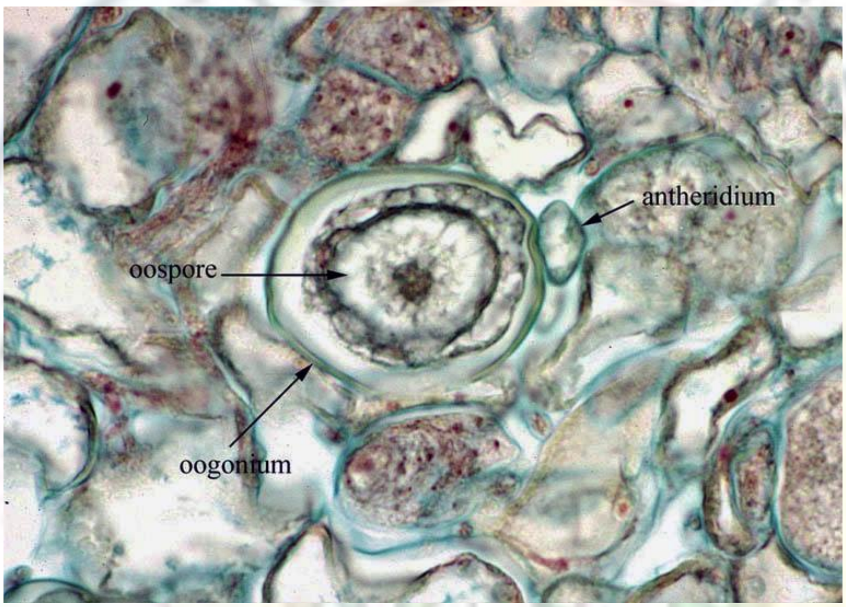


Figure: Transverse section of the leaf showing oospore of *Albugo*.

Source:https://atrium.lib.uoguelph.ca/xmlui/bitstream/handle/10214/5933/Albugo_candida_sexual_oospore_oogonium_antheridium.jpg?sequence=3

Albugo candida is thought to be **heterothallic**. Sometimes *Albugo* and *Peronospora* both occur on the same host. It is seen that large number of oospores are formed when both the parasites coexist in the host. It is suggested that the other pathogen produces same kind of stimulus inducing self fertilization in ***Albugo*.**

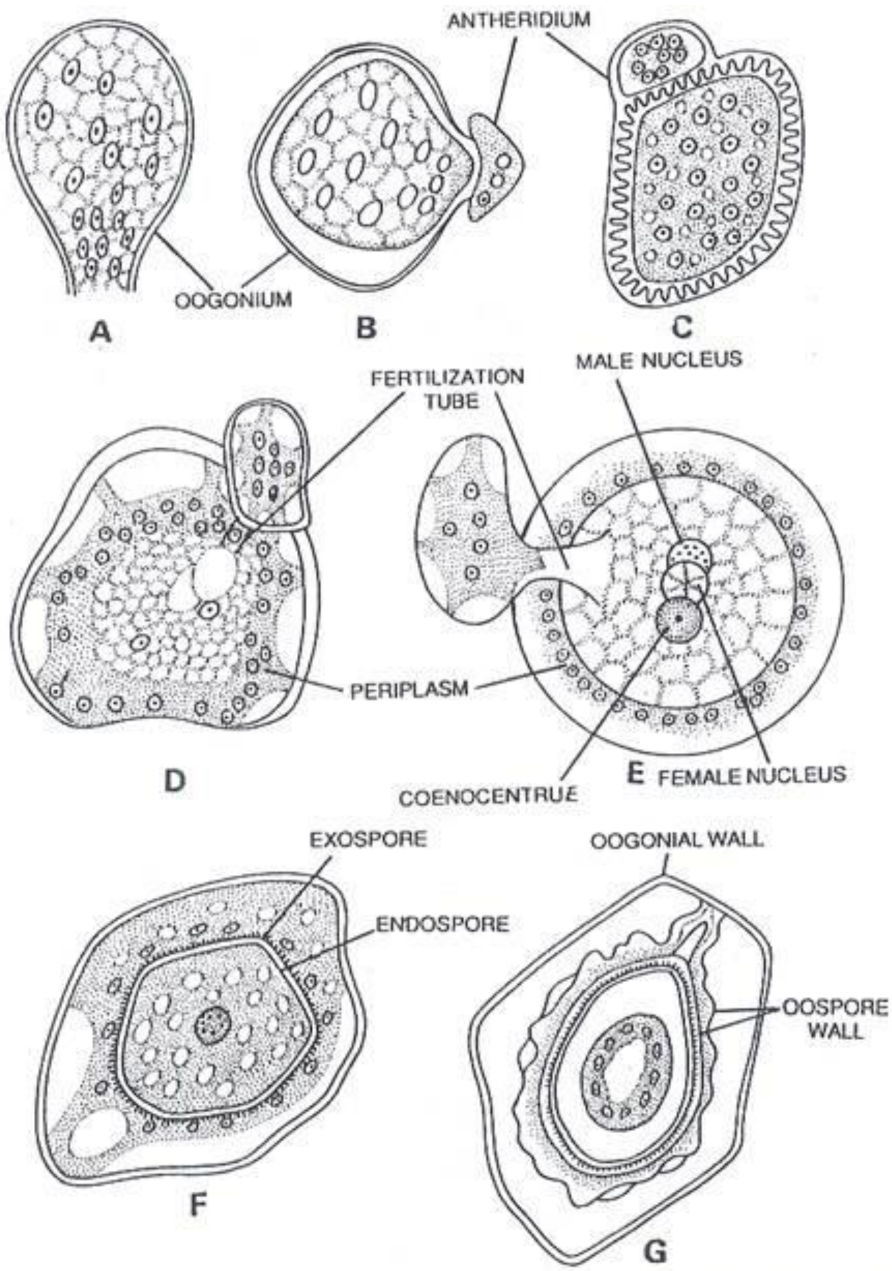


Figure: *Albugo candida* : (A) oogonium with many nuclei; (B) formation of receptive spot towards the attached antheridium; (C) nuclei of both antheridium and oogonium; (D) formation of fertilization tube and migration of the nuclei of oogonium to the peripheral region leaving behind a single female nucleus in the center of oogonium; (E) fusion of male and female nuclei; (F) karyogamy and oospore formation; (G) mature oospore.

Source:http://www.yourarticlelibrary.com/reproduction/2-methods-of-reproduction-in-genus-albugo-or-cystopus/3903/

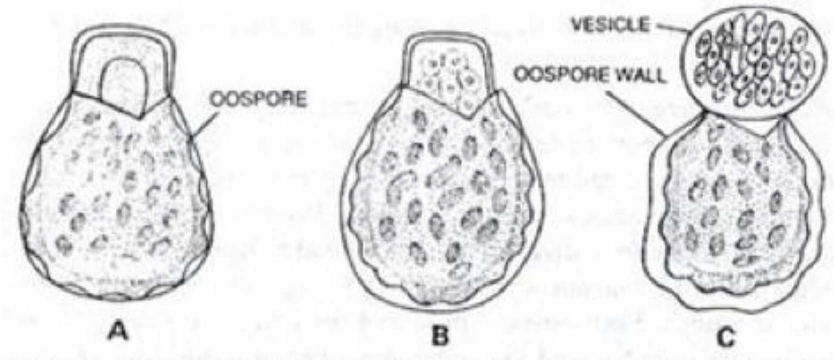


Figure: *Albugo sp* (A-B) germination of oospore; (C) the zoospores are formed in vesicle

Source:http://www.yourarticlelibrary.com/reproduction/2-methods-of-reproduction-in-genus-albugo-or-cystopus/3903/

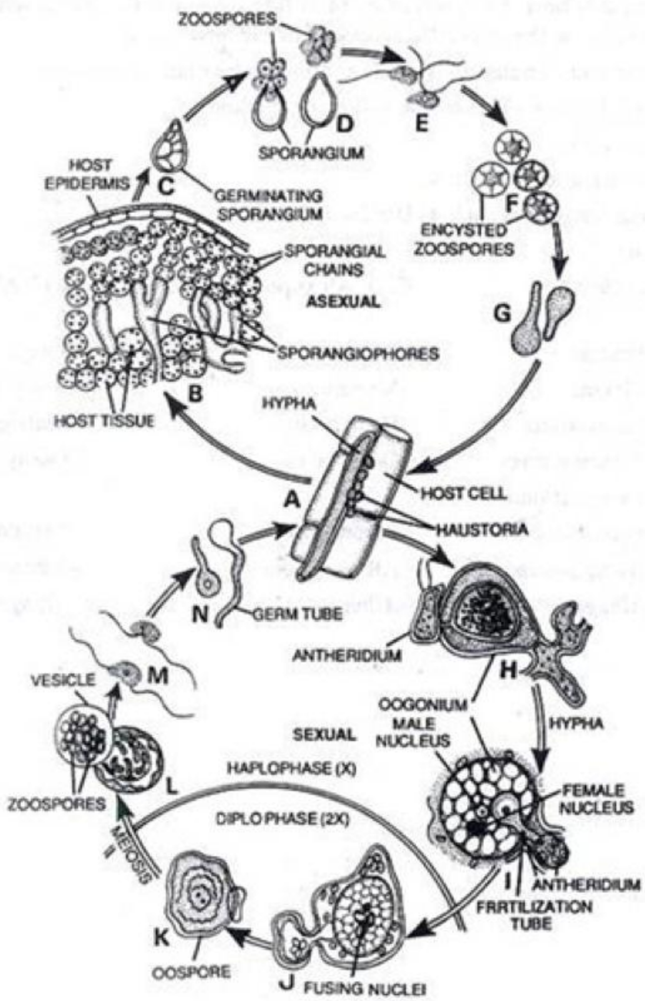


Figure: *Albugo* :Diagramatic life cycle. (A) hyphae within host cell showing globular haustoria; (B) infected leaf in vertical section showing sporangiophores and sporangial chains; (C) germinating sporangium; (D) sporangia releasing zoospores; (E) zoospores; (F) encysted zoospores; (G) germination of encysted zoospores; (H) antheridium and oogonium; (I) plasmogamy; (J) karyogamy; (K) oospore; (L) germination of oospore producing zoospores within vesicles; (M) Zoospores; (N) germination of encysted zoospores.

Source:http://www.yourarticlelibrary.com/reproduction/2-methods-of-reproduction-in-genus-albugo-or-cystopus/3903/

Disease Cycle of White rust of Crucifers

Albugo candida causes white rust of crucifers. The pathogen survives as oospores in the diseased plant debries. The oospores are the persistant resting spores.

Decomposition of the residues of the crop along with stagheads after harvesting, return oospores in the soil where they can remain dormant and infect the plants next year forming white pustules. Pustules produce spores which are released into the air and spread the infection to other plants.

Disease Control

- Field sanitation and crop rotation helps in reducing the soil borne primary inoculum (oospore).
- Use of healthy, clean seeds and eradication of weed hosts help in reducing the disease incidence.
- Foliage sprays whith chemicals are helpful in seed crops. These sprays are the fungicides such as Bordeaux mixture (0.8%), Bliton- 50 (0.3%), Daconil (0.1%), Difolatan (0.3%), Dithane M-45 (0.2%), Dithane Z-78 (0.2%) and Didomil (0.1%) can be used in 8-10 days intervals.
- Sources of resistance have also been identified in *Brassica napus* and *B. juncea* for development of disease resistant cultivars of the crops.

Summary

Albugo is an obligate, parasitic fungus which attacks several species of crucifers causing the white rust. The haplophase is represented by a well developed profusely branched mycelium which conists of aseptate, coenocytic hyphae. The hyphae are intercellular and they penetrate the host cells with the help of specialized structures called haustoria which are globose or spherical and help in absorbing nutrition from the host cells.

Asexual reproduction takes place usually by wind born sporangia produced in chains from the tips of short, club-shaped hyphae called as sporangiophores. The sporangiophores are packed in a palisade like manner beneath the epidermis of the host. Each sporangiophore cuts off sporangia at its tip in a basipetal fashion i.e. youngest sporangia at its base and oldest at its tip. The developing sporangia as well as sporangiophores exert pressure on the host epidermis due to which epidermis bulges and finally bursts over the sporangial sorus. Sporangia are then exposed as crust of white, blister-like patches. The mature globose, multinucleate sporangia thus exposed are disseminated by wind or washed away by splashes of rain water to the host where they germinate to spread the disease. The germination of sporangium depends on the moisture content and temperature conditions. In presence of moisture and low temperature the sporangium germinates by zoospores. In dry air and at high temperature it germinates as conidium by formation of germ tube. The zoospores are biflagellate, reniform, uninucleate structures. One flagellum is tinsel whereas other is whiplash type. They are inserted in depression. The liberated zoospore settles down on the host, withdraws its flagella, rounds off and secretes a wall around itself and germinates by producing germ tube. The germ tube infects the host tissue by entering through a stoma, it grows and forms the mycelium which is intercellular.

Sexual reproduction is oogamous and *Albugo* is heterothallic. The antheridia and oogonia are produced near each other towards the end of the growing season. Both antheridia and oogonia are multinucleate but only have one functional nucleus. The antheridium comes in contact with the oogonia at the side, the wall at the point of contact dissolves, a fertilization tube from the antheridium enters the oogonium and introduces a single male nucleus with some cytoplasm. The male and female nuclei fuses and fertilized egg becomes an oospore by screting a thick, warty wall around it. The oospore nucleus divides several times to produce about 32 nuclei. The oospore acts as resting spore and overcomes the unfavourable period. After the resting period the oospore germinates. The nuclei undergo mitotic division and the protoplast divides to form uninucleate daughter protoplasts which metamorphose into biflagellate, reniform zoospores. The oospore wall cracks and the zoospores pass into a thin vesicle which soon bursts open. The liberated zoospores swim in water for sometime and on coming in contact with suitable host it settles down. The quiescent zoospore withdraws it flagella , rounds off and secretes a wall around it. Soon it gives rise to germ tube which enters the host through a stoma and within the host tissue it grows vigorously and forms the mycelium.

Glossary

Amphigynous: having an antheridium through which the oogonial initial grows.

Auxillary (or primary) zoospores: pear shaped zoospores with the flagella attached at the anterior end of the spore.

Basipetal: A chain of conidia, the oldest conidium is at the apex and the youngest is at the base.

Biotrophic: Obtaining nutrients from living host cells without killing them

Blight: a general name for many diseases of plants esp. when leaf damage is sudden and serious, e.g., potato blight, late blight (*Phytophorainfestans*); early blight (*Alternariasolani*).

Coenocytic: (Gr. koinos = common + kylos = a hollow vessel):nonseptate; referring to the fact that the nuclei are embedded in the cytoplasm without being separated by cross-walls. i.e., the (: nucleilic in a common matrix

Deciduous : falling off, as leaves that are shed in the autumn; used to describe the sporangia of downy mildew fungi (Peronosporales: Oomycota).

Dimorphic: Having two different morphological forms.

Diplanetic: (Gr. dis = twice + planetes = wanderer): refers to a species which produces two types of zo&pores and in which two swarming periods

Disjunctor cell: An empty cell that fragments and/or undergoes lysis to release a conidium.

Downy mildew: A disease of plants caused by the fungi of the order Peronosporales and characterised by grey ,velvety patches of spores on the lower surface of leaves.

Encystment: Formation of a thick wall, (for example, around a zoospore after it settles and loses its flagellum/flagella)

Eucarpic: (Gr. Eu= good + karpos: fruit): forming reproductive structures on certain portions of the thallus, the thallus itself continuing to perform its somatic functions.

Facultative aerobe: An organism that can live in the absence as well as in the presence of oxygen.

Facultative anaerobe: An organism that carries aerobic metabolism when oxygen is present but shifts to anaerobic metabolism when oxygen is absent

Fertilization tube:(L. fertilis = fertile) a tube originating from the male gametangium and penetrating into the female through which the male gametes (nuclei) are transferred.

Gametangial contact: (Gr. gametes = husband + angeion = vessel): a method of sexual reproduction in which two gametangia come in contact but do not fuse. The male nucleus migrates through a fertilization tube into tile female gametangium

Haustorium: (L. haustor = drinker): absorbing organ originating on a hyphae of a parasite and penetrating into a cell of the host. Most often associated with obligate parasites, but also produced by some facultative parasites.

Holocarpic: (Gr. holo = entirely + kapos = fruit): refers to an organism whose thallus is entirely converted into one or more reproductive structures

Hyperplasia : (Gr. hyper: over + plasis. = molding, formation): excessive multiplication of cells; abnormal rate of cell division.

Hypertrophy: (Gr. hyper = over t trophe = food): excessive enlargement of cells.

Mastigoneme: in a tinsel flagellum, flimmer; tinsel; a fine hair-like projection that extends laterally from undulipodia.

Monophyletic: (Gr. monon = alone, single + Phylon = stock, race): of a single line of descent.

Monoplanetic: (Gr. monvs = alone, only + planetes = wanderer) refers to a species which produces only one type of zoospore and in which there is but one swarming period.

Obligate aerobe: Organism that must have oxygen to grow.

Obligate anaerobe: Organism that is killed by free oxygen.

Ooplasm: In*Oomycota*, the protoplasm, at the centre of the oogonium, this becomes the oosphere

Ooplast: The spherical, translucent to granular inclusion of the oospores

Oosphere:(Gr. oon = egg + sphaira = sphere): a large, naked, non-motile, female gamete.

Oospore: Gr. oon = egg + sporos = seed, spore): a thick-walled spore which develops from an oospherc through either fertilization or parthenogenesis.

Paragynous: having the antheridium at the side of the oogonium

Penetration: initial invasion of a host by a pathogen

Periplasm: peripheral cytoplasm; in *Oomycota*, the outer, non-functioning protoplasm of an oogonium or antheridium

Polyplanetic: Species having many swarming periods and only one type of zoospore.

Primary Inoculum: Inoculum that initiates disease in the field following a dormant stage in its Life cycle (called overwintering or oversummering).

Pustule: A blister-like spore mass breaking through a plant epidermis.

Vesicle: (L. vesicula = small bladder): a thin, bubble.like structure in which zoospores are released or in which zoospores are differentiated; also the bulbous head terminating the conidiophore of *Aspergillus.*

Excersises

Q1. Fill up the blanks.

1. The most common species of *Albugo* ………………which causes…………….
2. When the antheridia is paragynous, they are attached…………………to the oogonium.
3. The conidia in *Albugo* develop in ………………………succession.
4. The mycelium in *Albugo* is ………………………. and ……………………..
5. Haustoria in *Albugo* are……………………….
6. Cell wall of oomycetous fungi is made up of…………………………….
7. The zoospore of oomycetes are……………………
8. Sexual reproduction in oomycetes is……………………..
9. ……………………..fungicide is effective in controlling white rusts of crucifers.
10. Protoplasm at the center of oogonium is called as…………………….

Q2. Define the following terms.

- Amphigynous
- Paragynous
- Homothallic
- Coenocytic
- Diplanetic
- Periplasm
- Fertilization tube
- Coenocentrum
- Straminipilous flagellum
- Hypertrophy
- Hyperplasia

Q3. Answer the following questions

1. Write down the causal organism, symptoms and control measures of white rust of crucifers.
2. Briefly describe the asexual reproduction in *Albugo*.
3. What changes occur in the floral parts of crucifers due to infection of *Albugo.*
4. Briefly describe the germination of sporangium during reproduction of *Albugo.*
5. Briefly describe sexual reproduction in *Albugo.*
6. Describe the ecological significance of the group oomycota.
7. Describe the different views for systematic position of Oomycota.

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Web Links

https://www.apsnet.org/edcenter/intropp/LabExercises/Pages/Oomycetes.aspx

http://www.indiancropdiseases.com/mustard.aspx