

# Plant Pathology

Shavonne Meza

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## Chapter- 1

# Plant Pathology

**Plant pathology** (also **phytopathology**) is the scientific study of plant diseases caused by pathogens (infectious diseases) and environmental conditions (physiological factors). Organisms that cause infectious disease include fungi, oomycetes, bacteria, viruses, viroids, virus-like organisms, phytoplasmas, protozoa, nematodes and parasitic plants. Not included are ectoparasites like insects, mites, vertebrate or other pests that affect plant health by consumption of plant tissues. Plant pathology also involves the study of pathogen identification, disease etiology, disease cycles, economic impact, plant disease epidemiology, plant disease resistance, how plant diseases affect humans and animals, pathosystem genetics, and management of plant diseases.

### ***Plant pathogens***

#### **Fungi**

The majority of phytopathogenic fungi belong to the Ascomycetes and the Basidiomycetes.

The fungi reproduce both sexually and asexually via the production of spores. These spores may be spread long distances by air or water, or they may be soil borne. Many soil borne spores, normally zoospores, are capable of living saprotrophically, carrying out the first part of their lifecycle in the soil.

Fungal diseases can be controlled through the use of fungicides in agriculture, however new races of fungi often evolve that are resistant to various fungicides.



Powdery mildew, a Biotrophic Fungus



Rice blast, a necrotrophic fungus

Biotrophic fungal pathogens colonize living plant tissue and obtain nutrients from living host cells. Necrotrophic fungal pathogens infect and kill host tissue and extract nutrients from the dead host cells.

Significant fungal plant pathogens include:

### **Ascomycetes**

- *Fusarium* spp. (causal agents of Fusarium wilt disease)
- *Thielaviopsis* spp. (causal agents of: canker rot, black root rot, *Thielaviopsis* root rot)
- *Verticillium* spp.
- *Magnaporthe grisea* (causal agent of blast of rice and gray leaf spot in turfgrasses)

### **Basidiomycetes**

- *Rhizoctonia* spp.
- *Phakospora pachyrhizi* (causal agent of soybean rust)
- *Puccinia* spp. (causal agents of severe rusts of virtually all cereal grains and cultivated grasses)

### **Oomycetes**

The oomycetes are not true fungi but are fungal-like organisms. They include some of the most destructive plant pathogens including the genus *Phytophthora* which includes the causal agents of potato late blight and sudden oak death.

Despite not being closely related to the fungi, the oomycetes have developed very similar infection strategies and so many plant pathologists group them with fungal pathogens.

Significant oomycete plant pathogens

- *Pythium* spp.
- *Phytophthora* spp.; including the causal agent of the Great Irish Famine (1845–1849)

## Bacteria



Crown gall disease caused by *Agrobacterium*

Most bacteria that are associated with plants are actually saprotrophic, and do no harm to the plant itself. However, a small number, around 100 species, are able to cause disease. Bacterial diseases are much more prevalent in sub-tropical and tropical regions of the world.

Most plant pathogenic bacteria are rod shaped (bacilli). In order to be able to colonize the plant they have specific pathogenicity factors. Five main types of bacterial pathogenicity factors are known:

1. **Cell wall degrading enzymes** – used to break down the plant cell wall in order to release the nutrients inside. Used by pathogens such as *Erwinia* to cause soft rot.
2. **Toxins** These can be non-host specific, and damage all plants, or host specific and only cause damage on a host plant.
3. **Effector proteins** These can be secreted into the extracellular environment or directly into the host cell, often via the Type three secretion system. Some effectors are known to suppress host defense processes.
4. **Phytohormones** – for example *Agrobacterium* changes the level of auxins to cause tumours.

5. **Exopolysaccharides** – these are produced by bacteria and block xylem vessels, often leading to the death of the plant.

Bacteria control the production of pathogenicity factors via quorum sensing.

Significant bacterial plant pathogens

- Burkholderia
- Proteobacteria
  - *Xanthomonas* spp.
  - *Pseudomonas* spp.

### **Phytoplasmas ('Mycoplasma-like organisms') and spiroplasmas**



*Vitis vinifera* with "Ca. *Phytoplasma vitis*" infection

*Phytoplasma* and *Spiroplasma* are a genre of bacteria that lack cell walls, and are related to the mycoplasmas which are human pathogens. Together they are referred to as the mollicutes. They also tend to have smaller genomes than true bacteria. They are normally transmitted by sap-sucking insects, being transferred into the plants phloem where it reproduces.

### **Viruses, viroids and virus-like organisms**

There are many types of plant virus, and some are even asymptomatic. Normally plant viruses only cause a loss of crop yield. Therefore it is not economically viable to try to control them, the exception being when they infect perennial species, such as fruit trees.

Most plant viruses have small, single stranded RNA genomes. These genomes may only encode three or four proteins: a replicase, a coat protein, a movement protein to allow cell

to cell movement through plasmodesmata and sometimes a protein that allows transmission by a vector.

Plant viruses must be transmitted from plant to plant by a vector. This is often by an insect (for example, aphids), but some fungi, nematodes and protozoa have been shown to be viral vectors.

## **Nematodes**



Root-knot nematode galls

Nematodes are small, multicellular wormlike creatures. Many live freely in the soil, but there are some species which parasitize plant roots. They are a problem in tropical and subtropical regions of the world, where they may infect crops. Potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*) are widely distributed in Europe and North and South America and cause \$300 million worth of damage in Europe every year. Root knot nematodes have quite a large host range, whereas cyst nematodes tend to only be able to infect a few species. Nematodes are able to cause radical changes in root cells in order to facilitate their lifestyle.

## **Protozoa**

There are a few examples of plant diseases caused by protozoa. They are transmitted as zoospores which are very durable, and may be able to survive in a resting state in the soil for many years. They have also been shown to transmit plant viruses.

When the motile zoospores come into contact with a root hair they produce a plasmodium and invade the roots.

## **Parasitic plants**

Parasitic plants such as mistletoe and dodder are included in the study of phytopathology. Dodder, for example, is used as a conduit for the transmission of viruses or virus-like agents from a host plant to either a plant that is not typically a host or for an agent that is not graft-transmissible.

## ***Physiological plant disorders***

Significant abiotic disorders can be caused by:

### ***Natural***

Drought

Frost damage, and breakage by snow and hail

Flooding and poor drainage

Nutrient deficiency

Salt deposition and other soluble mineral excesses (e.g. gypsum)

Wind (windburn, and breakage by hurricanes and tornadoes)

Lightning and wildfire (also often man-made)

***Man-made*** (arguably not abiotic, but usually regarded as such)

Soil compaction

Pollution of air, soil, or both

Salt from winter road salt application or irrigation

Herbicide over-application

Poor education and training of people working with plants (e.g. lawnmower damage to trees)

Vandalism

## ***Management***

### Quarantine

Wherein a diseased patch of vegetation or individual plants are isolated from other, healthy growth. Specimens may be destroyed or relocated into a greenhouse for treatment/study. Another option is to avoid introduction of harmful non-native organisms by controlling all human traffic and activity (for e.g., AQIS) although legislation and enforcement are key in order to ensure lasting effectiveness.

### Cultural

Farming in some societies is kept on a small scale, tended by peoples whose culture includes farming traditions going back to ancient times. (An example of such traditions would be lifelong training in techniques of plot terracing, weather anticipation and response, fertilization, grafting, seed care, and dedicated gardening.) Plants that are intently monitored often benefit not only from active external protection, but a greater overall vigor as well. While primitive in the sense of being the most labor-intensive solution by far, where practical or necessary it is more than adequate.

### Plant resistance

Sophisticated agricultural developments now allow growers to choose from among systematically cross-bred species to ensure the greatest hardiness in their crops, as suited for a particular region's pathological profile. Breeding practices have been perfected over centuries, but with the advent of genetic manipulation even finer control of a crop's immunity traits is possible. The engineering of foodplants may be less rewarding however, as higher output is frequently offset by popular suspicion and negative opinion about this "tampering" with nature.

#### Chemical

(See: pesticide application) Many natural and synthetic compounds exist that could be employed to combat the above threats. This method works by directly eliminating disease-causing organisms or curbing their spread; however it has been shown to have too broad an effect, typically, to be good for the local ecosystem. From an economic standpoint all but the simplest natural additives may disqualify a product from "organic" status, potentially reducing the value of the yield.

#### Biological

Crop rotation may be an effective means to prevent a parasitic population from becoming well established, as an organism affecting leaves would be starved when the leafy crop is replaced by a tuberous type, etc. Other means to undermine parasites without attacking them directly may exist.

#### Integrated

The use of two or more of these methods in combination offers a higher chance of effectiveness.

## Chapter- 2

# Plant Disease Epidemiology

**Plant Disease epidemiology** is the study of disease in plant populations. Much like diseases of humans and animals, plant diseases occur due to pathogens such as bacteria, viruses, fungi, oomycetes, nematodes, phytoplasmas, protozoa, and parasitic plants. Plant disease epidemiologists strive for an understanding of the cause and effects of disease and develop strategies to intervene in situations where crop losses may occur. Typically successful intervention will lead to a low enough level of disease to be acceptable, depending upon the value of the crop.

Plant disease epidemiology is often looked at from a multi-disciplinary approach, requiring biological, statistical, agronomic and ecological perspectives. Biology is necessary for understanding the pathogen and its life cycle. It is also necessary for understanding the physiology of the crop and how the pathogen is adversely affecting it. Agronomic practices often influence disease incidence for better or for worse. Ecological influences are numerous. Native species of plants may serve as reservoirs for pathogens that cause disease in crops. Statistical models are often applied in order to summarize and describe the complexity of plant disease epidemiology, so that disease processes can be more readily understood. For example, comparisons between patterns of disease progress for different diseases, cultivars, management strategies, or environmental settings can help in determining how plant diseases may best be managed. Policy can be influential in the occurrence of diseases, through actions such as restrictions on imports from sources where a disease occurs.

In 1963 J. E. van der Plank published "Plant Diseases: Epidemics and Control", a seminal work that created a theoretical framework for the study of the epidemiology of plant diseases. This book provides a theoretical framework based on experiments in many different host pathogen systems and moved the study of plant disease epidemiology forward rapidly, especially for fungal foliar pathogens. Using this framework we can now model and determine thresholds for epidemics that take place in a homogeneous environment such as a mono-cultural crop field.

## ***Elements of an epidemic***

Disease epidemics in plants can cause huge losses in yield of crops as well threatening to wipe out an entire species such as was the case with Dutch Elm Disease and could occur with Sudden Oak Death. An epidemic of potato late blight, caused by *Phytophthora infestans*, led to the Great Irish Famine and the loss of many lives.

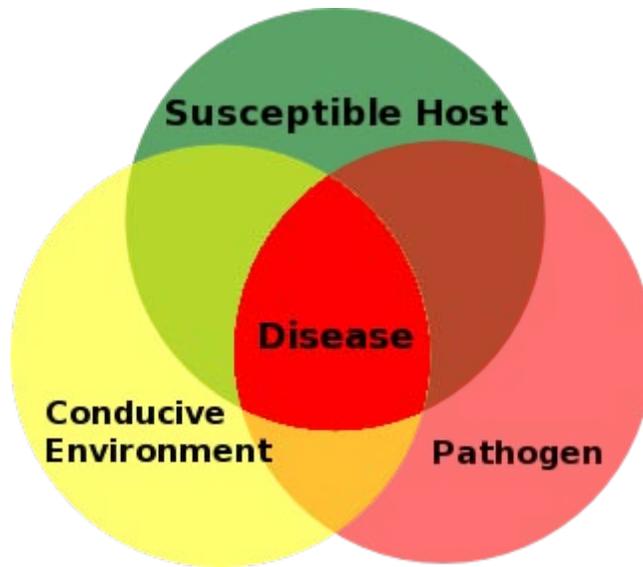
Commonly the elements of an epidemic are referred to as the “disease triangle”: a susceptible host, pathogen, and conducive environment. For disease to occur all three of these must be present. Below is an illustration of this point. Where all three items meet there is disease. The fourth element missing from this illustration for an epidemic to occur, is time. As long as all three of these elements are present disease can initiate, an epidemic will only ensue if all three continue to be present. Any one of the three might be removed from the equation though. The host might out-grow susceptibility as with high-temperature adult-plant resistance, the environment changes and is not conducive for the pathogen to cause disease, or the pathogen is controlled through a fungicide application for instance.

Sometimes a fourth factor of time is added as the time at which a particular infection occurs, and the length of time conditions remain viable for that infection, can also play an important role in epidemics. The age of the plant species can also play a role, as certain species change in their levels of disease resistance as they mature; a process known as ontogenic resistance.

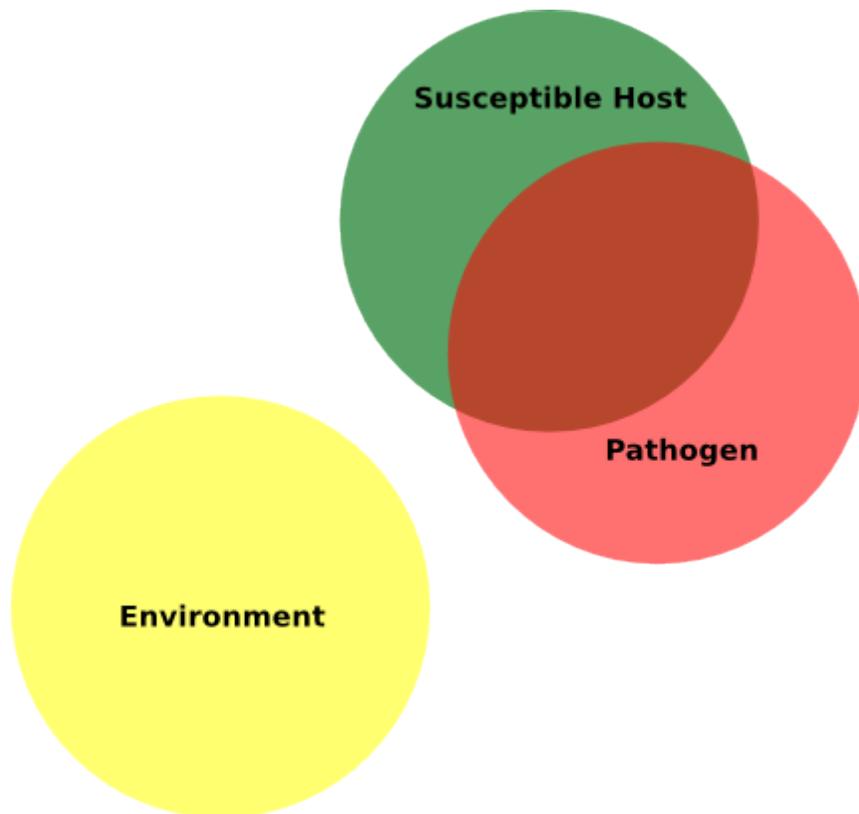
If all of the criteria are not met, such as a susceptible host and pathogen are present but the environment is not conducive to the pathogen infecting and causing disease, disease cannot occur. For example, corn is planted into a field with corn residue that has the fungus *Cercospora zea-maydis*, the causal agent of Grey leaf spot of corn, but if the weather is too dry and there is no leaf wetness the spores of the fungus in the residue cannot germinate and initiate infection.

Likewise, it stands to reason if the host is susceptible and the environment favours the development of disease but the pathogen is not present there is no disease. Taking the example above, the corn is planted into a ploughed field where there is no corn residue with the fungus *Cercospora zea-maydis*, the causal agent of Grey leaf spot of corn, present but the weather means long periods of leaf wetness, there is no infection initiated.

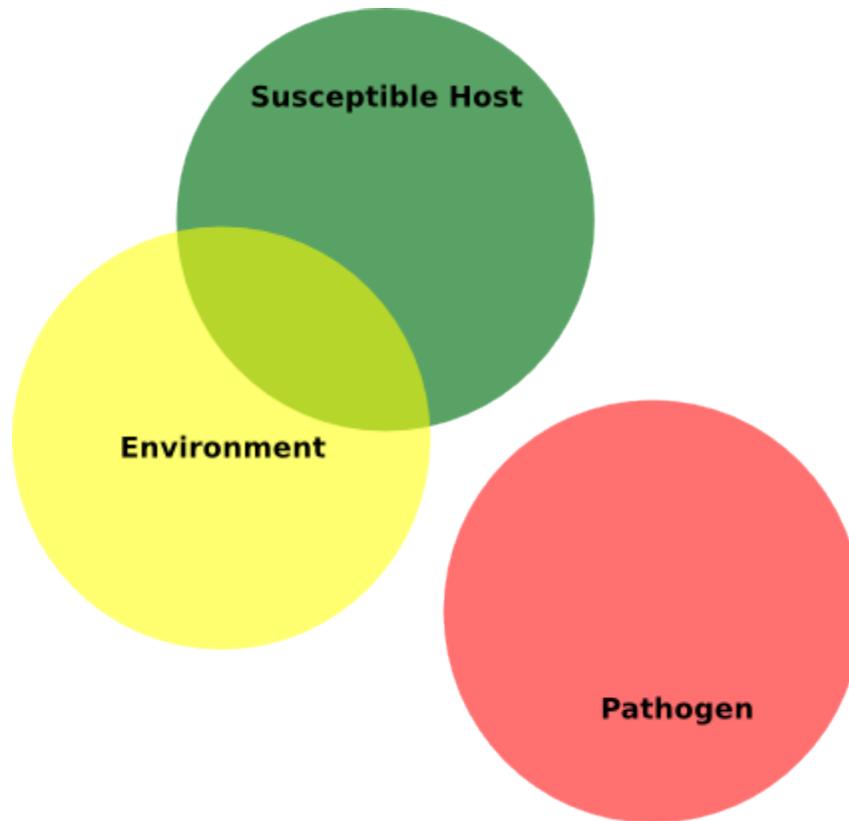
When a pathogen requires a vector to be spread then for an epidemic to occur the vector must be plentiful and active.



Plant disease triangle illustration



Plant disease triangle illustration, non-conductive environment



Plant disease triangle illustration, no pathogen

## Types of Epidemics

Monocyclic epidemics are caused by pathogens with a low birth rate and death rate meaning they only have one infection cycle per season. They are typical of soil born diseases such as Fusarium wilt of flax. Polycyclic epidemics are caused by pathogens capable of several infection cycles a season. These are most often caused by airborne diseases such as powdery mildew. Bimodal polycyclic epidemics can also occur. For example in brown rot of stone fruits the blossoms and the fruits are infected at different times.

For some diseases it is important to consider the disease occurrence over several growing seasons, especially if growing the crops in monoculture year after year or growing perennial plants. Such conditions can mean that the inoculum produced in one season can be carried over to the next leading to a build of an inoculum over the years. In the tropics there are no clear cut breaks between growing seasons as there are in temperate regions and this can lead to accumulation of inoculum.

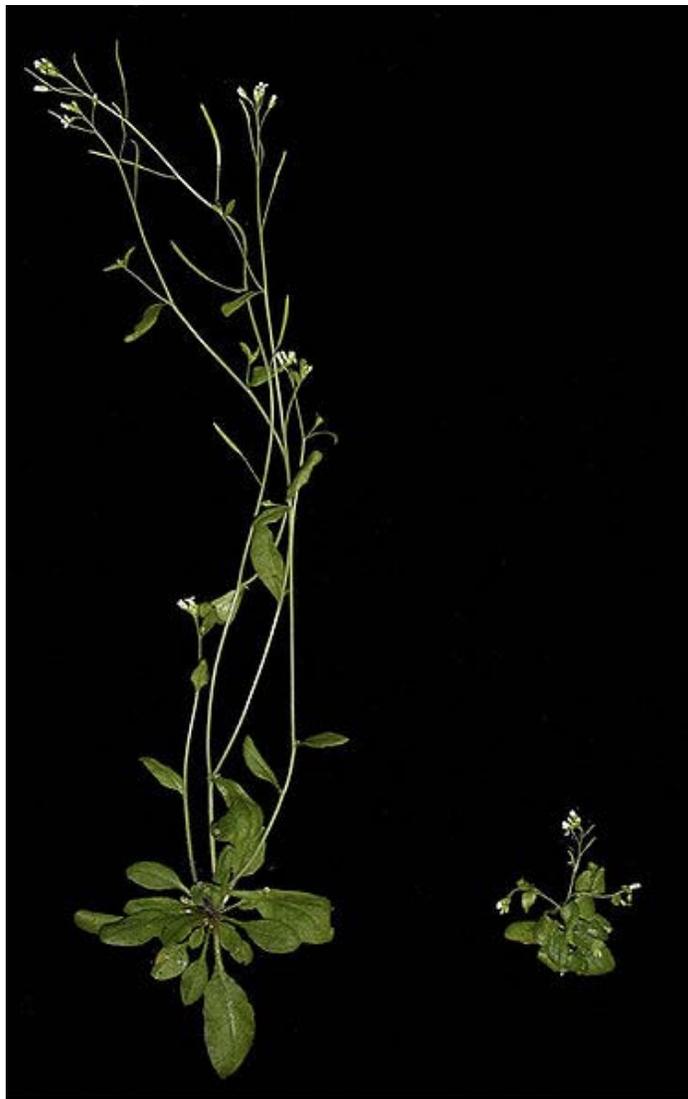
Epidemics that occur under these conditions are referred to as polyetic epidemics and can be caused by both monocyclic and polycyclic pathogens. Apple powdery mildew is an example of a polyetic epidemic caused by a polycyclic pathogen and Dutch Elm disease a polyetic epidemic caused by a monocyclic pathogen.

## ***Employment***

Plant disease epidemiologists are typically employed as researchers by universities, or governmental institutions such as the USDA. However, private companies in agricultural fields also employ epidemiologists.

## Chapter- 3

# Plant Hormone



Lack of the plant hormone auxin can cause abnormal growth (right)

**Plant hormones** (also known as **phytohormones**) are chemicals that regulate plant growth, which, in the UK, are termed 'plant growth substances'. Plant hormones are signal molecules produced within the plant, and occur in extremely low concentrations. Hormones regulate cellular processes in targeted cells locally and when moved to other locations, in other locations of the plant. Hormones also determine the formation of flowers, stems, leaves, the shedding of leaves, and the development and ripening of fruit. Plants, unlike animals, lack glands that produce and secrete hormones, instead each cell is capable of producing hormones. Plant hormones shape the plant, affecting seed growth, time of flowering, the sex of flowers, senescence of leaves and fruits. They affect which tissues grow upward and which grow downward, leaf formation and stem growth, fruit development and ripening, plant longevity, and even plant death. Hormones are vital to plant growth and lacking them, plants would be mostly a mass of undifferentiated cells.

### ***Characteristics***

The word hormone is derived from Greek, meaning 'set in motion.' Plant hormones affect gene expression and transcription levels, cellular division, and growth. They are naturally produced within plants, though very similar chemicals are produced by fungi and bacteria that can also effect plant growth. A large number of related chemical compounds are synthesized by humans, they are used to regulate the growth of cultivated plants, weeds, and in vitro-grown plants and plant cells; these manmade compounds are called **Plant Growth Regulators** or **PGRs** for short. Early in the study of plant hormones, "phytohormone" was the commonly used term, but its use is less widely applied now.

Plant hormones are not nutrients, but chemicals that in small amounts promote and influence the growth, development, and differentiation of cells and tissues. The biosynthesis of plant hormones within plant tissues is often diffuse and not always localized. Plants lack glands to produce and store hormones, because, unlike animals, which have two circulatory systems (lymphatic and cardiovascular) powered by a heart that moves fluids around the body, plants use more passive means to move chemicals around the plant. Plants utilize simple chemicals as hormones, which move more easily through the plant's tissues. They are often produced and used on a local basis within the plant body, plant cells even produce hormones that affect different regions of the cell producing the hormone.

Hormones are transported within the plant by utilizing four types of movements. For localized movement, cytoplasmic streaming within cells and slow diffusion of ions and molecules between cells are utilized. Vascular tissues are used to move hormones from one part of the plant to another; these include sieve tubes that move sugars from the leaves to the roots and flowers, and xylem that moves water and mineral solutes from the roots to the foliage.

Not all plant cells respond to hormones, but those cells that do are programmed to respond at specific points in their growth cycle. The greatest effects occur at specific stages during the cell's life, with diminished effects occurring before or after this period. Plants need hormones at very specific times during plant growth and at specific locations.

They also need to disengage the effects that hormones have when they are no longer needed. The production of hormones occurs very often at sites of active growth within the meristems, before cells have fully differentiated. After production they are sometimes moved to other parts of the plant where they cause an immediate effect or they can be stored in cells to be released later. Plants use different pathways to regulate internal hormone quantities and moderate their effects; they can regulate the amount of chemicals used to biosynthesize hormones. They can store them in cells, inactivate them, or cannibalise already-formed hormones by conjugating them with carbohydrates, amino acids or peptides. Plants can also break down hormones chemically, effectively destroying them. Plant hormones frequently regulate the concentrations of other plant hormones. Plants also move hormones around the plant diluting their concentrations.

The concentration of hormones required for plant responses are very low ( $10^{-6}$  to  $10^{-5}$  mol/L). Because of these low concentrations, it has been very difficult to study plant hormones, and only since the late 1970s have scientists been able to start piecing together their effects and relationships to plant physiology. Much of the early work on plant hormones involved studying plants that were genetically deficient in one or involved the use of tissue-cultured plants grown *in vitro* that were subjected to differing ratios of hormones, and the resultant growth compared. The earliest scientific observation and study dates to the 1880s; the determination and observation of plant hormones and their identification was spread-out over the next 70 years.

## ***Classes of plant hormones***

In general, it is accepted that there are five major classes of plant hormones, some of which are made up of many different chemicals that can vary in structure from one plant to the next. The chemicals are each grouped together into one of these classes based on their structural similarities and on their effects on plant physiology. Other plant hormones and growth regulators are not easily grouped into these classes; they exist naturally or are synthesized by humans or other organisms, including chemicals that inhibit plant growth or interrupt the physiological processes within plants. Each class has positive as well as inhibitory functions, and most often work in tandem with each other, with varying ratios of one or more interplaying to affect growth regulation.

The five major classes are:

### **Abscisic acid**

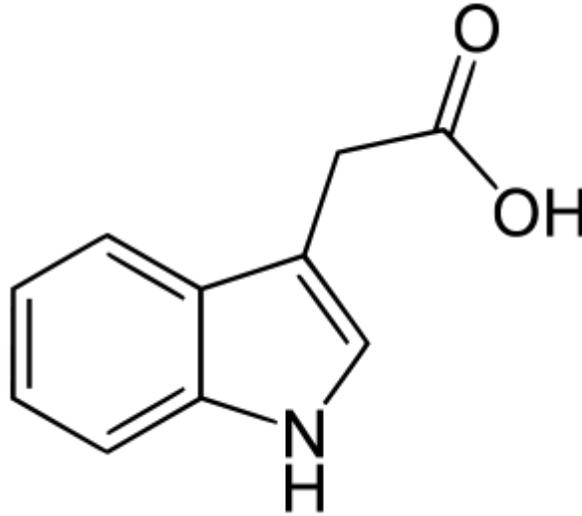
Abscisic acid also called ABA, was discovered and researched under two different names before its chemical properties were fully known, it was called *dormin* and *abscicin II*. Once it was determined that the two latter compounds were the same; it was named abscisic acid. The name "abscisic acid" was given because it was found in high concentrations in newly abscised or freshly fallen leaves.

This class of PGR is composed of one chemical compound normally produced in the leaves of plants, originating from chloroplasts, especially when plants are under stress. In

general, it acts as an inhibitory chemical compound that affects bud growth, seed and bud dormancy. It mediates changes within the apical meristem causing bud dormancy and the alteration of the last set of leaves into protective bud covers. Since it was found in freshly abscised leaves, it was thought to play a role in the processes of natural leaf drop but further research has disproven this. In plant species from temperate parts of the world it plays a role in leaf and seed dormancy by inhibiting growth, but, as it is dissipated from seeds or buds, growth begins. In other plants, as ABA levels decrease, growth then commences as gibberellin levels increase. Without ABA, buds and seeds would start to grow during warm periods in winter and be killed when it froze again. Since ABA dissipates slowly from the tissues and its effects take time to be offset by other plant hormones, there is a delay in physiological pathways that provide some protection from premature growth. It accumulates within seeds during fruit maturation, preventing seed germination within the fruit, or seed germination before winter. Abscisic acid's effects are degraded within plant tissues during cold temperatures or by its removal by water washing in out of the tissues, releasing the seeds and buds from dormancy.

In plants under water stress, ABA plays a role in closing the stomata. Soon after plants are water-stressed and the roots are deficient in water, a signal moves up to the leaves, causing the formation of ABA precursors there, which then move to the roots. The roots then release ABA, which is translocated to the foliage through the vascular system and modulates the potassium and sodium uptake within the guard cells, which then lose turgidity, closing the stomata. ABA exists in all parts of the plant and its concentration within any tissue seems to mediate its effects and function as a hormone; its degradation, or more properly catabolism, within the plant affects metabolic reactions and cellular growth and production of other hormones. Plants start life as a seed with high ABA levels, just before the seed germinates ABA levels decrease; during germination and early growth of the seedling, ABA levels decrease even more. As plants begin to produce shoots with fully functional leaves - ABA levels begin to increase, slowing down cellular growth in more "mature" areas of the plant. Stress from water or predation affects ABA production and catabolism rates, mediating another cascade of effects that trigger specific responses from targeted cells. Scientists are still piecing together the complex interactions and effects of this and other phytohormones.

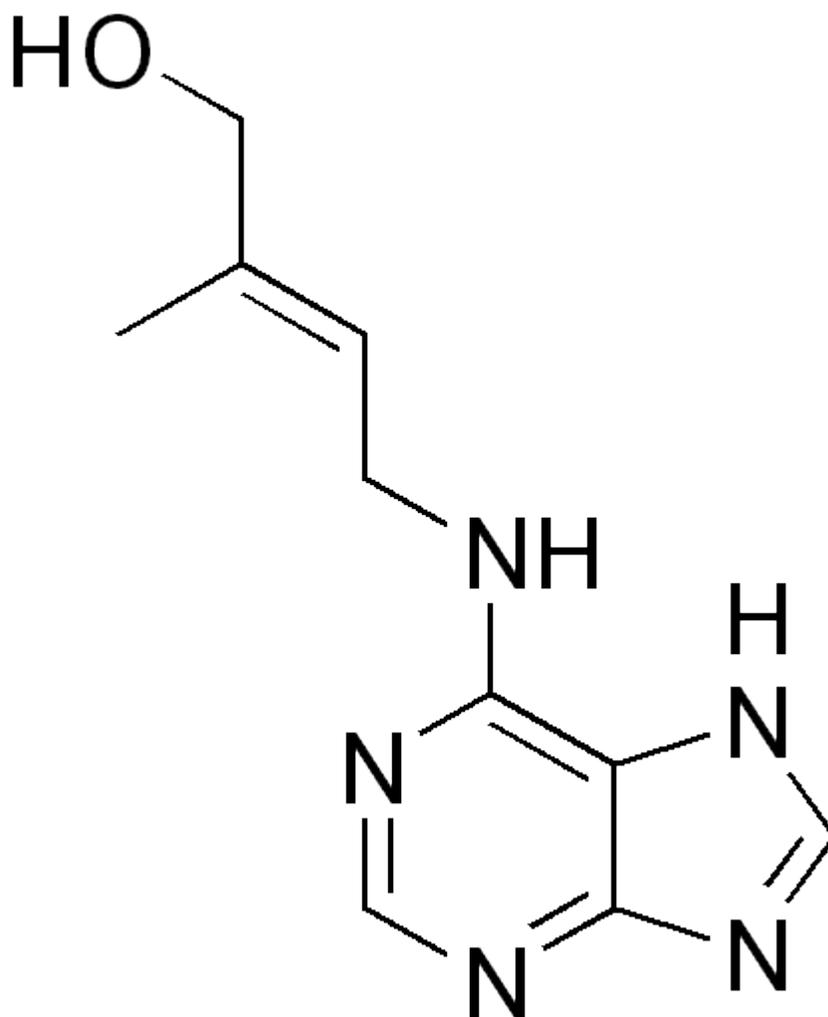
## Auxins



The auxin indoleacetic acid

Auxins are compounds that positively influence cell enlargement, bud formation and root initiation. They also promote the production of other hormones and in conjunction with cytokinins, they control the growth of stems, roots, and fruits, and convert stems into flowers. Auxins were the first class of growth regulators discovered. They affect cell elongation by altering cell wall plasticity. Auxins decrease in light and increase where it is dark. They stimulate cambium cells to divide and in stems cause secondary xylem to differentiate. Auxins act to inhibit the growth of buds lower down the stems (apical dominance), and also to promote lateral and adventitious root development and growth. Leaf abscission is initiated by the growing point of a plant ceasing to produce auxins. Auxins in seeds regulate specific protein synthesis, as they develop within the flower after pollination, causing the flower to develop a fruit to contain the developing seeds. Auxins are toxic to plants in large concentrations; they are most toxic to dicots and less so to monocots. Because of this property, synthetic auxin herbicides including 2,4-D and 2,4,5-T have been developed and used for weed control. Auxins, especially 1-Naphthaleneacetic acid (NAA) and Indole-3-butyric acid (IBA), are also commonly applied to stimulate root growth when taking cuttings of plants. The most common auxin found in plants is indoleacetic acid or IAA. The correlation of auxins and cytokinins in the plants is a constant ( $A/C = \text{const.}$ ).

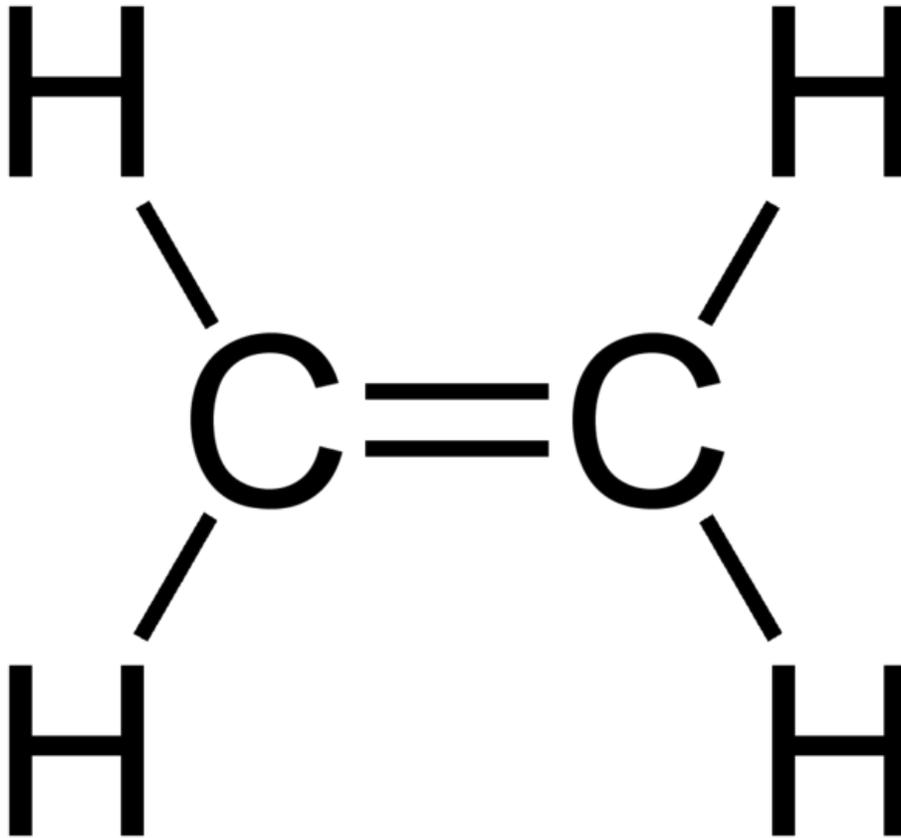
## Cytokinins



The cytokinin zeatin, *Zea*, in which it was first discovered in immature kernels.

Cytokinins or CKs are a group of chemicals that influence cell division and shoot formation. They were called kinins in the past when the first cytokinins were isolated from yeast cells. They also help delay senescence or the aging of tissues, are responsible for mediating auxin transport throughout the plant, and affect internodal length and leaf growth. They have a highly synergistic effect in concert with auxins and the ratios of these two groups of plant hormones affect most major growth periods during a plant's lifetime. Cytokinins counter the apical dominance induced by auxins; they in conjunction with ethylene promote abscission of leaves, flower parts and fruits. The correlation of auxins and cytokinins in the plants is a constant ( $A/C = \text{const.}$ ).

## Ethylene

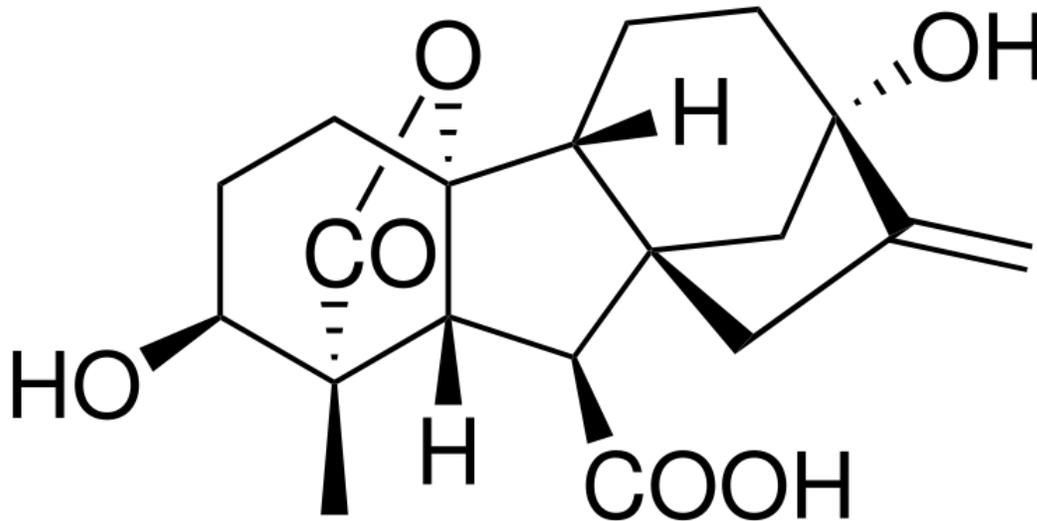


Ethylene

Ethylene is a gas that forms through the Yang Cycle from the breakdown of methionine, which is in all cells. Ethylene has very limited solubility in water and does not accumulate within the cell but diffuses out of the cell and escapes out of the plant. Its effectiveness as a plant hormone is dependent on its rate of production versus its rate of escaping into the atmosphere. Ethylene is produced at a faster rate in rapidly growing and dividing cells, especially in darkness. New growth and newly germinated seedlings produce more ethylene than can escape the plant, which leads to elevated amounts of ethylene, inhibiting leaf expansion. As the new shoot is exposed to light, reactions by phytochrome in the plant's cells produce a signal for ethylene production to decrease, allowing leaf expansion. Ethylene affects cell growth and cell shape; when a growing shoot hits an obstacle while underground, ethylene production greatly increases, preventing cell elongation and causing the stem to swell. The resulting thicker stem can exert more pressure against the object impeding its path to the surface. If the shoot does not reach the surface and the ethylene stimulus becomes prolonged, it affects the stems natural geotropic response, which is to grow upright, allowing it to grow around an object. Studies seem to indicate that ethylene affects stem diameter and height: When stems of trees are subjected to wind, causing lateral stress, greater ethylene production

occurs, resulting in thicker, more sturdy tree trunks and branches. Ethylene affects fruit-ripening: Normally, when the seeds are mature, ethylene production increases and builds-up within the fruit, resulting in a climacteric event just before seed dispersal. The nuclear protein Ethylene Insensitive2 (EIN2) is regulated by ethylene production, and, in turn, regulates other hormones including ABA and stress hormones.

## Gibberellins



Gibberellin A1

Gibberellins, or GAs, include a large range of chemicals that are produced naturally within plants and by fungi. They were first discovered when Japanese researchers, including Eiichi Kurosawa, noticed a chemical produced by a fungus called *Gibberella fujikuroi* that produced abnormal growth in rice plants. Gibberellins are important in seed germination, affecting enzyme production that mobilizes food production used for growth of new cells. This is done by modulating chromosomal transcription. In grain (rice, wheat, corn, etc.) seeds, a layer of cells called the aleurone layer wraps around the endosperm tissue. Absorption of water by the seed causes production of GA. The GA is transported to the aleurone layer, which responds by producing enzymes that break down stored food reserves within the endosperm, which are utilized by the growing seedling. GAs produce bolting of rosette-forming plants, increasing internodal length. They promote flowering, cellular division, and in seeds growth after germination. Gibberellins also reverse the inhibition of shoot growth and dormancy induced by ABA.

## Other known hormones

Other identified plant growth regulators include:

- Brassinosteroids, are a class of polyhydroxysteroids, a group of plant growth regulators. Brassinosteroids have been recognized as a sixth class of plant

hormones which stimulate cell elongation and division, gravitropism, resistance to stress and xylem differentiation. They inhibit root growth and leaf abscission.

Brassinolide was the first identified brassinosteroid and was isolated from organic extracts of rapeseed (*Brassica napus*) pollen in 1970.

- Salicylic acid - activates genes in some plants that produce chemicals that aid in the defense against pathogenic invaders.
- Jasmonates - are produced from fatty acids and seem to promote the production of defense proteins that are used to fend off invading organisms. They are believed to also have a role in seed germination, and affect the storage of protein in seeds, and seem to affect root growth.
- Plant peptide hormones - encompasses all small secreted peptides that are involved in cell-to-cell signaling. These small peptide hormones play crucial roles in plant growth and development, including defense mechanisms, the control of cell division and expansion, and pollen self-incompatibility.
- Polyamines - are strongly basic molecules with low molecular weight that have been found in all organisms studied thus far. They are essential for plant growth and development and affect the process of mitosis and meiosis.
- Nitric oxide (NO) - serves as signal in hormonal and defense responses.
- Strigolactones, implicated in the inhibition of shoot branching.
- Karrikins, a group of plant growth regulators found in the smoke of burning plant material that have the ability to stimulate the germination of seeds

### ***Potential medical applications***

Plant stress hormones activate cellular responses, including cell death, to diverse stress situations in plants. Researchers have found that some plant stress hormones share the ability to adversely affect human cancer cells. For example, sodium salicylate has been found to suppress proliferation of lymphoblastic leukemia, prostate, breast, and melanoma human cancer cells. Jasmonic acid, a plant stress hormone that belongs to the jasmonate family, induced death in lymphoblastic leukemia cells. Methyl jasmonate has been found to induce cell death in a number of cancer cell lines.

### ***Hormones and plant propagation***

Synthetic plant hormones or PGRs are commonly used in a number of different techniques involving plant propagation from cuttings, grafting, micropropagation, and tissue culture.

The propagation of plants by cuttings of fully developed leaves, stems, or roots is performed by gardeners utilizing auxin as a rooting compound applied to the cut surface; the auxins are taken into the plant and promote root initiation. In grafting, auxin promotes callus tissue formation, which joins the surfaces of the graft together. In micropropagation, different PGRs are used to promote multiplication and then rooting of new plantlets. In the tissue-culturing of plant cells, PGRs are used to produce callus growth, multiplication, and rooting.

## Seed dormancy

Plant hormones affect seed germinations and dormancy by affecting different parts of the seed.

Embryo dormancy is characterized by a high ABA/GA ratio, whereas the seed has a high ABA sensitivity and low GA sensitivity. To release the seed from this type of dormancy and initiate seed germination, an alteration in hormone biosynthesis and degradation towards a low ABA/GA ratio, along with a decrease in ABA sensitivity and an increase in GA sensitivity needs to occur.

ABA controls embryo dormancy, and GA embryo germination. Seed coat dormancy involves the mechanical restriction of the seed coat, this along with a low embryo growth potential, effectively produces seed dormancy. GA releases this dormancy by increasing the embryo growth potential, and/or weakening the seed coat so the radical of the seedling can break through the seed coat. Different types of seed coats can be made up of living or dead cells and both types can be influenced by hormones; those composed of living cells are acted upon after seed formation while the seed coats composed of dead cells can be influenced by hormones during the formation of the seed coat. ABA affects testa or seed coat growth characteristics, including thickness, and effects the GA-mediated embryo growth potential. These conditions and effects occur during the formation of the seed, often in response to environmental conditions. Hormones also mediate endosperm dormancy: Endosperm in most seeds is composed of living tissue that can actively respond to hormones generated by the embryo. The endosperm often acts as a barrier to seed germination, playing a part in seed coat dormancy or in the germination process. Living cells respond to and also affect the ABA/GA ratio, and mediate cellular sensitivity; GA thus increases the embryo growth potential and can promote endosperm weakening. GA also affects both ABA-independent and ABA-inhibiting processes within the endosperm.

## Chapter- 4

# Pathogen

A **pathogen**, (from Greek: πάθος pathos "suffering, passion", and γίγνομαι (γεν-) gignomai (gen-) "I give birth to") an **infectious agent**, or more commonly **germ**, is a biological agent such as a virus, bacteria, prion, or fungus that causes disease to its host. There are several substrates including *pathways* whereby pathogens can invade a host; the principal pathways have different episodic time frames, but soil contamination has the longest or most persistent potential for harboring a pathogen.

The body contains many natural orders of defense against some of the common pathogens (such as *Pneumocystis*) in the form of the human immune system and by some "helpful" bacteria present in the human body's normal flora. However, if the immune system or "good" bacteria is damaged in any way (such as by chemotherapy, human immunodeficiency virus (HIV), or antibiotics being taken to kill other pathogens), pathogenic bacteria that were being held at bay can proliferate and cause harm to the host. Such cases are called opportunistic infection.

Some pathogens (such as the bacterium *Yersinia pestis* which may have caused the Black Plague, the *Variola* virus, and the Malaria protozoa) have been responsible for massive numbers of casualties and have had numerous effects on afflicted groups. Of particular note in modern times is HIV, which is known to have infected several million humans globally, along with the Influenza virus. Today, while many medical advances have been made to safeguard against infection by pathogens, through the use of vaccination, antibiotics, and fungicide, pathogens continue to threaten human life. Social advances such as food safety, hygiene, and water treatment have reduced the threat from some pathogens. Not all pathogens are negative. In entomology, pathogens are one of the "Three P's" (predators, pathogens, and parasitoids) that serve as natural or introduced biological controls to suppress arthropod pest populations.

## **Types of pathogen**

### **Viral**

Pathogenic viruses are mainly those of the families of: Adenoviridae, Picornaviridae, Herpesviridae, Hepadnaviridae, Flaviviridae, Retroviridae, Orthomyxoviridae, Paramyxoviridae, Papovaviridae, Polyomavirus, Rhabdoviridae, Togaviridae. Some notable pathogenic viruses cause: smallpox, influenza, mumps, measles, chickenpox, ebola, and rubella. Viruses typically range between 20-300 nanometers in length.

### **Bacterial**

Although the vast majority of bacteria are harmless or beneficial, a few pathogenic bacteria can cause infectious diseases. The most common bacterial disease is tuberculosis, caused by the bacterium *Mycobacterium tuberculosis*, which affects about 2 million people mostly in sub-Saharan Africa. Pathogenic bacteria contribute to other globally important diseases, such as pneumonia, which can be caused by bacteria such as *Streptococcus* and *Pseudomonas*, and foodborne illnesses, which can be caused by bacteria such as *Shigella*, *Campylobacter* and *Salmonella*. Pathogenic bacteria also cause infections such as tetanus, typhoid fever, diphtheria, syphilis and Hansen's disease. Bacteria can often be killed by antibiotics because the cell wall in the outside is destroyed and then the D.N.A. They typically range between 1-5 micrometers in length.

### **Fungal**

Fungi comprise a eukaryotic kingdom of microbes that are usually saprophytes but can cause diseases in humans, animals and plants. Fungi are the most common cause of diseases in crops and other plants. Life threatening fungal infections in humans most often occur in immunocompromised patients or vulnerable people with a weakened immune system, although fungi are common problems in the immunocompetent population as the causative agents of skin, nail or yeast infections. Most antibiotics that function on bacterial pathogens cannot be used to treat fungal infections because fungi and their hosts both have eukaryotic cells. Most clinical fungicides belong to the azole group. The typical fungal spore size is 1-40 micrometer in length.

### **Other parasites**

Some eukaryotic organisms, such as protists and helminths, cause disease. One of the best known diseases caused by protists in the genus *Plasmodium* is malaria. These can range from 3-200 micrometers in length.

### **Prionic**

Prions are infectious pathogens that do not contain nucleic acids. Prions are abnormal proteins whose presence causes some diseases such as scrapie, bovine spongiform encephalopathy (mad cow disease) and Creutzfeldt–Jakob disease. The discovery of

prion as a new class of pathogen has led Stanley B. Prusiner to receive Nobel Prize in Physiology or Medicine in 1997.

## **Potency**

One hypothesis regarding pathogens states that the longer a pathogen can survive outside of the body, the more dangerous it can be to a potential host. For example, the smallpox virus (*variola virus*) can survive outside the human body for approximately 885 days. It is also one of the most deadly pathogenic viruses, as it kills between 20-50% of the people it infects. The tuberculosis bacterium kills 1 in 5 of the people it infects, but only survives 244 days outside of its host. However, research into the basis of the ability of pathogens to cause disease provides evidence from multiple and diverse species of the existence of pathogenicity or virulence factors, encoded within the pathogens' genetic material, that facilitate microbes to cause disease.

In countries that have higher sanitation standards, pathogens cannot survive for as long outside of the human. This is seen as encouragement to mutations to the pathogen which would make it less deadly, as such mutations would allow the pathogen to survive in the host for longer periods of time.

## **Transmission**

One of the primary pathways by which food or water become contaminated is from the release of untreated sewage into a drinking water supply or onto cropland, with the result that people who eat or drink contaminated sources become infected. In developing countries most sewage is discharged into the environment or on cropland; even in developed countries there are periodic system failures resulting in a sanitary sewer overflow.

## **Examples of pathogens**

### **Major human pathogens**

- *Mycobacterium tuberculosis* — the causative agent of most cases of tuberculosis
- *Mycobacterium leprae* — the bacterium that causes leprosy (Hansen's disease)
- *Yersinia pestis* — pneumonic, septicemic, and the notorious bubonic plagues (aka "Black Death")
- *Rickettsia prowazekii* — the etiologic agent of typhus fever
- *Bartonella* spp.
- Spanish influenza virus

## Chapter- 5

# Phytoplasma

*Phytoplasma*



Phyllody induced by phytoplasma infection on a coneflower

### Scientific classification

Division: Firmicutes  
Class: Mollicutes  
Order: Acholeplasmatales  
Family: Acholeplasmataceae  
Genus: ***Candidatus Phytoplasma***

### Species

"*Ca. Phytoplasma allocasuarinae*"  
"*Ca. Phytoplasma asteris*"  
"*Ca. Phytoplasma aurantifolia*"  
"*Ca. Phytoplasma australiense*"  
"*Ca. Phytoplasma brasiliense*"  
"*Ca. Phytoplasma castaneae*"  
"*Ca. Phytoplasma cocostanzaniae*"  
"*Ca. Phytoplasma cocosnigeriae*"  
"*Ca. Phytoplasma cynodontis*"  
"*Ca. Phytoplasma fraxini*"  
"*Ca. Phytoplasma japonicum*"  
"*Ca. Phytoplasma luffae*"  
"*Ca. Phytoplasma mali*"  
"*Ca. Phytoplasma oryzae*"  
"*Ca. Phytoplasma palmae*"  
"*Ca. Phytoplasma phoenicium*"  
"*Ca. Phytoplasma pruni*"  
"*Ca. Phytoplasma prunorum*"  
"*Ca. Phytoplasma pyri*"  
"*Ca. Phytoplasma rhamni*"  
"*Ca. Phytoplasma solani*"  
"*Ca. Phytoplasma spartii*"  
"*Ca. Phytoplasma trifolii*"  
"*Ca. Phytoplasma ulmi*"  
"*Ca. Phytoplasma vitis*"  
"*Ca. Phytoplasma ziziphi*"

**Phytoplasma** are specialised bacteria that are obligate parasites of plant phloem tissue and transmitting insects (vectors). They were first discovered by scientists in 1967 and were named mycoplasma-like organisms or **MLOs**. They cannot be cultured in vitro in cell-free media. They are characterised by their lack of a cell wall, a pleiomorphic or filamentous shape, normally with a diameter less than 1 micrometer, and their very small genomes.

Phytoplasmas are pathogens of important crops, including coconuts and sugarcane, causing a wide variety of symptoms that range from mild yellowing to death of infected plants. They are most prevalent in tropical and sub-tropical regions of the world. Phytoplasmas require a vector to be transmitted from plant to plant, and this normally

takes the form of sap sucking insects such as leaf hoppers in which they are also able to replicate.

## **History**

There are references to diseases now known to be caused by phytoplasmas as far back as 1603 for Mulberry dwarf disease in Japan. Such diseases were originally thought to be caused by viruses, which, like phytoplasmas, require insect vectors, cannot be cultured, and have some symptom similarity. In 1967 phytoplasmas were discovered in ultrathin sections of plant phloem tissue and named mycoplasma-like organisms (MLOs), because they physically resembled mycoplasmas. The organisms were renamed phytoplasmas in 1994 at the 10th congress of the International Organization of Mycoplasmaology.

## **Morphology**

Being mollicutes, phytoplasmas lack cell walls and instead are bound by a triple layered membrane. The cell membranes of all phytoplasmas studied so far usually contain a single immunodominant protein (of unknown function) that makes up the majority of the protein content of the cell membrane. The typical phytoplasma exhibits a pleiomorphic or filamentous shape and is less than 1 micrometer in diameter. Like other prokaryotes, DNA is free in the cytoplasm.

## **Symptoms**

A common symptom caused by phytoplasma infection is phyllody, the production of leaf-like structures in place of flowers. Evidence suggests that the phytoplasma downregulates a gene involved in petal formation (*AP3* and its orthologues) and genes involved in the maintenance of the apical meristem (*Wus* and *CLV1*). Other symptoms, such as the yellowing of leaves, are thought to be caused by the phytoplasma's presence in the phloem, affecting its function and changing the transport of carbohydrates.

Phytoplasma infected plants may also suffer from virescence, the development of green flowers due to the loss of pigment in the petal cells. Sometimes sterility of the flowers is also seen.

Many phytoplasma infected plants gain a bushy or witch's broom appearance due to changes in normal growth patterns caused by the infection. Most plants show apical dominance, but phytoplasma infection can cause the proliferation of auxiliary (side) shoots and an increase in size of the internodes. Such symptoms are actually useful in the commercial production of poinsettia. The infection produces more axillary shoots, which enables production of poinsettia plants that have more than one flower.

Phytoplasmas may cause many other symptoms that are induced because of the stress placed on the plant by infection rather than specific pathogenicity of the phytoplasma. Photosynthesis, especially photosystem II, is inhibited in many phytoplasma infected

plants. Phytoplasma infected plants often show yellowing which is caused by the breakdown of chlorophyll, whose biosynthesis is also inhibited.

## ***Transmission***

### **Movement between plants**

The phytoplasmas are mainly spread by insects of the families Cicadellidea (leafhoppers), Fulgoridea (planthoppers) and Psyllidae (jumping plant lice), which feed on the phloem tissues of infected plants, picking up the phytoplasmas and transmitting them to the next plant they feed on. For this reason the host range of phytoplasmas is strongly dependent upon its insect vector. Phytoplasmas contain a major antigenic protein that makes up the majority of their cell surface proteins. This protein has been shown to interact with insect microfilament complexes and is believed to be the determining factor in insect-phytoplasma interaction. Phytoplasmas may overwinter in insect vectors or perennial plants. Phytoplasmas can have varying effects on their insect hosts; examples of both reduced and increased fitness have been seen.

Phytoplasmas enter the insect's body through the stylet, move through the intestine, and are then absorbed into the haemolymph. From here they proceed to colonise the salivary glands, a process that can take up to three weeks. Once established, phytoplasmas will be found in most major organs of an infected insect host. The time between being taken up by the insect and reaching an infectious titre in the salivary glands is called the latency period.

Phytoplasmas can also be spread via dodders *Cuscutaceae* or vegetative propagation such as the grafting of a piece of infected plant onto a healthy plant.

### **Movement within plants**

Phytoplasmas are able to move within the phloem from source to sink, and they are able to pass through sieve tube elements. But since they spread more slowly than solutes, for this and other reasons, movement by passive translocation is not supported.

## ***Detection and Diagnosis***

Before molecular techniques were developed, the diagnosis of phytoplasma diseases was difficult because they could not be cultured. Thus classical diagnostic techniques, such as observation of symptoms, were used. Ultrathin sections of the phloem tissue from suspected phytoplasma infected plants would also be examined for their presence. Treating infected plants with antibiotics such as tetracycline to see if this cured the plant was another diagnostic technique employed.

Molecular diagnostic techniques for the detection of phytoplasma began to emerge in the 1980s and included ELISA based methods. In the early 1990s, PCR-based methods were

developed that were far more sensitive than those that used ELISA, and RFLP analysis allowed the accurate identification of different strains and species of phytoplasma.

More recently, techniques have been developed that allow for assessment of the level of infection. Both QPCR and bioimaging have been shown to be effective methods of quantifying the titre of phytoplasmas within the plant.

## **Control**

Phytoplasmas are normally controlled by the breeding and planting of disease resistance varieties of crops (believed to be the most economically viable option) and by the control of the insect vector.

Tissue culture can be used to produce clones of phytoplasma infected plants that are healthy. The chances of gaining healthy plants in this manner can be enhanced by the use of cryotherapy, freezing the plant samples in liquid nitrogen, before using them for tissue culture.

Work has also been carried out investigating the effectiveness of plantibodies targeted against phytoplasmas.

Tetracyclines are bacteriostatic to phytoplasmas, that is they inhibit their growth. However, without continuous use of the antibiotic, disease symptoms will reappear. Thus, tetracycline is not a viable control agent in agriculture, but it is used to protect ornamental coconut trees.

## **Genetics**

The genomes of three phytoplasmas have been sequenced: Aster Yellows Witches Broom, Onion Yellows (*Ca. Phytoplasma asteris*) and *Ca. Phytoplasma australiense*. Phytoplasmas have very small genomes, which also have extremely low levels of the nucleotides G and C, sometimes as little as 23% which is thought to be the threshold for a viable genome. In fact Bermuda grass white leaf phytoplasma has a genome size of just 530Kb, one of the smallest known genomes of living organisms. Larger phytoplasma genomes are around 1350 Kb. The small genome size associated with phytoplasmas is due to their being the product of reductive evolution from Bacillus/Clostridium ancestors. They have lost 75% or more of their original genes, and this is why they can no longer survive outside of insects or plant phloem. Some phytoplasmas contain extrachromosomal DNA such as plasmids.

Despite their very small genomes, many predicted genes are present in multiple copies. Phytoplasmas lack many genes for standard metabolic functions and have no functioning homologous recombination pathways, but do have a *sec* transport pathway. Many phytoplasmas contain 2 rRNA operons. Unlike the rest of the Mollicutes, the triplet code of UGA is used as a stop codon in phytoplasmas, rather than to code for tryptophan.

Phytoplasma genomes contain large numbers of transposon genes and insertion sequences. They also contain a unique family of repetitive extragenic palindromes (REPs) called PhREPS whose role is unknown though it is theorised that the stem loop structures the PhREPS are capable of forming may play a role in transcription termination or genome stability.

## **Taxonomy**

Phytoplasmas are mollicutes and within this group belong to the monophyletic order *Acholeplasmatales*. In 1992 the *Subcommittee on the Taxonomy of Mollicutes* proposed the use of the name *Phytoplasma* in place of the use of the term MLO (Mycoplasma-like organism) "for reference to the phytopathogenic mollicutes". In 2004 the genus name *Phytoplasma* was adopted and is currently at Candidatus status which is used for bacteria that can not be cultured. It's taxonomy is complicated by the fact that it can not be cultured and thus methods normally used for classification of prokaryotes are not possible. Phytoplasma taxonomic groups are based on differences in the fragment sizes produced by the restriction digest of the 16S rRNA gene sequence (Called RFLP) or by comparison of DNA sequences from the 16s/23s spacer regions. There is some disagreement over how many taxonomic groups the phytoplasmas fall into, recent work involving computer simulated restriction digests of the 16Sr gene suggest there maybe up to 28 groups where as other papers argue for less groups, but more sub-groups. Each group includes at least one *Ca. Phytoplasma* species, characterised by distinctive biological, phytopathological and genetic properties. The table below summaries some of the major taxonomic groups and the *candidatus* species that belong in them.

<b>16Sr Group</b>	<b>Group Name</b>	<b>Species</b>
16SrI	Aster yellows	<i>Ca. Phytoplasma asteris</i> <i>Ca. Phytoplasma japonicum</i>
16SrII	Peanut witch's broom	<i>Ca. Phytoplasma aurantifolia</i>
16SrIII	X-disease	<i>Ca. Phytoplasma pruni</i>
16SrIV	Coconut lethal yellowing	<i>Ca. Phytoplasma palmae</i> <i>Ca. Phytoplasma castaneae</i> <i>Ca. Phytoplasma cocosnigeriae</i>
16SrV	Elm yellows	<i>Ca. Phytoplasma ziziphi</i> <i>Ca. Phytoplasma vitis</i> <i>Ca. Phytoplasma ulmi</i>
16SrVI	Clover proliferation	<i>Ca. Phytoplasma trifolii</i>
16SrVII	Ash yellows	<i>Ca. Phytoplasma fraxini</i>
16SrVIII	Luffa witch's-broom	<i>Ca. Phytoplasma luffae</i>
16SrIX	Pidgeon pea witch's broom	<i>Ca. Phytoplasma phoenicium</i>

16SrX	Apple proliferation	<i>Ca. Phytoplasma mali</i> <i>Ca. Phytoplasma pyri</i> <i>Ca. Phytoplasma prunorum</i> <i>Ca. Phytoplasma spartii</i> <i>Ca. Phytoplasma rhamnii</i> <i>Ca. Phytoplasma allocasuarinae</i>
16SrXI	Rice Yellow Dwarf Sugarcane Grassy Shoot Disease (SCGS)	<i>Ca. Phytoplasma oryzae</i>
16SrXII	Stolbur	<i>Ca. Phytoplasma solani</i> <i>Ca. Phytoplasma australiense</i>
16SrXIII	Mexican periwinkle virescence	Undefined
16SrXIV	Bermuda white leaf	<i>Ca. Phytoplasma cynodontis</i>
16SrXV	Hibiscus witch's-broom	<i>Ca. Phytoplasma brasiliense</i>



A grape vine with phytoplasma disease



Coconut palms dying of lethal yellowing disease



Symptoms of Aster yellows on marigold



Symptoms of elm phloem necrosis phytoplasma



Trees dying of Ash yellows phytoplasma



Symptoms of sweet potato little leaf phytoplasma on *Catharanthus roseus*



Phyllody caused by phytoplasma infection on *Cosmos spp.*



Phyllody of goldenrod



Sugarcane Grassy Shoot (SCGS) disease caused by Phytoplasma



A palm tree dying of lethal yellowing phytoplasma

## Chapter- 6

# Plant Virus

**Plant viruses** are viruses affecting plants. Like all other viruses, plant viruses are obligate intracellular parasites that do not have the molecular machinery to replicate without a host. Plant viruses are pathogenic to higher plants.

### **Overview**

Although plant viruses are not nearly as well understood as the animal counterparts, one plant virus has become iconic. The first virus to be discovered (see below) was *Tobacco mosaic virus* (TMV). This and other viruses cause an estimated US\$60 billion loss in crop yields worldwide each year. Plant viruses are grouped into 73 genera and 49 families.

In order to transmit themselves from one plant to another and from one plant cell to another, plant viruses must use strategies which are usually different than those of animal viruses. Plants do not move, and so plant-to-plant transmission usually involves vectors (such as insects). Plant cells are surrounded by solid cell walls, therefore transport through plasmodesmata is the preferred path for virions to move between plant cells. Plants probably have specialized mechanisms for transporting mRNAs through plasmodesmata, and these mechanisms are thought to be utilized by RNA viruses in order to spread from one cell to another.

Plant defenses against viral infection include, among other measures, the use of siRNA in response to dsRNA. Most plant viruses encode a protein to suppress this response. Plants also reduce transport through plasmodesmata in response to injury.

### **History**

The discovery of plant viruses causing disease is often accredited to Martinus Beijerinck who determined, in 1898, that plant sap obtained from tobacco leaves with the "mosaic disease" remained infectious when passed through a porcelain filter. This was in contrast to bacteria microorganisms, which were retained by the filter. Beijerinck referred to the

infectious filtrate as a "contagium vivum fluidum", thus the coinage of the modern term "virus".

After the initial discovery of the 'viral concept' there was need to classify any other known viral diseases based on the mode of transmission even though microscopic observation proved fruitless. In 1939 Holmes published a classification list of 129 plant viruses. This was expanded and in 1999 there were 977 officially recognized, and some provisional, plant virus species.

The purification (crystallization) of TMV was first performed by Wendell Stanley, who published his findings in 1935, although he did not determine that the RNA was the infectious material. However, he received the Nobel Prize in Chemistry in 1946. In the 1950s a discovery by two labs simultaneously proved that the purified RNA of the TMV was infectious which reinforced the argument. The RNA carries genetic information to code for the production of new infectious particles.

More recently virus research has been focused on understanding the genetics and molecular biology of plant virus genomes, with a particular interest in determining how the virus can replicate, move and infect plants. Understanding the virus genetics and protein functions has been used to explore the potential for commercial use by biotechnology companies. In particular, viral-derived sequences have been used to provide an understanding of novel forms of resistance. The recent boom in technology allowing humans to manipulate plant viruses may provide new strategies for production of value-added proteins in plants.

## **Structure**

Viruses are extremely small and can only be observed with an electron microscope. The structure of a virus is given by its coat of proteins, which surround the viral genome. Assembly of viral particles takes place spontaneously.

Over 50% of known plant viruses are rod-shaped (flexuous or rigid). The length of the particle is normally dependent on the genome but it is usually between 300–500 nm with a diameter of 15–20 nm. Protein subunits can be placed around the circumference of a circle to form a disc. In the presence of the viral genome, the discs are stacked, then a tube is created with room for the nucleic acid genome in the middle.

The second most common structure amongst plant viruses are isometric particles. They are 40–50 nm in diameter. In cases when there is only a single coat protein, the basic structure consists of 60 T subunits, where T is an integer. Some viruses may have 2 coat proteins are the associate to form a icosahedral shaped particle.

There are three genera of *Geminiviridae* that possess geminate particles which are like two isometric particles stuck together.

A very small number of plant viruses have, in addition to their coat proteins, a lipid envelope. This is derived from the plant cell membrane as the virus particle buds off from the cell.

## ***Transmission of plant viruses***

### **Through sap**

Viruses can be spread by direct transfer of sap by contact of a wounded plant with a healthy one. Such contact may occur during agricultural practices, as by damage caused by tools or hands, or naturally, as by an animal feeding on the plant. Generally TMV, potato viruses and cucumber mosaic viruses are transmitted via sap.

### **Insects**

Plant viruses need to be transmitted by a vector, most often insects such as leafhoppers. One class of viruses, the Rhabdoviridae, has been proposed to actually be insect viruses that have evolved to replicate in plants. The chosen insect vector of a plant virus will often be the determining factor in that virus's host range: it can only infect plants that the insect vector feeds upon. This was shown in part when the old world white fly made it to the USA, where it transferred many plant viruses into new hosts. Depending on the way they are transmitted, plant viruses are classified as non-persistent, semi-persistent and persistent. In non-persistent transmission, viruses become attached to the distal tip of the stylet of the insect and on the next plant it feeds on, it inoculates it with the virus. Semi-persistent viral transmission involves the virus entering the foregut of the insect. Those viruses that manage to pass through the gut into the haemolymph and then to the salivary glands are known as persistent. There are two sub-classes of persistent viruses: propagative and circulative. Propagative viruses are able to replicate in both the plant and the insect (and may have originally been insect viruses), whereas circulative can not. Circulative viruses are protected inside aphids by the chaperone protein symbionin produced by bacterial symbionts. Many plant viruses encode within their genome polypeptides with domains essential for transmission by insects. In non-persistent and semi-persistent viruses, these domains are in the coat protein and another protein known as the helper component. A bridging hypothesis has been proposed to explain how these proteins aid in insect-mediated viral transmission. The helper component will bind to the specific domain of the coat protein, and then the insect mouthparts — creating a bridge. In persistent propagative viruses, such as tomato spotted wilt virus (TSWV), there is often a lipid coat surrounding the proteins that is not seen in the other classes of plant viruses. In the case of TSWV, 2 viral proteins are expressed in this lipid envelope. It has been proposed that the viruses bind via these proteins and are then taken into the insect cell by receptor-mediated endocytosis.

### **Nematodes**

Soil-borne nematodes also have been shown to transmit viruses. They acquire and transmit them by feeding on infected roots. Viruses can be transmitted both non-

persistently and persistently, but there is no evidence of viruses being able to replicate in nematodes. The virions attach to the stylet (feeding organ) or to the gut when they feed on an infected plant and can then unattach during later feeding to infect other plants. Examples of viruses that can be transmitted by nematodes include tobacco ringspot virus and tobacco rattle virus.

## **Plasmodiophorids**

A number of virus genera are transmitted, both persistently and non-persistently, by soil borne zoosporic protozoa. These protozoa are not phytopathogenic themselves, but parasitic. Transmission of the virus takes place when they become associated with the plant roots. Examples include *Polymyxa graminis*, which has been shown to transmit plant viral diseases in cereal crops and *Polymyxa betae* which transmits Beet necrotic yellow vein virus. Plasmodiophorids also create wounds in the plant's root through which other viruses can enter.

## **Seed and pollen borne viruses**

Plant virus transmission from generation to generation occurs in about 20% of plant viruses. When viruses are transmitted by seeds, the seed is infected in the generative cells and the virus is maintained in the germ cells and sometimes, but less often, in the seed coat. When the growth and development of plants is delayed because of situations like unfavourable weather, there is an increase in the amount of virus infections in seeds. There does not seem to be a correlation between the location of the seed on the plant and its chances of being infected. Little is known about the mechanisms involved in the transmission of plant viruses via seeds, although it is known that it is environmentally influenced and that seed transmission occurs because of a direct invasion of the embryo via the ovule or by an indirect route with an attack on the embryo mediated by infected gametes. These processes can occur concurrently or separately depending on the host plant. It is unknown how the virus is able to directly invade and cross the embryo and boundary between the parental and progeny generations in the ovule. Many plants species can be infected through seeds including but not limited to the families Leguminosae, Solanaceae, Compositae, Rosaceae, Curcubitaceae, Gramineae.

## **Direct plant-to-human transmission**

Researchers from the University of the Mediterranean in Marseille, France have found evidence that suggest a virus common to peppers (the Pepper Mild Mottle Virus) may have moved on to infect humans. This is a very rare and highly unlikely event, as in order to enter a cell and replicate a virus must "bind to a receptor on its surface, and a plant virus would be highly unlikely to recognize a receptor on a human cell. One possibility is that the virus does not infect human cells directly. Instead, the naked viral RNA may alter the function of the cells through a mechanism similar to RNA interference, in which the presence of certain RNA sequences can turn genes on and off", according to Virologist Robert Garry from the Tulane University in New Orleans, Louisiana.

Symptoms include being more likely to have fever, abdominal pain, and itching.

### ***Translation of plant viral proteins***

75% of plant viruses have genomes that consist of single stranded RNA (ssRNA). 65% of plant viruses have +ssRNA, meaning that they are in the same sense orientation as messenger RNA but 10% have -ssRNA, meaning they must be converted to +ssRNA before they can be translated. 5% are double stranded RNA and so can be immediately translated as +ssRNA viruses. 3% require a reverse transcriptase enzyme to convert between RNA and DNA. 17% of plant viruses are ssDNA and very few are dsDNA, in contrast a quarter of animal viruses are dsDNA and three quarters of bacteriophage are dsDNA. Viruses use the plant ribosomes to produce the 4-10 proteins encoded by their genome. However, since all of the proteins are encoded on a single strand (that is, they are polycistronic) this will mean that the ribosome will either only produce one protein, as it will terminate translation at the first stop codon, or that a polyprotein will be produced. Plant viruses have had to evolve special techniques to allow the production of viral proteins by plant cells.

### **5' Cap**

In order for translation to occur, eukaryotic mRNAs require a 5' Cap structure. This means that viruses must also have one. This normally consists of 7MeGpppN where N is normally adenine or guanine. The viruses encode a protein, normally a replicase, with a methyltransferase activity to allow this.

Some viruses are cap-snatchers. During this process, a <sup>7m</sup>G-capped host mRNA is recruited by the viral transcriptase complex and subsequently cleaved by a virally encoded endonuclease. The resulting capped leader RNA is used to prime transcription on the viral genome.

However some plant viruses do not use cap, yet translate efficiently due to cap-independent translation enhancers present in 5' and 3' untranslated regions of viral mRNA.

### **Readthrough**

Some viruses (e.g. tobacco mosaic virus (TMV) have RNA sequences that contain a "leaky" stop codon. In TMV 95% of the time the host ribosome will terminate the synthesis of the polypeptide at this codon but the rest of the time it continues past it. This means that 5% of the proteins produced are larger than and different from the others normally produced, which can be thought of as a crude form of transcriptional regulation. In TMV this extra sequence of polypeptide is an RNA polymerase which replicates its genome.

## **Production of sub-genomic RNAs**

Some viruses use the production of subgenomic RNAs to ensure the translation of all proteins within their genomes. In this process the first protein encoded on the genome, and this the first to be translated, is a replicase. This protein will act on the rest of the genome producing negative strand sub-genomic RNAs then act upon these to form positive strand sub-genomic RNAs that are essentially mRNAs ready for translation.

## **Segmented genomes**

Some viral families, such as the *Bromoviridae* instead opt to have multi-partite genomes, genomes split between multiple viral particles. For infection to occur, the plant must be infected with all particles across the genome. For instance *Brome mosaic virus* has a genome split between 3 viral particles, and all 3 particles with the different RNAs are required for infection to take place.

## **Polyprotein processing**

This strategy is adopted by viral genera such as the Potyviridae and Tymovirus. The ribosome translates a single protein from the viral genome. Within the polyprotein is an enzyme (or enzymes) with proteinase function that is able to cleave the polyprotein into the various single proteins or just cleave away the protease, which can then cleave other polypeptides producing the mature proteins.

## ***Well understood plant viruses***

Tobacco mosaic virus (TMV) and Cauliflower mosaic virus (CaMV) are frequently used in plant molecular biology. Of special interest is the CaMV 35S promoter, which is a very strong promoter most frequently used in plant transformations.

## Chapter- 7

# Nematode

### Nematodes



Unidentified roundworm from wet soil.  
The mouth is at the top left corner.

### Scientific classification

Kingdom:	Animalia
Subkingdom:	Eumetazoa
(unranked):	Bilateria
Phylum:	<b>Nematoda</b> Diesing, 1861

### Classes

Chromadorea (disputed)  
Enoplea (disputed)  
Secernentea

### Synonyms

Adenophorea  
Aphasmidia  
Nematoidea Rudolphi, 1808  
Nematodes Burmeister, 1837  
Nemates Cobb, 1919  
Nemata Cobb, 1919

The **nematodes** or **roundworms** (phylum **Nematoda**) are the most diverse phylum of pseudocoelomates, and one of the most diverse of all animals. Nematode species are very difficult to distinguish; over 28,000 have been described, of which over 16,000 are parasitic. It has been estimated that the total number of nematode species might be approximately 1,000,000. Unlike cnidarians or flatworms, roundworms have a digestive system that is like a tube with openings at both ends.

### ***Habitats***

Nematodes have successfully adapted to nearly every ecosystem from marine to fresh water, from the polar regions to the tropics, as well as the highest to the lowest of elevations. They are ubiquitous in freshwater, marine, and terrestrial environments, where they often outnumber other animals in both individual and species counts, and are found in locations as diverse as mountains, deserts and oceanic trenches. They represent, for example, 90% of all life on the seafloor of the Earth. Their many parasitic forms include pathogens in most plants and animals (including humans). Some nematodes can undergo cryptobiosis.

One group of carnivorous fungi, the nematophagous fungi, are predators of soil nematodes. They set enticements for the nematodes in the form of lassos or adhesive structures.

### ***Taxonomy and systematics***



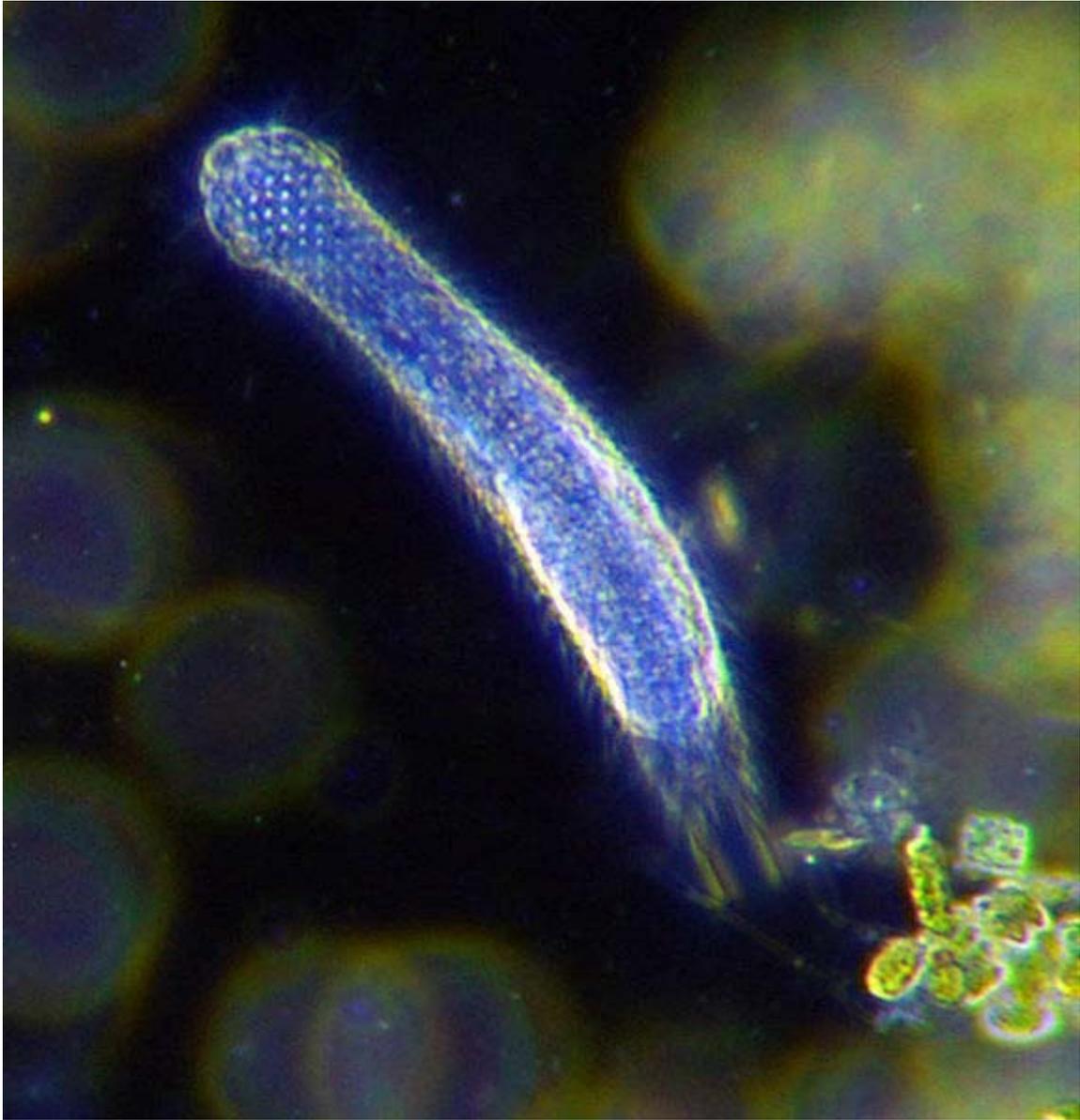
*Eophasma jurasicum*, a fossilized nematode

The group was originally defined by Karl Rudolphi in 1808 under the name **Nematoidea**, from Ancient Greek νῆμα (*nêma*, *nêmatos*, 'thread') and -ειδής (*-eidēs*, 'like'). The

vernacular word "nematode" is a corruption of this taxon, reclassified as family **Nematodes** by Burmeister in 1837 and order **Nematoda** by K. M. Diesing in 1861.

At the origin, the "Nematoidea" included both roundworms and horsehair worms. Along with Acanthocephala, Trematoda and Cestoidea, it formed the group Entozoa. The first differentiation of roundworms from horsehair worms, though erroneous, is due to von Siebold (1843) with orders Nematoidea and Gordiacei (Gordiacea). They were classed along with Acanthocephala in the new phylum Nemathelminthes (today obsolete) by Gegenbaur (1859). Then the taxon Nematoidea has been promoted to the rank of phylum by Ray Lankester (1877) including the family Gordiidae (horsehair worms). In 1919, Nathan Cobb proposed that roundworms should be recognized alone as a phylum. He argued that they should be called **nema(s)** in English rather than "nematodes" and defined the taxon **Nemates** (Latin plural of *nema*). For ITIS, the taxon Nematoda is invalid. Since Cobb was the first to exclude all but nematodes from the group, the valid taxon should be *Nemates* Cobb 1919 or *Nemata* Cobb 1919.

## Phylogeny



The mysterious Gastrotricha seem to hold the key to the "Ecdysozoa debate", but they have been little studied.

Whether they are relatives of the nematodes is still unknown.

The relationships of the nematodes and their close relatives among the protostomian Metazoa are unresolved. Traditionally, they were held to be a lineage of their own, but in the 1990s it was proposed that they form a clade together with moulting animals such as arthropods. This group has been named Ecdysozoa. However, the monophyly of the Ecdysozoa was never unequivocally accepted: while most researchers consider at least the placement of arthropods as more distant relatives of annelids — with which they were formerly united — to be warranted, the presumed close relationships of the nematodes and relatives with the arthropods has been a major point of contention.

Even though the amount of data since accumulated in regard to this problem is staggering, the situation seems if anything less clear these days. DNA sequence data, initially strongly supporting the Ecdysozoa hypothesis, has become rather equivocal on ecdysozoan monophyly, and is simply unable to refute either a close or a more distant relationship between the arthropod and nematode lineages. That the roundworms have a large number of peculiar apomorphies and in many cases a parasitic lifestyle confounds morphological analyses. Genetic analyses of roundworms suggest that — as is also indicated by their unique morphological features — the group has been under intense selective pressure during its early radiation, resulting apparently in accelerated rates of both morphological and molecular evolution. Furthermore, no distinctive apomorphies of Ecdysozoa are known; even moulting has recently been confirmed to occur outside the presumed clade.

Conversely, the identity of the closest living relatives of the Nematoda has always been considered to be well resolved. Morphological characters and molecular phylogenies agree with placement of the roundworms as sister taxon to the parasitic horsehair worms (Nematomorpha); together they make up the Nematoida. Together with the Scalidophora (formerly Cephalorhyncha), the Nematoida form the Introverta. It is entirely unclear whether the Introverta are, in turn, the closest living relatives of the enigmatic Gastrotricha; if so, they are considered a clade Cycloneuralia, but there is much disagreement both between and among the available morphological and molecular data. The Cycloneuralia or the Introverta — depending on the validity of the former — are often ranked as a superphylum.

## **Nematode systematics**

Due to the lack of knowledge regarding many nematodes, their systematics is contentious. Traditionally, they are divided into two classes, the Adenophorea and the Secernentea, and initial DNA sequence studies suggested the existence of five clades:

- Dorylaimia
- Enoplia
- Spirurina
- Tylenchina
- Rhabditina

As it seems, the Secernentea are indeed a natural group of closest relatives. But the "Adenophorea" appear to be a paraphyletic assemblage of roundworms simply retaining a good number of ancestral traits. The old Enoplia do not seem to be monophyletic either but to contain two distinct lineages. The old group "Chromadoria" seem to be another paraphyletic assemblage, with the Monhysterida representing a very ancient minor group of nematodes. Among the Secernentea, the Diplogasteria may need to be united with the Rhabditia. While the Tylenchia might be paraphyletic with the Rhabditia.

The understanding of roundworm systematics and phylogeny as of 2002 is summarised below:

## Phylum Nematoda

- Basal order Monhysterida
- Class Dorylaimea
- Class Enoplea
- Class Secernentea
  - Subclass Diplogasteria (disputed)
  - Subclass Rhabditia (paraphyletic?)
  - Subclass Spiruria
  - Subclass Tylenchia (disputed)
- "Chromadorea" assemblage

## Anatomy

Nematodes are slender, worm-like animals, typically less than 2.5 millimetres (0.10 in) long. The smallest nematodes are microscopic, while free-living species can reach as much as 5 centimetres (2.0 in) and some parasitic species are larger still. The body is often ornamented with ridges, rings, warts, bristles or other distinctive structures.

The head of a nematode is relatively distinctive. Whereas the rest of the body is bilaterally symmetrical, the head is radially symmetrical, with sensory bristles and, in many cases, solid *head-shields* radiating outwards around the mouth. The mouth has either three or six lips, which often bear a series of teeth on their inner edge. An adhesive *caudal gland* is often found at the tip of the tail.

The epidermis is either a syncytium or a single layer of cells, and is covered by a thick collagenous cuticle. The cuticle is often of complex structure, and may have two or three distinct layers. Underneath the epidermis lies a layer of muscle cells. Projections run from the inner surface of these cells towards the nerve cords; this is a unique arrangement in the animal kingdom, in which nerve cells normally extend fibres into the muscles rather than *vice versa*.

The muscle layer surrounds the body cavity, which is filled with a fluid that lacks any form of blood cells. The gut runs down the centre of the cavity.

## Digestive system

The oral cavity is lined with cuticle, which is often strengthened with ridges or other structures, and, especially in carnivorous species, may bear a number of teeth. The mouth often includes a sharp stylet which the animal can thrust into its prey. In some species, the stylet is hollow, and can be used to suck liquids from plants or animals.

The oral cavity opens into a muscular sucking pharynx, also lined with cuticle. Digestive glands are found in this region of the gut, producing enzymes that start to break down the food. In stylet-bearing species, these may even be injected into the prey.

There is no stomach, with the pharynx connecting directly to the intestine that forms the main length of the gut. This produces further enzymes, and also absorbs nutrients through its lining. The last portion of the intestine is lined by cuticle, forming a rectum which expels waste through the anus just below and in front of the tip of the tail. The intestine also has valves or sphincters at either end to help control the movement of food through the body.

## **Excretory system**

Nitrogenous waste is excreted in the form of ammonia through the body wall, and is not associated with any specific organs. However, the structures for excreting salt to maintain osmoregulation are typically more complex.

In many marine nematodes, there are one or two unicellular *renette glands* that excrete salt through a pore on the underside of the animal, close to the pharynx. In most other nematodes, these specialised cells have been replaced by an organ consisting of two parallel ducts connected by a single transverse duct. This transverse duct opens into a common canal that runs to the excretory pore.

## **Nervous system**

Four nerves run the length of the body on the dorsal, ventral, and lateral surfaces. Each nerve lies within a cord of connective tissue lying beneath the cuticle and between the muscle cells. The ventral nerve is the largest, and has a double structure forward of the excretory pore. The dorsal nerve is responsible for motor control, while the lateral nerves are sensory, and the ventral combines both functions.

At the anterior end of the animal, the nerves branch from a dense circular nerve ring surrounding the pharynx, and serving as the brain. Smaller nerves run forward from the ring to supply the sensory organs of the head.

The body of nematodes is covered in numerous sensory bristles and papillae that together provide a sense of touch. Behind the sensory bristles on the head lie two small pits, or *amphids*. These are well supplied with nerve cells, and are probably chemoreception organs. A few aquatic nematodes possess what appear to be pigmented eye-spots, but it is unclear whether or not these are actually sensory in nature.

## **Reproduction**

Most nematode species are dioecious, with separate male and female individuals. Both sexes possess one or two tubular gonads. In males, the sperm are produced at the end of the gonad, and migrate along its length as they mature. The testes each open into a relatively wide sperm duct and then into a glandular and muscular ejaculatory duct associated with the cloaca. In females, the ovaries each open into an oviduct and then a glandular uterus. The uteri both open into a common vagina, usually located in the middle of the ventral surface.

Reproduction is usually sexual. Males are usually smaller than females (often much smaller) and often have a characteristically bent tail for holding the female for copulation. During copulation, one or more chitinized spicules move out of the cloaca and are inserted into genital pore of the female. Amoeboid sperm crawl along the spicule into the female worm. Nematode sperm is thought to be the only eukaryotic cell without the globular protein G-actin.

Eggs may be embryonated or unembryonated when passed by the female, meaning that their fertilized eggs may not yet be developed. A few species are known to be ovoviviparous. The eggs are protected by an outer shell, secreted by the uterus. In free-living roundworms, the eggs hatch into larvae, which appear essentially identical to the adults, except for an under-developed reproductive system; in parasitic roundworms, the life cycle is often much more complicated.

Nematodes as a whole possess a wide range of modes of reproduction. Some nematodes, such as *Heterorhabditis* spp., undergo a process called *endotokia matricida*: intrauterine birth causing maternal death. Some nematodes are hermaphroditic, and keep their self-fertilized eggs inside the uterus until they hatch. The juvenile nematodes will then ingest the parent nematode. This process is significantly promoted in environments with a low or reducing food supply.

The nematode model species *Caenorhabditis elegans* and *C. briggsae* exhibit androdioecy, which is very rare among animals. The single genus *Meloidogyne* (root-knot nematodes) exhibit a range of reproductive modes including sexual reproduction, facultative sexuality (in which most, but not all, generations reproduce asexually), and both meiotic and mitotic parthenogenesis.

The genus *Mesorhabditis* exhibits an unusual form of parthenogenesis, in which sperm-producing males copulate with females, but the sperm do not fuse with the ovum. Contact with the sperm is essential for the ovum to begin dividing, but because there is no fusion of the cells, the male contributes no genetic material to the offspring, which are essentially clones of the female.

### ***Free-living species***

In free-living species, development usually consists of four molts of the cuticle during growth. Different species feed on materials as varied as algae, fungi, small animals, fecal matter, dead organisms and living tissues. Free-living marine nematodes are important and abundant members of the meiobenthos. They play an important role in the decomposition process, aid in recycling of nutrients in marine environments and are sensitive to changes in the environment caused by pollution. One roundworm of note is *Caenorhabditis elegans*, which lives in the soil and has found much use as a model organism. *C. elegans* has had its entire genome sequenced, as well as the developmental fate of every cell determined, and every neuron mapped.

## ***Parasitic species***

Nematodes commonly parasitic on humans include ascarids (*Ascaris*), filarias, hookworms, pinworms (*Enterobius*) and whipworms (*Trichuris trichiura*). The species *Trichinella spiralis*, commonly known as the *trichina worm*, occurs in rats, pigs, and humans, and is responsible for the disease trichinosis. *Baylisascaris* usually infests wild animals but can be deadly to humans as well. *Dirofilaria immitis* are Heartworms known for causing Heartworm disease by inhabiting the hearts, arteries, and lungs of dogs and some cats. *Haemonchus contortus* is one of the most abundant infectious agents in sheep around the world, causing great economic damage to sheep farms. In contrast, entomopathogenic nematodes parasitize insects and are considered by humans to be beneficial.

One form of nematode is entirely dependent upon fig wasps, which are the sole source of fig fertilization. They prey upon the wasps, riding them from the ripe fig of the wasp's birth to the fig flower of its death, where they kill the wasp, and their offspring await the birth of the next generation of wasps as the fig ripens.

A newly discovered parasitic tetradonematid nematode, *Myrmeconema neotropicum*, apparently induces fruit mimicry in the tropical ant *Cephalotes atratus*. Infected ants develop bright red gasters, tend to be more sluggish, and walk with their gasters in a conspicuous elevated position. These changes likely cause frugivorous birds to confuse the infected ants for berries and eat them. Parasite eggs passed in the bird's feces are subsequently collected by foraging *Cephalotes atratus* and are fed to their larvae, thus completing the life cycle of *Myrmeconema neotropicum*.



Colorized electron micrograph of soybean cyst nematode (*Heterodera* sp.) and egg

Plant parasitic nematodes include several groups causing severe crop losses. The most common genera are *Aphelenchoides* (foliar nematodes), *Ditylenchus*, *Globodera* (potato cyst nematodes), *Heterodera* (soybean cyst nematodes), *Longidorus*, *Meloidogyne* (root-knot nematodes), *Nacobbus*, *Pratylenchus* (lesion nematodes), *Trichodorus* and *Xiphinema* (dagger nematodes). Several phytoparasitic nematode species cause histological damages to roots, including the formation of visible galls (e.g. by root-knot nematodes), which are useful characters for their diagnostic in the field. Some nematode species transmit plant viruses through their feeding activity on roots. One of them is *Xiphinema index*, vector of GFLV (Grapevine Fanleaf Virus), an important disease of grapes.

Other nematodes attack bark and forest trees. The most important representative of this group is *Bursaphelenchus xylophilus*, the pine wood nematode, present in Asia and America and recently discovered in Europe.

## Agriculture and horticulture

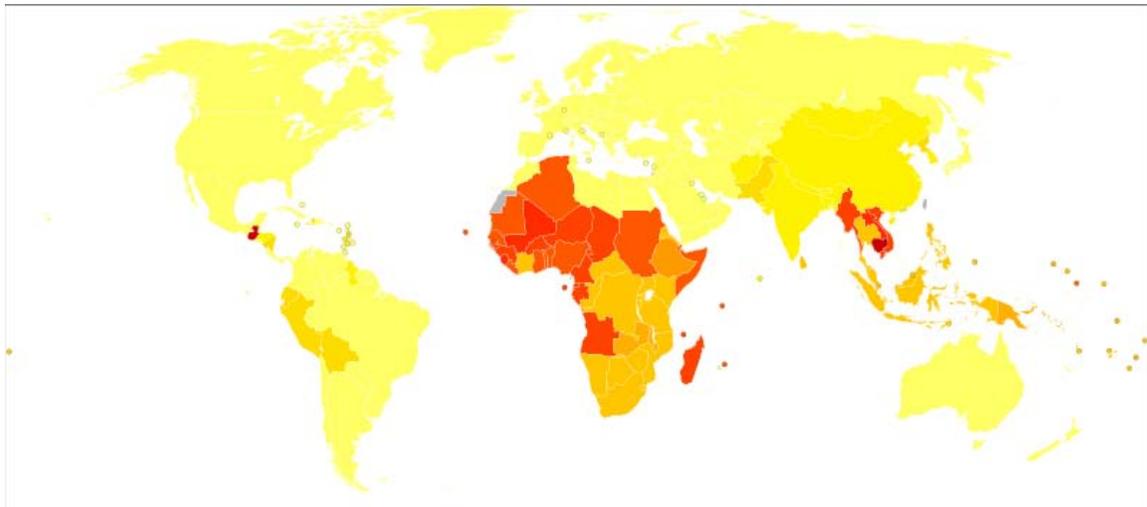
Depending on the species, a nematode may be beneficial or detrimental to plant health. From agricultural and horticulture perspectives, there are two categories of nematode: predatory ones, which will kill garden pests like cutworms, and pest nematodes, like the root-knot nematode, which attack plants and those that act as vectors spreading plant viruses between crop plants. Predatory nematodes can be bred by soaking a specific recipe of leaves and other detritus in water, in a dark, cool place, and can even be purchased as an organic form of pest control.

Rotations of plants with nematode resistant species or varieties is one means of managing parasitic nematode infestations. For example, marigolds, grown over one or more seasons (the effect is cumulative), can be used to control nematodes. Another is treatment with natural antagonists such as the fungus *Gliocladium roseum*. Chitosan is a natural biocontrol that elicits plant defense responses to destroy parasitic cyst nematodes on roots of soybean, corn, sugar beets, potatoes and tomatoes without harming beneficial nematodes in the soil. Furthermore soil steaming is an efficient method to kill nematodes before planting crop.

CSIRO has found that there was 13- to 14-fold reduction of nematode population densities in plots having Indian mustard (*Brassica juncea*) green manure or seed meal in the soil.

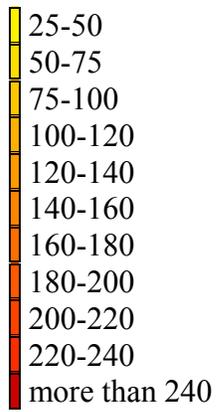
Hundreds of *Caenorhabditis elegans* were featured in a research project on NASA's STS-107 space mission (which ended in the Space Shuttle Columbia Disaster).

## Epidemiology



Disability-adjusted life year for intestinal nematode infections per 100,000 inhabitants in 2002.

no data  
less than 25



A number of intestinal nematodes affect human beings. These include ascariasis, trichuriasis and hookworm disease.

## Chapter- 8

# Physiological Plant Disorders and Parasitic Plant

## Physiological Plant Disorders

**Physiological plant disorders** are caused by non-pathological disorders such as poor light, weather damage, water-logging or a lack of nutrients, and affect the functioning of the plant system. Physiological disorder are distinguished from plant diseases caused by pathogens, such as a virus or fungus. Whilst the symptoms of physiological disorders may appear disease-like, they can usually be prevented by altering environmental conditions. However, once a plant shows symptoms of nutrient deficiency it is likely that that season's yields will be reduced.

Causes of physiological disorders can be identified by examining:

- Where symptoms first appear on a plant—on new leaves, old leaves or all over?
- The pattern of any discolouration or yellowing—is it all over, between the veins or around the edges? If only the veins are yellow deficiency is probably not involved.
- Note general patterns rather than looking at individual plants—are the symptoms distributed throughout a group of plants of the same type growing together. In the case of a deficiency all of the plants should be similarly effected, although distribution will depend on past treatments applied to the soil.
- Soil analysis, such as determining pH, can help to confirm the presence of physiological disorders. Recent conditions, such as heavy rains, dry spells, frosts, etc., may also help to determine the cause of plant disorders.

## **Weather damage**



Frosted seedhead of *Echinacea purpurea*

Frost and cold are major causes of crop damage to tender plants, although hardy plants can also suffer if new growth is exposed to a hard frost following a period of warm weather. Symptoms will often appear overnight, affecting many types of plants. Leaves and stems may turn black, and buds and flowers may be discoloured, and frosted blooms may not produce fruit. Many annual plants, or plants grown in frost free areas, can suffer from damage when the air temperature drops below 40 degrees Fahrenheit (4 degrees Celsius). Tropical plants may begin to experience cold damage when the temperature is 42 to 48 °F (5 to 9 °C), symptoms include wilting of the top of the stems and/or leaves, and blackening or softening of the plant tissue.

Frost or cold damage can be avoided by ensuring that tender plants are properly hardened before planting, and that they are not planted too early in the season, before the risk of frost has passed. Avoid planting susceptible plants in frost pockets, or where they will receive early morning sun. Protect young buds and bloom with horticultural fleece if frost is forecast. Cold, drying easterly winds can also severely inhibit spring growth even without an actual frost, thus adequate shelter or the use of windbreaks is important.

Drought can cause plants to suffer from water stress and wilt. Adequate irrigation is required during prolonged hot, dry periods. Rather than shallow daily watering, during a drought water should be directed towards the roots, ensuring that the soil is thoroughly soaked two or three times a week. Mulches also help preserve soil moisture and keep roots cool.

Heavy rains, particularly after prolonged dry periods, can also cause roots to split, onion saddleback (splitting at the base), tomatoes split and potatoes to become deformed or hollow. Using mulches or adding organic matter such as leaf mold, compost or well rotted manure to the soil will help to act as a 'buffer' between sudden changes in conditions. Water-logging can occur on poorly drained soils, particularly following heavy rains. Plants can become yellow and stunted, and will tend to be more prone to drought and diseases. Improving drainage will help to alleviate this problem.

Hail can cause damage to soft skinned fruits, and may also allow brown rot or other fungi to penetrate the plant. Brown spot markings or lines on one side of a mature apple are indicative of a spring hailstorm.

Plants affected by salt stress are unable to take water from soil, due to an osmotic imbalance between soil and plant.

### ***Nutrient deficiencies***

Poor growth and a variety of complaints such as leaf discolouration (chlorosis) can be caused by a lack of plant foods. This may be due to shortages of necessary nutrients, or because the nutrients are present but not available to the plant. The latter can be caused by incorrect pH, shortages of water or an excess of another nutrient. Generally, the key to avoiding nutrient deficiencies is to ensure that the soil is healthy and contains plenty of well rotted organic matter rather than by feeding or treating individual plants.

Nutrient (or mineral) deficiencies include:

- Boron deficiency
- Calcium deficiency
- Iron deficiency
- Magnesium deficiency
- Manganese deficiency
- Nitrogen deficiency
- Phosphorus deficiency

- Potassium deficiency
- Shortage of trace elements such as molybdenum can also cause disorders such as whiptail in cauliflower.

## Parasitic Plant



Cuscuta Parasite Plant

A **parasitic plant** is one that derives some or all of its sustenance from another plant. About 4,100 species in approximately 19 families of flowering plants are known. Parasitic plants have a modified root, the haustorium, that penetrates the host plant and connects to the xylem, phloem, or both. Parasitic plants are characterized as follows:

- 1a. Obligate parasite – a parasite that cannot complete its life cycle without a host.
- 1b. Facultative parasite – a parasite that can complete its life cycle independent of a host.
- 2a. Stem parasite – a parasite that attaches to the host stem.
- 2b. Root parasite – a parasite that attaches to the host root.
- 3a. Holoparasite – a plant that is completely parasitic on other plants and has virtually no chlorophyll.
- 3b. Hemiparasite – a plant that is parasitic under natural conditions and is also photosynthetic to some degree. Hemiparasites may just obtain water and mineral nutrients from the host plant. Many obtain at least part of their organic nutrients from the host as well.

For hemiparasites, one from each of the three sets of terms can be applied to the same species, e.g.

- *Nuytsia floribunda* (Western Australian Christmas tree) is an obligate root hemiparasite.
- *Rhinanthus* (Yellow rattle) is a facultative root hemiparasite.
- Mistletoe is an obligate stem hemiparasite.

Holoparasites are always obligate so only two terms are needed, e.g.

- Dodder is a stem holoparasite.
- *Hydnora* spp. are root holoparasites.

Plants usually considered holoparasites include broomrape, dodder, *Rafflesia*, and Hydnoraceae. Plants usually considered hemiparasites include *Castilleja*, mistletoe, Western Australian Christmas tree and yellow rattle.



**3.a Holoparasite** *Hyobanche sanguinea*, Richtersfeld, Namaqualand, northern cape, South Africa



**3.a Holoparasite** *Hyobanche sanguinea*, Richtersfeld, Namaqualand, northern cape, South Africa



**3.a Holoparasite** *Hyobanche sanguinea*, Goegap N.R., Namaqualand, northern cape, South Africa

### **Seeds Germination**

**Seed germination** of parasitic plants occurs in a variety of ways. These means can either be chemical or mechanical and the means used by seeds often depends on whether or not the parasites are root parasites or stem parasites. Both root and stem parasitic plants have evolved to use one or more means of finding their hosts in order to germinate. Most parasitic plants need to germinate in close proximity to their host plants because their seeds are limited in the amount of resources necessary to survive without nutrients from their host plants. Resources are limited due in part to the fact that most parasitic plants aren't able to use autotrophic nutrition to establish the early stages of seeding. Root parasitic plant seeds tend to use chemical cues for germination. In order for germination to occur, seeds need to be fairly close to their host plant. For example, the seeds of the parasitic plant Witchweed (*Striga asiatica*) need to be within 3 to 4 millimeters (mm) of its host in order to pick up chemical signals in the soil to signal germination. This range is important because *Striga asiatica* will only grow about 4 mm after germination. Chemical compound cues sensed by parasitic plant seeds are from host plant root exudates that are leached in close proximity from the host's root system into the surrounding soil. These chemical cues are a variety of compounds that are unstable and rapidly degraded in soil and are present within a radius of a few meters of the plant.

exuding them. Parasitic plants germinate and follow a concentration gradient of these compounds in the soil toward the host plants if close enough. These compounds are called strigolactones. Strigolactone stimulates ethylene biosynthesis in seeds causing them to germinate.

There are a variety of chemical germination stimulants. Strigol was the first of the germination stimulants to be isolated. It was isolated from a non-host cotton plant and has been found in true host plants such as corn and millets. The stimulants are usually plant specific, examples of other germination stimulants include sorgolactone from sorghum, orobanchol and alectrol from red clover, and 5-deoxystrigol from *Lotus japonicas*.

Strigolactones are apocarotenoids that are produced via the carotenoid pathway of plants. Strigolactones and mycorrhizal fungi have a relationship in which Strigolactone also cues the growth of mycorrhizal fungus.

Stem parasitic plants unlike most root plants germinate using the resources inside its endosperm and are able to survive for a small amount of time. An example, Dodder (*Cuscuta* spp.) is a parasitic plant whose seed falls to the ground and may remain dormant for up to five years before it is able to sense a host plants nearby. Using the resources in the seed endosperm, Dodder is able to germinate. Once germinated, the plant has 6 days to find and establish a connection with its host plant before its resources run out.

Dodder seeds germinate above ground and then the plant sends out stems in search of its host plant reaching up to 6cm before it dies. It is believed that the plant uses two methods of finding a host. The stem is able to pick up its host plant's scent whereby it then is able to orient itself in the direction of its host. Scientists used volatiles from tomato plants ( $\alpha$ -pinene,  $\beta$ -myrcene, and  $\beta$ -phellandrene) to test the reaction of *C. pentagona* and found that the stem will oriented itself in the direction of the odor. Some studies suggest that by using light reflecting off of near by plants dodders are able to select host with higher sugar because of the levels of chlorophyll in the leaves. Once Dodder finds its host, it wraps itself around the host plants stem. Using adventitious roots, Dodder taps into the host plant's stem and creates a haustoria, which is a special connection into the host plant vascular tissue. Dodder makes several of these connections with the host as it moves up the plant.

### **Host range**

Some parasitic plants are generalists and parasitize many different species, even several different species at once. Dodder (*Cuscuta* spp., *Cassytha* spp.) and red rattle (*Odontites verna*) are generalist parasites. Other parasitic plants are specialists that parasitize a few or even just one species. Beech drops (*Epifagus virginiana*) is a root holoparasite only on American Beech (*Fagus grandifolia*). *Rafflesia* is a holoparasite on the vine *Tetrastigma*.

### **Importance**

- Witchweed, broomrape and dodder cause huge economic losses in a variety of herbaceous crops. Mistletoes cause economic damage to forest and ornamental trees.
- *Rafflesia arnoldii* produces the world's largest flowers at about one meter in diameter. It is a tourist attraction in its native habitat.

- Indian paintbrush (*Castilleja linariaefolia*) is the state flower of Wyoming.
- The Oak Mistletoe (*Phoradendron serotinum*) is the floral emblem of Oklahoma.
- A few other parasitic plants are occasionally cultivated for their attractive flowers, such as *Nuytsia* and broomrape.
- Parasitic plants are important in research, especially on the loss of photosynthesis during evolution.
- A few dozen parasitic plants have occasionally been used as food by people.
- Western Australian Christmas tree (*Nuytsia floribunda*) sometimes damages underground cables. It mistakes the cables for host roots and tries to parasitize them using its sclerenchymatic guillotine



Newly emergent snow plant (*Sarcodes sanguinea*), a fungus parasite

### ***Plants parasitic on fungi***

About 400 species of flowering plants and one gymnosperm (*Parasitaxus usta*), are parasitic on mycorrhizal fungi. They are termed myco-heterotrophs rather than parasitic plants. Some myco-heterotrophs are Indian pipe (*Monotropa uniflora*), snow plant (*Sarcodes sanguinea*), underground orchid (*Rhizanthella gardneri*), bird's nest orchid (*Neottia nidus-avis*) and sugarstick (*Allotropa virgata*).

## Chapter- 9

# Ascomycota

### Ascomycota



*Sarcoscypha coccinea*

### Scientific classification

Domain:	Eukarya
Kingdom:	Fungi
Subkingdom:	Dikarya
Phylum:	<b>Ascomycota</b> (Berk. 1857) Caval.-Sm. 1998

### Subphyla/Classes

Pezizomycotina  
Arthoniomycetes  
Dothideomycetes  
Eurotiomycetes  
Geoglossomycetes  
Laboulbeniomycetes

- Lecanoromycetes
- Leotiomycetes
- Lichinomycetes
- Orbiliomycetes
- Pezizomycetes
- Sordariomycetes
- "Unplaced orders"
- Lahmiales
- Medeolariales
- Triblidiales
- Saccharomycotina
- Saccharomycetes
- Taphrinomycotina
- Neoelectomycetes
- Pneumocystidomycetes
- Schizosaccharomycetes
- Taphrinomycetes

The **Ascomycota** are a Division/Phylum of the kingdom Fungi, and subkingdom Dikarya. Its members are commonly known as the **Sac fungi**. They are the largest phylum of Fungi, with over 64,000 species. The defining feature of this fungal group is the "ascus" (from Greek: *ἄσκος* (*askos*), meaning "sac" or "wineskin"), a microscopic sexual structure in which nonmotile spores, called ascospores, are formed. However, some species of the Ascomycota are asexual, meaning that they do not have a sexual cycle and thus do not form asci or ascospores. Previously placed in the Deuteromycota along with asexual species from other fungal taxa, asexual (or anamorphic) ascomycetes are now identified and classified based on morphological or physiological similarities to ascus-bearing taxa, and by phylogenetic analyses of DNA sequences.

The ascomycetes are a monophyletic group, i.e., all of its members trace back to one common ancestor. This group is of particular relevance to humans as sources for medicinally important compounds, such as antibiotics and for making bread, alcoholic beverages, and cheese, but also as pathogens of humans and plants. Familiar examples of sac fungi include morels, truffles, brewer's yeast and baker's yeast, Dead Man's Fingers, and cup fungi. The fungal symbionts in the majority of lichens (loosely termed "ascolichens") such as *Cladonia* belong to the Ascomycota. There are many plant-pathogenic ascomycetes, including apple scab, rice blast, the ergot fungi, black knot, and the powdery mildews. Several species of ascomycetes are biological model organisms in laboratory research. Most famously *Neurospora crassa*, several species of yeasts, and *Aspergillus* species are used in many genetics and cell biology studies. *Penicillium* species on cheeses and those producing antibiotics for treating bacterial infectious diseases are examples of taxa that belong to the Ascomycota.

## ***Ascomycetes versus Ascomycota***

Before the recognition of the fungal kingdom, the sac fungi were considered to be a *Class*, not a *Phylum*. The original collective term for these taxa was "Ascomycetes", which was first coined in the 1800s for a rankless nonlichenized taxon that possessed asci. The names Ascomycota, Ascomycetes, and others with the same root are based upon the term "ascus". "Ascomycetes" was soon used to include lichenized taxa, and became the standard term, at the class level, for all ascus-bearing species, just as the term "Basidiomycetes" became used for their basidium-bearing counterparts. Elevation of the taxonomic rank of the Ascomycetes resulted in the names Ascomycetae, Ascomycotina, and finally Ascomycota. Together, the Ascomycota and the Basidiomycota form the subkingdom Dikarya. The more familiar term, Ascomycetes, is still loosely used, e.g. at fungal forays it is often said of a fungus, such as *Peziza*, "It is an ascomycete, not a basidiomycete" in reference to their sexual reproductive mode. The terms are further abbreviated to "ascos" and "basidos" which are not officially sanctioned technical names.

## ***Modern classification of Ascomycota***

There are three subphyla that are described and accepted:

- The *Pezizomycotina* is the largest subphylum and contains all ascomycetes that produce ascocarps (fruiting bodies), except for one genus, *Neolecta*, in the Taphrinomycotina. It is roughly equivalent to the previous taxon, *Euascomycetes*. The *Pezizomycotina* includes most macroscopic "ascos" such as truffles, ergot, ascolichens, cup fungi (discomycetes), pyrenomycetes, lorchels, and caterpillar fungus. It also contains microscopic fungi such as powdery mildews, dermatophytic fungi, and Laboulbeniales.
- The *Saccharomycotina* comprises most of the "true" yeasts, such as baker's yeast and *Candida* which are single-celled (unicellular) fungi, which reproduce vegetatively by budding. Most of these species were previously classified in a taxon called *Hemiascomycetes*.
- The *Taphrinomycotina* includes a disparate and basal group within the Ascomycota that was recognized following molecular (DNA) analyses. The taxon was originally named *Archiascomycetes* (or *Archaeascomycetes*). It includes both hyphal fungi (*Neolecta*, *Taphrina*), fission yeasts (*Schizosaccharomyces*), and the mammalian lung parasite, *Pneumocystis*.

Ribosomal RNA gene sequencing of soil suggests that there may be a fourth subphylum of Ascomycota (termed Soil Clone Group I or SCGI), that has not been described in cultures or based on fruiting bodies. SCGI organisms are only known from DNA sequences found in soils worldwide and are placed between the Taphrinomycotina and the Saccharomycotina.

## **Outdated taxon names**

Several outdated taxon names—based on morphological features—are still occasionally used for species of the Ascomycota. These include the following sexual (teleomorphic) groups, defined by the structures of their sexual fruiting bodies: the Discomycetes, which included all species forming apothecia; the Pyrenomycetes, which included all sac fungi that formed perithecia or pseudothecia, or any structure resembling these morphological structures; and the Plectomycetes, which included those species that form cleistothecia. Hemiascomycetes included the yeasts and yeast-like fungi that have now been placed into the Saccharomycotina or Taphrinomycotina, while the Euascomycetes included the remaining species of the Ascomycota which are now in the Pezizomycotina, and the Neolecta which are in the Taphrinomycotina.

Some ascomycetes do not reproduce sexually or are not known to produce asci and are therefore anamorphic species. Those anamorphs that produce conidia (mitospores) were previously described as Mitosporic Ascomycota. Some taxonomists placed this group into a separate artificial phylum, the Deuteromycota (or "Fungi Imperfecti"). Where recent molecular analyses have identified close relationships with ascus-bearing taxa, anamorphic species have been grouped into the Ascomycota, despite the absence of the defining ascus. Sexual and asexual isolates of the same species commonly carry different binomial species names, as, for example, *Aspergillus nidulans* and *Emericella nidulans*, for asexual and sexual isolates, respectively, of the same species.

Species of the Deuteromycota were classified as Coelomycetes if they produced their conidia in minute flask- or saucer-shaped conidiomata, known technically as *pycnidia* and *acervuli*. The Hyphomycetes were those species where the conidiophores (*i.e.*, the hyphal structures that carry conidia-forming cells at the end) are free or loosely organized. They are mostly isolated but sometimes also appear as bundles of cells aligned in parallel (described as *synnematal*) or as cushion-shaped masses (described as *sporodochial*).

## **Morphology**

Most species grow as filamentous, microscopic structures called hyphae. Many interconnected hyphae form a mycelium, which—when visible to the naked eye (macroscopic)—is commonly called mold (or, in botanical terminology, thallus). During sexual reproduction, many Ascomycota typically produce large numbers of asci. The ascus is often contained in a multicellular, occasionally readily visible fruiting structure, the ascocarp (also called an *ascoma*). Ascocarps come in a very large variety of shapes: cup-shaped, club-shaped, potato-like, spongy, seed-like, oozing and pimple-like, coral-like, nit-like, golf-ball-shaped, perforated tennis ball-like, cushion-shaped, plated and feathered in miniature (Laboulbeniales), microscopic classic Greek shield-shaped, stalked or sessile. They can appear solitary or clustered. Their texture can likewise be very variable, including fleshy, like charcoal (carbonaceous), leathery, rubbery, gelatinous, slimy, powdery, or cob-web-like. Ascocarps come in multiple colors such as red, orange, yellow, brown, black, or, more rarely, green or blue. Some ascomycetous fungi, such as

*Saccharomyces cerevisiae*, grow as single-celled yeasts, which—during sexual reproduction—develop into an ascus, and do not form fruiting bodies.



The "candlesnuff fungus", *Xylaria hypoxylon*

In lichenized species, the thallus of the fungus defines the shape of the symbiotic colony. Some dimorphic species, such as *Candida albicans*, can switch between growth as single cells and as filamentous, multicellular hyphae. Other species are pleomorphic, exhibiting asexual (anamorphic) as well as a sexual (teleomorphic) growth forms.

Except for lichens, the non-reproductive (vegetative) mycelium of most ascomycetes is usually inconspicuous because it is commonly embedded in the substrate, such as soil, or grows on or inside a living host, and only the ascoma may be seen when fruiting. Pigmentation, such as melanin in hyphal walls, along with prolific growth on surfaces can result in visible mold colonies; examples include *Cladosporium* species, which form black spots on bathroom caulking and other moist areas. Many ascomycetes cause food spoilage, and, therefore, the pellicles or moldy layers that develop on jams, juices, and other foods are the mycelia of these species or occasionally Mucoromycotina and almost never Basidiomycota. Sooty molds that develop on plants, especially in the tropics are the thalli of many species.



The ascocarp of a morel contains numerous apothecia

Large masses of yeast cells, asci or ascus-like cells, or conidia can also form macroscopic structures. For example, *Pneumocystis* species can colonize lung cavities (visible in x-rays), causing a form of pneumonia. Asci of *Ascospaera* fill honey bee larvae and pupae causing mummification with a chalk-like appearance, hence the name "chalkbrood". Yeasts form small colonies in vitro and in vivo, and excessive growth of *Candida* species in the mouth or vagina causes "thrush", a form of candidiasis.

The cell walls of the ascomycetes almost always contain chitin and  $\beta$ -glucans, and divisions within the hyphae, called "septa", are the internal boundaries of individual cells (or compartments). The cell wall and septa give stability and rigidity to the hyphae and may prevent loss of cytoplasm in case of local damage to cell wall and cell membrane.

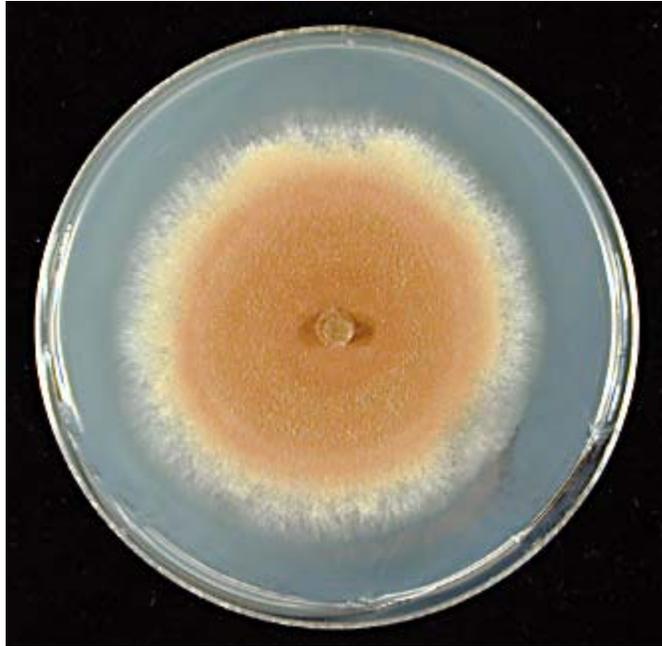
The septa commonly have a small opening in the center, which functions as a cytoplasmic connection between adjacent cells, also sometimes allowing cell-to-cell movement of nuclei within a hypha. Vegetative hyphae of most ascomycetes contain only one nucleus per cell (*uninucleate* hyphae), but multinucleate cells—especially in the apical regions of growing hyphae—can also be present.

## **Metabolism**

In common with other fungal phyla, the Ascomycota are heterotrophic organisms that require organic molecules as energy sources. These are obtained by feeding on a variety of organic substrates including dead matter, foodstuffs, or as symbionts in or on other living organisms. To obtain these nutrients from their surroundings, ascomycetous fungi secrete powerful digestive enzymes which break down organic substances into smaller molecules, which are then taken up into the cell. Many species live on dead plant material such as leaves, twigs, or logs. Several species colonize plants, animals, or other fungi as parasites or mutualistic symbionts and derive all their metabolic energy in form of nutrients from the tissues of their hosts.

Owing to their long evolutionary history, the Ascomycota have evolved the capacity to break down almost every organic substance. Unlike most organisms, they are able to use their own enzymes to digest plant biopolymers such as cellulose or lignin. Collagen, an abundant structural protein in animals, and keratin—a protein that forms hair and nails—, can also serve as food sources. Unusual examples include *Aureobasidium pullulans*, which feeds on wall paint, and the kerosene fungus *Amorphotheca resinae*, which feeds on aircraft fuel (causing occasional problems for the airline industry), and may sometimes block fuel pipes. Other species can resist high osmotic stress and grow, for example, on salted fish, and a few ascomycetes are aquatic.

The Ascomycota is characterized by a high degree of specialization; for instance, certain species of Laboulbeniales attack only one particular leg of one particular insect species. Many Ascomycota engage in symbiotic relationships such as in lichens—symbiotic associations with green algae or cyanobacteria—in which the fungal symbiont directly obtains products of photosynthesis. In common with many basidiomycetes and Glomeromycota, some ascomycetes form symbioses with plants by colonizing the roots to form mycorrhizal associations. The Ascomycota also represents several carnivorous fungi, which have developed hyphal traps to capture small protists such as amoebae, as well as roundworms (*Nematoda*), rotifers, tardigrades, and small arthropods such as springtails (*Collembola*).



*Hypomyces completus* on culture medium

### ***Distribution and living environment***

The Ascomycota are represented in all land ecosystems worldwide, occurring on all continents including Antarctica. Spores and hyphal fragments are dispersed through the atmosphere and freshwater environments, as well as ocean beaches and tidal zones. The distribution of species is variable; while some are found on all continents, others, as for example the white truffle *Tuber magnatum*, only occur in isolated locations in Italy and Eastern Europe. The distribution of plant-parasitic species is often restricted by host distributions; for example, *Cyttaria* is only found on *Nothofagus* (Southern Beech) in the Southern Hemisphere.

### ***Reproduction***

#### **Asexual reproduction**

Asexual reproduction is the dominant form of propagation in the Ascomycota, and is responsible for the rapid spread of these fungi into new areas. It occurs through vegetative reproductive spores, the conidia. The conidiospores commonly contain one nucleus and are products of mitotic cell divisions and thus are sometimes called mitospores, which are genetically identical to the mycelium from which they originate. They are typically formed at the ends of specialized hyphae, the *conidiophores*. Depending on the species they may be dispersed by wind or water, or by animals.

## Asexual spores

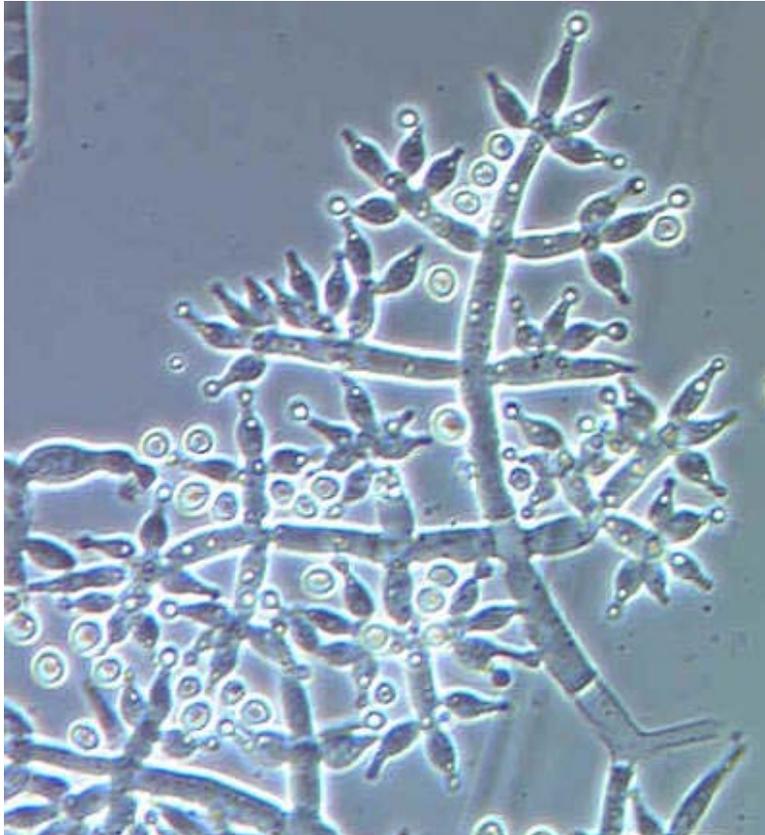
Different types of asexual spores can be identified by colour, shape, and how they are released as individual spores. Spore types can be used as taxonomic characters in the classification within the Ascomycota. The most frequent types are the single-celled spores, which are designated *ameroconidia*. If the spore is divided into two by a cross-wall (septum), it is called a *didymospore*.



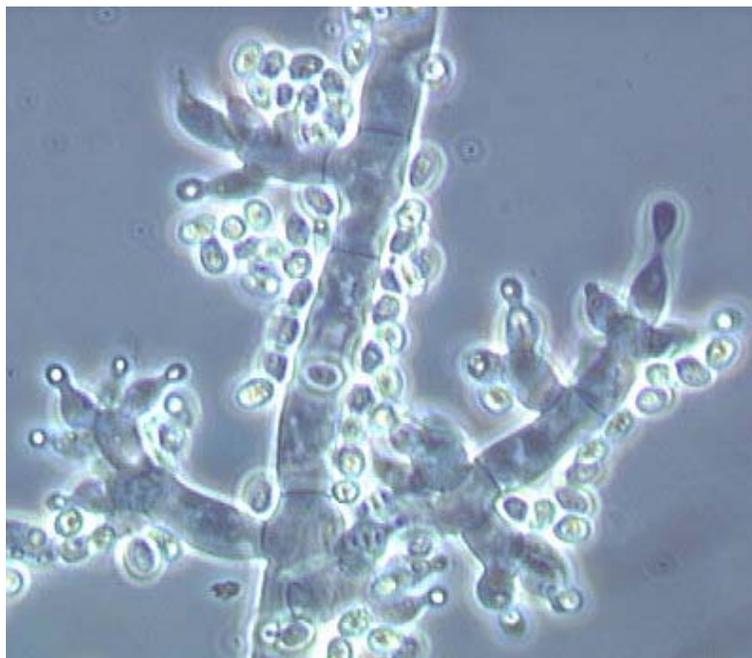
Conidiospores of *Trichoderma aggressivum*, Diameter approx. 3 $\mu$ m



Conidiophores of molds of the genus *Aspergillus*, conidiogenesis is blastic-phialidic



Conidiophores of *Trichoderma harzianum*, conidiogenesis is blastic-phialidic



Conidiophores of *Trichoderma fertile* with vase-shaped phialides and newly formed conidia on their ends (bright points)

When there are two or more cross-walls, the classification depends on spore shape. If the septa are *transversal*, like the rungs of a ladder, it is a *phragmospore*, and if they possess a net-like structure it is a *dictyospore*. In *staurospores* ray-like arms radiate from a central body; in others (*helicospores*) the entire spore is wound up in a spiral like a spring. Very long worm-like spores with a length-to-diameter ratio of more than 15:1, are called *scoleospores*.

## Conidiogenesis and dehiscence

Important characteristics of the anamorphs of the Ascomycota are *conidiogenesis*, which includes spore formation and dehiscence (separation from the parent structure). Conidiogenesis corresponds to Embryology in animals and plants and can be divided into two fundamental forms of development: *blastic* conidiogenesis, where the spore is already evident before it separates from the conidiogenic hypha, and *thallic* conidiogenesis, during which a cross-wall forms and the newly created cell develops into a spore. The spores may or may not be generated in a large-scale specialized structure which helps to spread them.

These two basic types can be further classified as follows:

- *blastic-acropetal* (repeated budding at the tip of the conidiogenic hypha, so that a chain of spores is formed with the youngest spores at the tip),
- *blastic-synchronous* (simultaneous spore formation from a central cell, sometimes with secondary acropetal chains forming from the initial spores),
- *blastic-sympodial* (repeated sideways spore formation from behind the leading spore, so that the oldest spore is at the main tip),
- *blastic-annellidic* (each spore separates and leaves a ring-shaped scar which is inside the scar left by the previous spore),
- *blastic-phialidic* (the spores arise and are ejected from the open ends of special conidiogenic cells called phialides which remain constant in length),
- *basauxic* (where a chain of conidia, in successively younger stages of development, is emitted from the mother cell),
- *blastic-retrogressive* (spores separate by formation of crosswalls near the tip of the conidiogenic hypha, which thus becomes progressively shorter),
- *thallic-arthric* (double cell walls split the conidiogenic hypha into cells which develop into short, cylindrical spores called *arthroconidia*; sometimes every second cell dies off, leaving the arthroconidia free),
- *thallic-solitary* (a large bulging cell separates from the conidiogenic hypha, forms internal walls, and develops to a *phragmospore*).

Sometimes the conidia are produced in structures visible to the naked eye, which help to distribute the spores. These structures are called "conidiomata" (singular: conidioma), and may take the form of *pycnidia* (which are flask-shaped and arise in the fungal tissue) or *acervuli* (which are cushion-shaped and arise in host tissue).

Dehiscence happens in two ways. In *schizolytic* dehiscence, a double-dividing wall with a central lamella (layer) forms between the cells; the central layer then breaks down thereby releasing the spores. In *rhizolytic* dehiscence, the cell wall which joins the spores on the outside degenerates and releases the conidia.

## Heterokaryosis and parasexuality

Several Ascomycota species are not known to have a sexual cycle. Such asexual species may be able to undergo genetic recombination between individuals by processes involving *heterokaryosis* and *parasexual* events.

Parasexuality refers to the process of heterokaryosis, caused by merging of two hyphae belonging to different individuals, by a process called *anastomosis*, followed by a series of events resulting in genetically different cell nuclei in the mycelium. The merging of nuclei is not followed by meiotic events, such as gamete formation and results in an increased number of chromosomes per nuclei. *Mitotic crossover* may enable recombination, i.e., an exchange of genetic material between homologous chromosomes. The chromosome number may then be restored to its haploid state by nuclear division, with each daughter nuclei being genetically different from the original parent nuclei. Alternatively, nuclei may lose some chromosomes, resulting in aneuploid cells.

## Sexual reproduction



Ascus of *Hypocrea virens* with eight two-celled Ascospores

Sexual reproduction in the Ascomycota leads to the formation of the *ascus*, the structure that defines this fungal group and distinguishes it from other fungal phyla. The ascus is a tube-shaped vessel, a *meiosporangium*, which contains the sexual spores produced by meiosis and which are called *ascospores*.

Apart from a few exceptions, such as *Candida albicans*, most ascomycetes are haploid, i.e., they contain one set of chromosomes per nuclei. During sexual reproduction there is a diploid phase which commonly is very short, and meiosis restores the haploid state.

### **Formation of sexual spores**

The sexual part of the life cycle commences when two hyphal structures mate. In the case of *homothallic* species, mating is enabled between hyphae of the same fungal clone, whereas in *heterothallic* species, the two hyphae must originate from fungal clones that differ genetically, i.e., those that are of a different mating type. Mating types are typical of the fungi and correspond roughly to the sexes in plants and animals; however one species may have more than two mating types, resulting in sometimes complex vegetative incompatibility systems.

Gametangia are sexual structures formed from hyphae, and are the generative cells. A very fine hypha, called trichogyne emerges from one gametangium, the *ascogonium*, and merges with a gametangium (the *antheridium*) of the other fungal isolate. The nuclei in the antheridium then migrate into the ascogonium, and plasmogamy—the mixing of the cytoplasm—occurs. Unlike in animals and plants, plasmogamy is not immediately followed by the merging of the nuclei (called *karyogamy*). Instead, the nuclei from the two hyphae form pairs, initiating the *dikaryophase* of the sexual cycle, during which time the pairs of nuclei synchronously divide. Fusion of the paired nuclei leads to mixing of the genetic material and recombination and is followed by meiosis. A similar sexual cycle is present in the red algae (Rhodophyta).



Unitunicate-inoperculate Asci of *Hypomyces chrysospermus*

From the fertilized ascogonium, *dinucleate* hyphae emerge in which each cell contains two nuclei. These hyphae are called *ascogenous* or fertile hyphae. They are supported by the vegetative mycelium containing uni- (or mono-) nucleate hyphae, which are sterile. The mycelium containing both sterile and fertile hyphae may grow into fruiting body, the *ascocarp*, which may contain millions of fertile hyphae.

The sexual structures are formed in the fruiting layer of the ascocarp, the hymenium. At one end of ascogenous hyphae, characteristic U-shaped hooks develop, which curve back opposite to the growth direction of the hyphae. The two nuclei contained in the apical part of each hypha divide in such a way that the threads of their mitotic spindles run parallel, creating two pairs of genetically different nuclei. One daughter nucleus migrates close to the hook, while the other daughter nucleus locates to the basal part of the hypha. The formation of two parallel cross-walls then divides the hypha into three sections: one at the hook with one nucleus, one at the basal of the original hypha that contains one nucleus, and one that separates the U-shaped part which contains the other two nuclei.

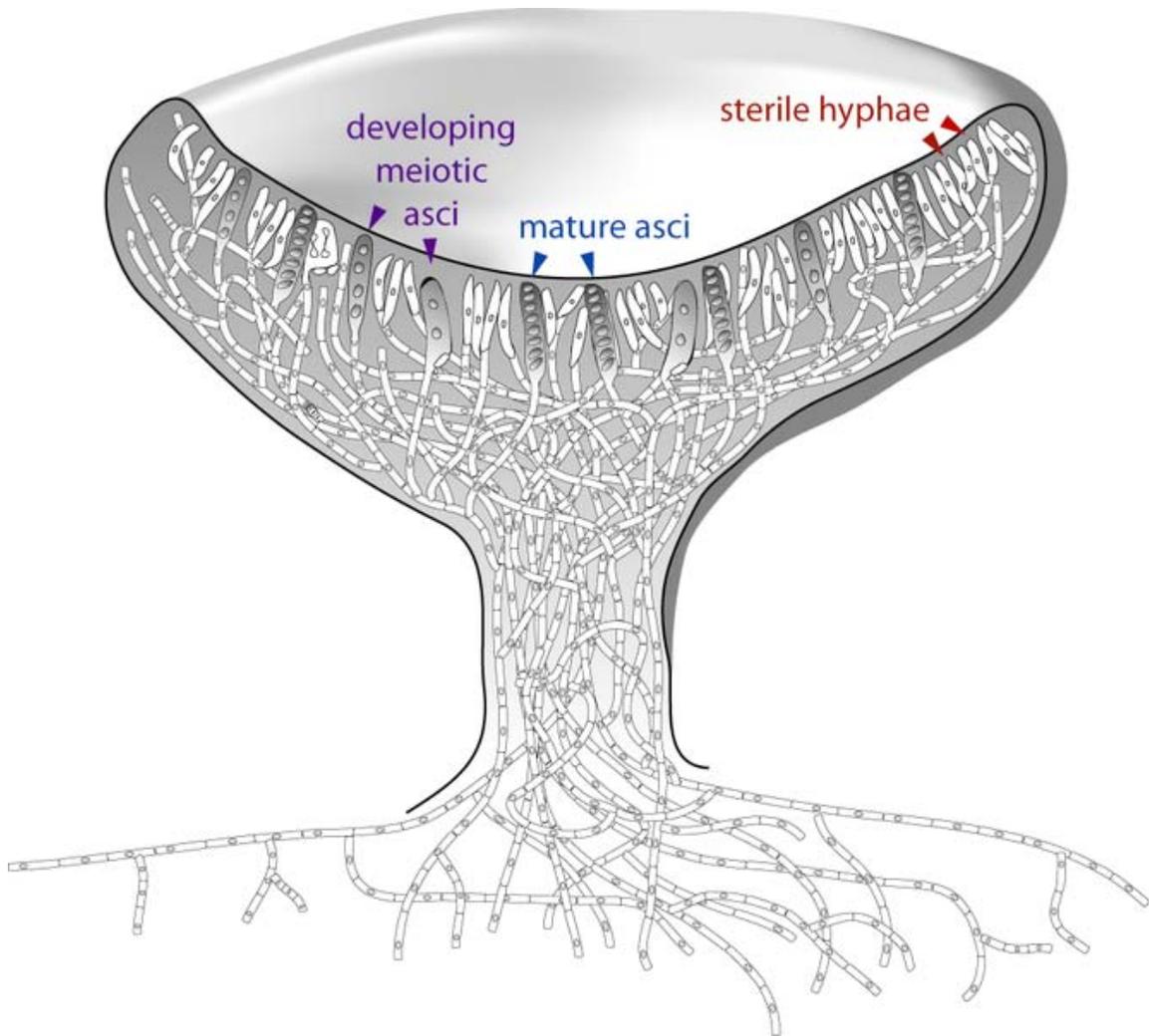


Diagram of an apothecium (the typical cup-like reproductive structure of Ascomycetes) showing sterile tissues as well as developing and mature asci.

Fusion of the nuclei (karyogamy) takes place in the U-shaped cells in the hymenium, and results in the formation of a diploid zygote. The zygote grows into the ascus, an elongated tube-shaped or cylinder-shaped capsule. Meiosis then gives rise to four haploid nuclei, usually followed by a further mitotic division that results in eight nuclei in each ascus. The nuclei along with some cytoplasm become enclosed within membranes and a cell wall to give rise to ascospores that are aligned inside the ascus like peas in a pod.

Upon opening of the ascus, ascospores may be dispersed by the wind, while in some cases the spores are forcibly ejected from the ascus; certain species have evolved spore cannons, which can eject ascospores up to 30 cm. away. When the spores reach a suitable substrate, they germinate, form new hyphae, which restarts the fungal life cycle.

The form of the ascus is important for classification and is divided into four basic types: unitunicate-operculate, unitunicate-inoperculate, bitunicate, or prototunicate.

## **Ecology**

The Ascomycota fulfil a central role in most land-based ecosystems. They are important decomposers which break down organic materials, such as dead leaves and animals, and help the detritivores (animals which feed on decomposing material) to obtain their nutrients. Ascomycetes along with other fungi can break down large molecules such as cellulose or lignin, and thus have important roles in nutrient cycling such as the carbon cycle.

The fruiting bodies of the Ascomycota provide food for many animals ranging from insects and slugs and snails (*Gastropoda*) to rodents and larger mammals such as deer and wild boars.

Many ascomycetes also form symbiotic relationships with other organisms, including plants and animals.

## **Lichens**

Probably since early in their evolutionary history, the Ascomycota have formed symbiotic associations with green algae (*Chlorophyta*), and other types of algae and cyanobacteria. These mutualistic associations are commonly known as lichens, and can grow and persist in terrestrial regions of the earth that are inhospitable to other organisms and characterized by extremes in temperature and humidity, including the Arctic, the Antarctic, deserts, and mountaintops. While the photoautotrophic algal partner generates metabolic energy through photosynthesis, the fungus offers a stable, supportive matrix and protects cells from radiation and dehydration. Around 42% of the Ascomycota (about 18,000 species) form lichens, and almost all the fungal partners of lichens belong to the Ascomycota.

## **Mycorrhizal fungi and endophytes**

Members of the Ascomycota form two important types of relationship with plants: as mycorrhizal fungi and as endophytes. Mycorrhiza are symbiotic associations of fungi with the root systems of the plants, which can be of vital importance for growth and persistence for the plant. The fine mycelial network of the fungus enables the increased uptake of mineral salts that occur at low levels in the soil. In return, the plant provides the fungus with metabolic energy in the form of photosynthetic products.

Endophytic fungi live inside plants, and those that form mutualistic or commensal associations with their host, do not damage their hosts. The exact nature of the relationship between endophytic fungus and host depends on the species involved, and in some cases fungal colonization of plants can bestow a higher resistance against insects, roundworms (nematodes), and bacteria; in the case of grass endophytes the fungal symbiont produces poisonous alkaloids, which can affect the health of plant-eating (herbivorous) mammals and deter or kill insect herbivores.

## Symbiotic relationships with animals

Several ascomycetes of the genus *Xylaria* colonize the nests of leafcutter ants and other fungus-growing ants of the tribe Attini, and the fungal gardens of termites (Isoptera). Since they do not generate fruiting bodies until the insects have left the nests, it is suspected that, as confirmed in several cases of Basidiomycota species, they may be cultivated.

Bark beetles (family Scolytidae) are important symbiotic partners of ascomycetes. The female beetles transport fungal spores to new hosts in characteristic tucks in their skin, the *mycetangia*. The beetle tunnels into the wood and into large chambers in which they lay their eggs. Spores released from the mycetangia germinate into hyphae, which can break down the wood. The beetle larvae then feed on the fungal mycelium, and, on reaching maturity, carry new spores with them to renew the cycle of infection. A well-known example of this is Dutch elm disease, caused by *Ophiostoma ulmi*, which is carried by the European elm bark beetle, *Scolytus multistriatus*.

## Importance for humans



Tree attacked by the Bluestain fungus, *Ophiostoma minus*

Ascomycetes make many contributions to the good of humanity, and also have many ill effects.

## Harmful interactions

One of their most harmful roles is as the agent of many plant diseases. For instance:

- Dutch Elm Disease, caused by the closely related species *Ophiostoma ulmi* and *Ophiostoma novo-ulmi*, has led to the death of many elms in Europe and North America.



*Claviceps purpurea* on rye (*Secale cereale*)

- The originally Asian *Cryphonectria parasitica* is responsible for attacking Sweet Chestnuts (*Castanea sativa*), and virtually eliminated the once-widespread American Chestnut (*Castanea dentata*),
- A disease of Maize (*Zea mays*), which is especially prevalent in North America, is brought about by *Cochliobolus heterostrophus*.
- *Taphrina deformans* causes leaf curl of peach.
- *Uncinula necator* is responsible for the disease Powdery mildew, which attacks grapevines.
- Species of *Monilia* cause brown rot of stone fruit such as peaches (*Prunus persica*) and sour cherries (*Prunus cerasus*).

- Members of the Ascomycota such as *Stachybotrys chartarum* are responsible for fading of woollen textiles, which is a common problem especially in the tropics.
- Blue-green, red and brown moulds attack and spoil foodstuffs - for instance *Penicillium italicum* rots oranges.
- Cereals infected with *Fusarium graminearum* contain mycotoxins like deoxynivalenol (DON), which can lead to skin and mucous membrane lesions when eaten by pigs.
- Ergot (*Claviceps purpurea*) is a direct menace to humans when it attacks wheat or rye and produces highly poisonous and carcinogenic alkaloids, causing ergotism if consumed. Symptoms include hallucinations, stomach cramp, and a burning sensation in the limbs ("Saint Anthony's Fire").
- *Aspergillus flavus*, which grows on peanuts and other hosts, generates aflatoxin, which damages the liver and is highly carcinogenic.
- *Candida albicans*, a yeast which attacks the mucous membranes, can cause an infection of the mouth or vagina called thrush or candidiasis, and is also blamed for "yeast allergies".
- Fungi like *Epidermophyton* cause skin infections but are not very dangerous for people with healthy immune systems. However if the immune system is damaged they can be life-threatening; for instance, *Pneumocystis jiroveci* is responsible for severe lung infections which occur in AIDS patients.

## Positive effects

On the other hand, ascus fungi have brought some important benefits to humanity.

- The most famous case may be that of the mould *Penicillium chrysogenum* (formerly *Penicillium notatum*), which, probably to attack competing bacteria, produces an antibiotic which, under the name of Penicillin, triggered a revolution in the treatment of bacterial infectious diseases in the 20th century.
- The medical importance of *Tolypocladium niveum* as an immunosuppressor can hardly be exaggerated. It excretes Cyclosporin, which, as well as being given during organ transplants to prevent rejection, is also prescribed for auto-immune diseases such as multiple sclerosis, although there is some doubt over the long-term side-effects of the treatment.



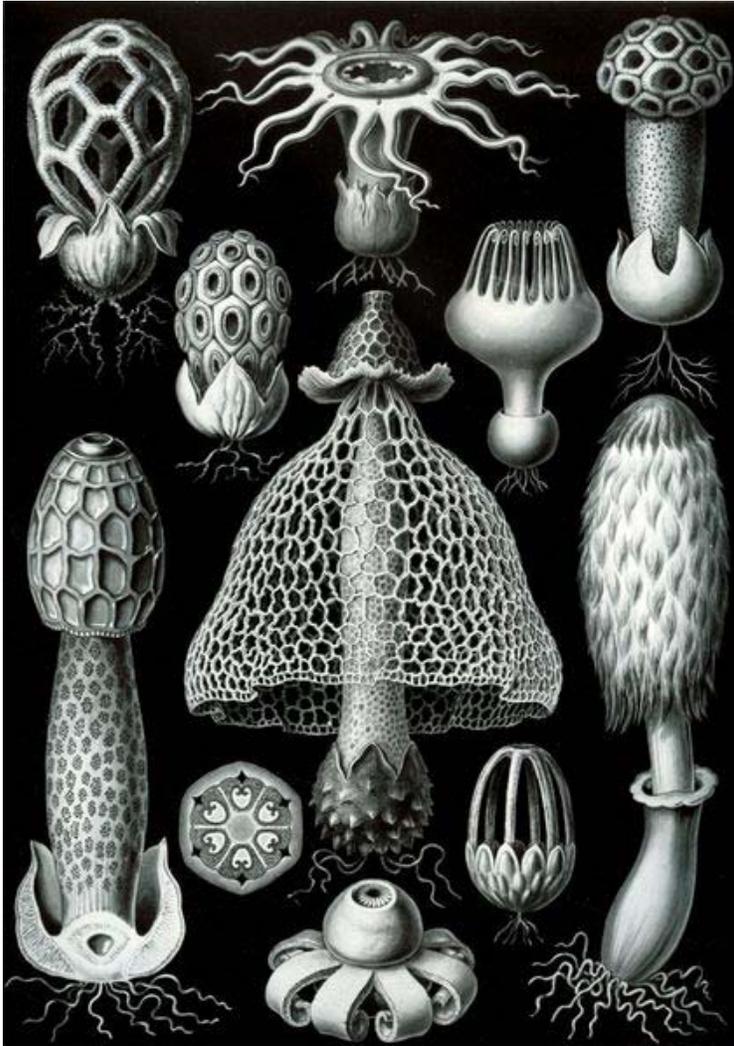
Stilton cheese veined with *Penicillium roqueforti*

- Some ascomycete fungi can be altered relatively easily through genetic engineering procedures. They can then produce useful proteins such as insulin, human growth hormone, or TPA, which is employed to dissolve blood clots.
- Several species are common model organisms in biology, including *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Neurospora crassa*. The genomes of a number of ascomycete fungi have been fully sequenced.
- Baker's Yeast (*Saccharomyces cerevisiae*) is used to make bread, beer and wine, during which process sugars such as glucose or sucrose are fermented to make alcohol and carbon dioxide. In the case of bread-making, the alcohol evaporates and the carbon dioxide serves to make the dough rise.
- Enzymes of *Penicillium camemberti* play a role in the manufacture of the cheeses Camembert and Brie, while those of *Penicillium roqueforti* do the same for Gorgonzola, Roquefort and Stilton.
- In Asia *Aspergillus oryzae* is added to a pulp of soaked soya beans to make soy sauce.
- Finally, some members of the Ascomycota are eaten with relish; morels (*Morchella*) and truffles (*Tuber*) are some of the most sought-after fungus delicacies.

## Chapter- 10

# Basidiomycota

### Basidiomycota



Basidiomycetes from Ernst Haeckel's 1904 *Kunstformen der Natur*

### Scientific classification

Domain: Eukarya  
Kingdom: Fungi  
Subkingdom: Dikarya  
Phylum: **Basidiomycota**  
R.T. Moore, 1980

### Subphyla/Classes

Agaricomycotina  
Pucciniomycotina  
Ustilaginomycotina  
*Incertae sedis* (no phylum)

Wallemiomycetes

**Basidiomycota** is one of two large phyla that, together with the Ascomycota, comprise the subkingdom Dikarya (often referred to as the "**higher fungi**") within the Kingdom Fungi. More specifically the Basidiomycota include mushrooms, puffballs, stinkhorns, bracket fungi, other polypores, jelly fungi, boletes, chanterelles, earth stars, smuts, bunts, rusts, mirror yeasts, and the human pathogenic yeast *Cryptococcus*. Basically, Basidiomycota are filamentous fungi composed of hyphae (except for those forming yeasts), and reproducing sexually via the formation of specialized club-shaped end cells called basidia that normally bear external meiospores (usually four). These specialized spores are called basidiospores. However, some Basidiomycota reproduce asexually, and may or may not also reproduce sexually. Asexually reproducing Basidiomycota (discussed below) can be recognized as members of this phylum by gross similarity to others, by the formation of a distinctive anatomical feature (the clamp connection - see below), cell wall components, and definitively by phylogenetic molecular analysis of DNA sequence data.

### **Classification**

The most recent classification adopted by a coalition of 67 mycologists recognizes three subphyla (Pucciniomycotina, Ustilaginomycotina, Agaricomycotina) and two other class level taxa (Wallemiomycetes, Entorrhizomycetes) outside of these, among the Basidiomycota. As now classified, the subphyla join and also cut across various obsolete taxonomic groups (see below) previously commonly used to describe various Basidiomycota. According to a 2008 estimate, Basidiomycota comprises three subphyla (including six unassigned classes) 16 classes, 52 orders, 177 families, 1,589 genera, and 31,515 species.

The Basidiomycota had traditionally been divided into two obsolete classes, the Homobasidiomycetes (including true mushrooms); and the Heterobasidiomycetes (the jelly, rust and smut fungi). Previously the entire Basidiomycota were called **Basidiomycetes**, an invalid class level name coined in 1959 as a counterpart to the **Ascomycetes**, when neither of these taxa were recognized as phyla. The terms basidiomycetes and ascomycetes are frequently used loosely to refer to Basidiomycota

and Ascomycota. They are often abbreviated to "basidios" and "ascos" as mycological slang.

## **Agaricomycotina**

The Agaricomycotina include what had previously been called the Hymenomycetes (an obsolete morphological based class of Basidiomycota that formed hymenial layers on their fruitbodies), the Gasteromycetes (another obsolete class that included species mostly lacking hymenia and mostly forming spores in enclosed fruitbodies), as well as most of the jelly fungi. The three classes in the Agaricomycotina are the Agaricomycetes, the Dacrymycetes, and the Tremellomycetes.

## **Pucciniomycotina**

The Pucciniomycotina includes the rust fungi, the insect parasitic/symbiotic genus *Septobasidium*, a former group of smut fungi (in the Microbotryomycetes, which includes mirror yeasts), and a mixture of odd, infrequently seen or seldom recognized fungi, often parasitic on plants. The eight classes in the Pucciniomycotina are Agaricostilbomycetes, Atractiellomycetes, Classiculomycetes, Cryptomycocolacomycetes, Cystobasidiomycetes, Microbotryomycetes, Mixiomycetes, and Pucciniomycetes.

## **Ustilaginomycotina**

The Ustilaginomycotina are most (but not all) of the former smut fungi and along with the Exobasidiales. The classes of the Ustilaginomycotina are the Exobasidiomycetes, the Entorrhizomycetes, and the Ustilaginomycetes.

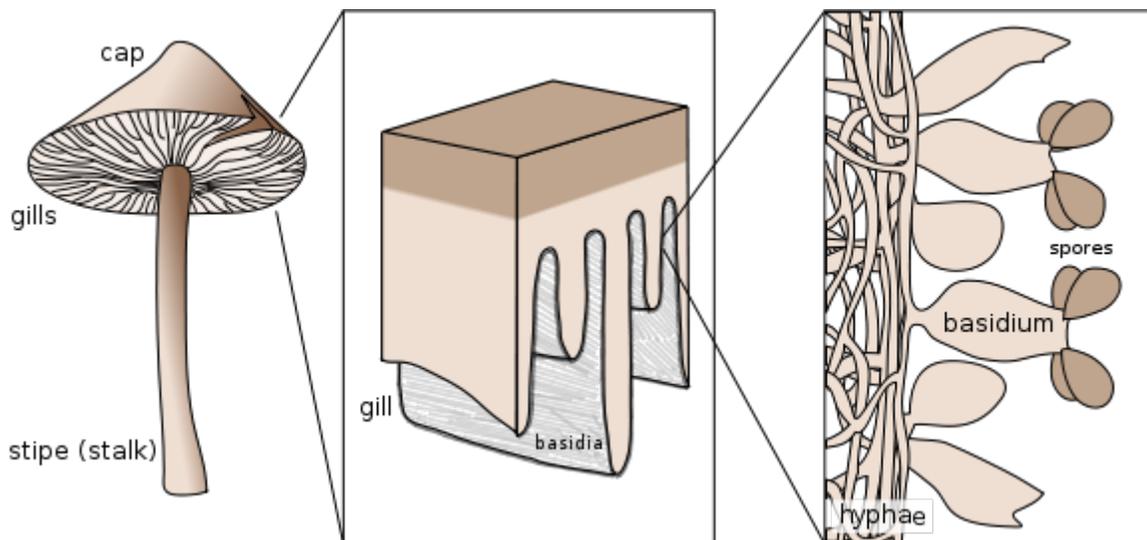
The class Wallemiomycetes is not yet placed in a subphylum.

## ***Typical life-cycle***

Unlike higher animals and plants which have readily recognizable male and female counterparts, Basidiomycota (except for the Rust (Pucciniales)) tend to have mutually indistinguishable, compatible haploids which are usually mycelia being composed of filamentous hyphae. Typically haploid Basidiomycota mycelia fuse via plasmogamy and then the compatible nuclei migrate into each other's mycelia and pair up with the resident nuclei. Karyogamy is delayed, so that the compatible nuclei remain in pairs, called a **dikaryon**. The hyphae are then said to be **dikaryotic**. Conversely, the haploid mycelia are called **monokaryons**. Often, the dikaryotic mycelium is more vigorous than the individual **monokaryotic** mycelia, and proceeds to take over the substrate in which they are growing. The dikaryons can be long-lived, lasting years, decades, or centuries. *The monokaryons are neither male nor female.* They have either a **bipolar (unifactorial)** or a **tetrapolar (bifactorial)** mating system. This results in the fact that following meiosis, the resulting haploid basidiospores and resultant monokaryons, have nuclei that are compatible with 50% (if bipolar) or 25% (if tetrapolar) of their sister basidiospores (and their resultant monokaryons) because the mating genes must differ for them to be

compatible. However, there are many variations of these genes in the population, and therefore, over 90% of monokaryons are compatible with each other. It is as if there were multiple sexes.

The maintenance of the dikaryotic status in dikaryons in many Basidiomycota is facilitated by the formation of clamp connections that physically appear to help coordinate and re-establish pairs of compatible nuclei following synchronous mitotic nuclear divisions. Variations are frequent and multiple. In a typical Basidiomycota lifecycle the long lasting dikaryons periodically (seasonally or occasionally) produce basidia, the specialized usually club-shaped end cells, in which a pair of compatible nuclei fuse (karyogamy) to form a diploid cell. Meiosis follows shortly with the production of 4 haploid nuclei that migrate into 4 external, usually apical basidiospores. Variations occur, however. Typically the basidiospores are ballistic, hence they are sometimes also called ballistospores. In most species, the basidiospores disperse and each can start a new haploid mycelium, continuing the lifecycle. Basidia are microscopic but they are often produced on or in multicelled large fructifications called basidiocarps or basidiomes, or fruitbodies), variously called mushrooms, puffballs, etc. Ballistic basidiospores are formed on **sterigmata** which are tapered spine-like projections on basidia, and are typically curved, like the horns of a bull. In some Basidiomycota the spores are not ballistic, and the sterigmata may be straight, reduced to stubbs, or absent. The basidiospores of these non-ballistosporic basidia may either bud off, or be released via dissolution or disintegration of the basidia.



Schematic of a typical basidiocarp, the diploid reproductive structure of a basidiomycete, showing fruiting body, hymenium and basidia.

In summary, meiosis takes place in a diploid basidium. Each one of the four haploid nuclei migrates into its own basidiospore. The basidiospores are ballistically discharged and start new haploid mycelia called monokaryons. There are no males or females, rather there are compatible thalli with multiple compatibility factors. Plasmogamy between compatible individuals leads to delayed karyogamy leading to establishment of a

dikaryon. The dikaryon is long lasting but ultimately gives rise to either fruitbodies with basidia or directly to basidia without fruitbodies. The paired dikaryon in the basidium fuse (i.e. karyogamy takes place). The diploid basidium begins the cycle again.

### **Variations in life-cycles**

Many variations occur. Some are self compatible and spontaneously form dikaryons without a separate compatible thallus being involved. These fungi are said to be *homothallic*, versus the normal *heterothallic* species with mating types. Others are **secondarily homothallic**, in that two compatible nuclei following meiosis migrate into each basidiospore, which is then dispersed as a pre-existing dikaryon. Often such species form only two spores per basidium, but that too varies. Following meiosis, mitotic divisions can occur in the basidium. Multiple numbers of basidiospores can result, including odd numbers via degeneration of nuclei, or pairing up of nuclei, or lack of migration of nuclei. For example, the chanterelle genus *Craterellus* often has 6-spored basidia, while some corticioid *Sistotrema* species can have 2-, 4-, 6-, or 8-spored basidia, and the cultivated button mushroom, *Agaricus bisporus*. can have 1-, 2-, 3- or 4-spored basidia under some circumstances. Occasionally monokaryons of some taxa can form morphologically fully formed basidiomes and anatomically correct basidia and ballistic basidiospores in the absence of dikaryon formation, diploid nuclei, and meiosis. A rare few number of taxa have extended diploid life-cycles, but can be common species. Examples exist in the mushroom genera *Armillaria* and *Xerula*, both in the Physalacriaceae. Occasionally basidiospores are not formed and parts of the "basidia" act as the dispersal agents, e.g. the peculiar mycoparasitic jelly fungus, *Tetragoniomyces* or the entire "basidium" acts as a "spore", e.g. in some false puffballs (*Scleroderma*). In the human pathogenic genus *Cryptococcus*, 4 nuclei following meiosis remain in the basidium but continually divide mitotically, each nucleus migrating into synchronously forming nonballistic basidiospores that are then pushed upwards by another set forming below them, resulting in 4 parallel chains of dry "basidiospores".

Other variations occur, some as standard life-cycles (that themselves have variations within variations) within specific orders.

### **Rusts**

Rusts (Pucciniales, previously known as Uredinales) at their greatest complexity produce five different types of spores on two different hosts in two unrelated host families. Such rusts are heteroecious (requiring 2 hosts) and macrocyclic (producing all 5 spores types). Wheat stem rust is an example. By convention the stages and spore states are numbered by Roman numerals. Typically, basidiospores infect host one, the mycelium forms pycnidia, called spermagonia, which are miniature, flask-shaped, hollow, submicroscopic bodies embedded in host tissue (such as a leaf). This stage, numbered "0", produces single-celled, minute spores that ooze out in a sweet liquid and that act as nonmotile spermatia, and also protruding receptive hyphae. Insects and probably other vectors such as rain carry the spermatia from spermagonia to spermagonia, cross inoculating the mating types. Neither thallus is male or female. Once crossed, the dikaryons are

established and a second spore stage is formed, numbered "I" and called aecia, which form dikaryotic aeciospores in dry chains in inverted cup-shaped bodies embedded in host tissue. These aeciospores then infect the second host genus and cannot infect the host on which they are formed (in macrocyclic rusts). On the second host a repeating spore stage is formed, numbered "II", the urediospores in dry pustules called uredinia. Urediospores are dikaryotic and can infect the same host that produced them. They repeatedly infect this host over the growing season. At the end of the season, a fourth spore type, the teliospore, is formed. It is thicker-walled and serves to overwinter or to survive other harsh conditions. It does not continue the infection process, rather it remains dormant for a period and then germinates to form basidia (stage "IV"), sometimes called a promycelium. In the Pucciniales, the basidia are cylindrical and become 3-septate after meiosis, with each of the 4 cells bearing one basidiospore each. The basidiospores disperse and start the infection process on host 1 again. Autoecious rusts complete their life-cycles on one host instead of two, and **microcyclic** rusts cut out one or more stages.

## Smuts

The characteristic part of the life-cycle of smuts is the thick-walled, often darkly pigmented, ornate, teliospore that serves to survive harsh conditions such as overwintering and also serves to help disperse the fungus as dry diaspores. The teliospores are initially dikaryotic but become diploid via karyogamy. Meiosis takes place at the time of germination. A promycelium is formed that consists to a short hypha (equated to a basidium). In some smuts such as *Ustilago maydis* the nuclei migrate into the promycelium that becomes septate, and haploid yeast-like conidia/basidiospores sometimes called sporidia, bud off laterally from each cell. In various smuts, the yeast phase may proliferate, or they may fuse, or they may infect plant tissue and become hyphal. In other smuts, such as *Tilletia caries*, the elongated haploid basidiospores form apically, often in compatible pairs that fuse centrally resulting in "H"-shaped diaspores which are by then dikaryotic. Dikaryotic conidia may then form. Eventually the host is infected by infectious hyphae. Teliospores form in host tissue. Many variations on these general themes occur.

Smuts with both a yeast phase and an infectious hyphal state are examples of dimorphic Basidiomycota. In plant parasitic taxa, the saprotrophic phase is normally the yeast while the infectious stage is hyphal. However, there are examples of animal and human parasites where the species are dimorphic but it is the yeast-like state that is infectious. The genus *Filobasidiella* forms basidia on hyphae but the main infectious stage is more commonly known by the anamorphic yeast name *Cryptococcus*, e.g. *Cryptococcus neoformans* and *Cryptococcus gattii*.

The dimorphic Basidiomycota with yeast stages and the pleiomorphic rusts are examples of fungi with anamorphs, which are the asexual stages. Some Basidiomycota are only known as anamorphs. Many are yeasts, collectively called basidiomycetous yeasts to differentiate them from ascomycetous yeasts in the Ascomycota. Aside from yeast anamorphs, and uredinia, aecia and pycnidia, some Basidiomycota form other distinctive

anamorphs as parts of their life-cycles. Examples are *Collybia tuberosa* with its apple-seed-shaped and coloured sclerotium, *Dendrocollybia racemosa* with its sclerotium and its *Tilachlidiopsis racemosa* conidia, *Armillaria* with their rhizomorphs, *Hohenbuehelia* with their *Nematoctonus* nematode infectious state and the coffee leaf parasite, *Mycena citricolor* and its *Decapitatus flavidus* propagules called gemmae.

## Chapter- 11

# Banana Diseases

## Black sigatoka

*Mycosphaerella fijiensis*

### Scientific classification

Kingdom: Fungi  
Division: Ascomycota  
Subdivision: Pezizomycotina  
Class: Dothideomycetes  
Order: Mycosphaerellales  
Genus: *Mycosphaerella*  
Species: *M. fijiensis*

### Binomial name

*Mycosphaerella fijiensis*  
Morelet

**Black Sigatoka** is a leaf spot disease of banana plants caused by ascomycete fungus *Mycosphaerella fijiensis* (Morelet). Plants with leaves damaged by the disease may have up to 50% lower yield of fruit. Black Sigatoka, also known as **black leaf streak**, was named for its similarities with the Yellow Sigatoka caused by *Mycosphaerella musicola* (Mulder), after the Sigatoka Valley in Fiji where an outbreak of this disease reached epidemic proportions from 1912 to 1923.

*M. fijiensis* reproduces both sexually and asexually, and both conidia and ascospores are important in its dispersal. The conidia are mainly water-borne to short distances, while ascospores are carried by wind to more remote places (the distances being limited by their susceptibility to ultraviolet light). Over sixty distinct strains with different pathogenic potentials have been isolated. In order to better understand the mechanisms of its variability, the "Genetic diversity of *Mycosphaerella fijiensis* Project" has been initiated.

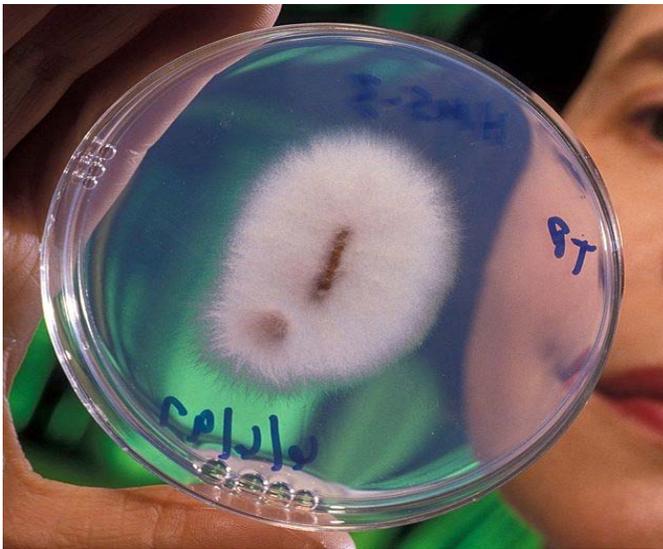
When spores of *M. fijiensis* are deposited on a susceptible banana leaf they germinate within three hours if there is a film of water present or if the humidity is very high. The optimal temperature for germination of the conidia is 27°C. The germ tube grows epiphytically over the epidermis for two to three days before penetrating the leaf via a stoma. Once inside the leaf the invasive hypha forms a vesicle and fine hyphae grow through the mesophyll layers into an air chamber. More hyphae then grow into the palisade tissue and continue on into other air chambers, eventually emerging through stomata in the streak that has developed. Further epiphytic growth occurs before the re-entry of the hypha into the leaf through another stoma repeats the process. The optimal conditions for *M. fijiensis* as compared with *M. musicola* are a higher temperatures and higher relative humidity and the whole disease cycle is much faster in *M. fijiensis*.

In commercial export plantations, Black Sigatoka is controlled by frequent applications of fungicides. Removal of affected leaves, good drainage, and sufficient spacing also help to fight the disease. Although fungicides improved over the years, the pathogen developed resistance. Therefore higher frequency of application is required, increasing the impact on the environment and health of the banana workers.

Small farmers growing bananas for local market cannot afford expensive measures to fight the disease. However, some cultivars of banana are resistant to the disease. Research is done to improve productivity and fruit properties of these cultivars. A genetically modified banana variety made more resistant to the fungus has recently been developed and will be soon field tested in Uganda.

## **Fusarium oxysporum f.sp. cubense**

### *Fusarium oxysporum* f.sp. *cubense*



Plant-pathogenic strain of *Fusarium oxysporum*

### **Scientific classification**

Kingdom: Fungi

Phylum: Ascomycota  
Class: Sordariomycetes  
Order: Hypocreales  
Family: Nectriaceae  
Genus: *Fusarium*  
Species: ***F. oxysporum f.sp. cubense***

#### Binomial name

***Fusarium oxysporum f.sp. cubense***  
(E.F.Sm.) W.C.Snyder & H.N.Hansen (1940)

#### Synonyms

*Fusarium cubense* E.F.Sm. (1910)  
*Fusarium oxysporum* var. *cubense* (E.F.Sm.) Wollenw.  
(1935)  
*Fusarium oxysporum* f. *cubense* (E.F.Sm.) W.C.Snyder &  
H.N.Hansen (1940)

***Fusarium oxysporum f.sp. cubense*** is a fungal plant pathogen that causes **Panama disease** of banana (*Musa* spp.), also known as **fusarium wilt of banana**.

## Overview

Although fruit of the wild banana, *Musa balbisiana* have large, hard seeds, these have been bred out of the modern culinary banana which is seedless. Banana plants are therefore propagated asexually from offshoots. Because these rhizomes are usually free of symptoms even when the plant is infected by *F. oxysporum* f. sp. *cubense*, they are a common means by which this pathogen is disseminated. It can also be spread in soil and running water, on farm implements or machinery.

Panama disease is one of the most destructive plant diseases of modern times. It is believed to have originated in Southeast Asia and was first reported in Australia in 1876. By 1950 it had spread to all the banana-producing regions of the world with the exception of some islands in the South Pacific, the Mediterranean, Melanesia and Somalia.

Panama disease affects a wide range of banana cultivars; however, it is best known for the damage it caused to a single cultivar in the early export plantations. Before 1960, a total reliance was put on the cultivar 'Gros Michel', and it supplied almost all the export trade. It proved susceptible to the disease and the use of infected rhizomes to establish new plantations caused widespread and severe losses. Some indication of the scale of the losses is demonstrated by the complete eradication of production on 30,000 hectares of plantation in the Ulua Valley of Honduras between 1940 and 1960. In Suriname, an entire operation of 4,000 hectares was out of business within eight years and in the Quepos area of Costa Rica, 6,000 hectares were destroyed in twelve years.



A Cavendish banana

By the mid-1900s, resistant cultivars in the 'Cavendish' subgroup were being used as a substitute for 'Gros Michel' in the export trade. These cultivars have proved resilient and grow well in the western tropics, remaining the clones on which the current export trades are based. Unfortunately, in several growing areas in the Eastern Hemisphere these cultivars are susceptible to the disease. It is considered inevitable that this susceptibility will spread to the Western Hemisphere, and this poses a significant threat to production because there are currently no acceptable replacement cultivars. To make things worse, this variant of the pathogen also affects plantains, *Musa acuminata* × *balbisiana*, which are an important staple food in tropical regions of the world. The average American eats 26.2 pounds of the Cavendish banana each year and the question is being asked as to whether this oft-consumed fruit is on course to extinction.

Apart from the export trade, 85% of banana production is for local consumption and many of the cultivars used for this purpose are also susceptible to infection.

## **Description**

*Fusarium oxysporum* is a common inhabitant of soil and produces three types of asexual spores; macroconidia, microconidia and chlamydospores.

The macroconidia are nearly straight, slender and thin-walled. They usually have three or four septa, a foot-shaped basal cell and a curved and tapered apical cell. They are generally produced from phialides on conidiophores by basipetal division. They are important in secondary infection.

The microconidia are ellipsoidal and have either a single septum or none at all. They are formed from phialides in false heads by basipetal division. They are important in secondary infection.

The chlamydospores are globose with thick walls. They are either formed from hyphae or by the modification of hyphal cells. They endure in soils for long periods and act as inocula in primary infection.

The macroconidia and chlamydospores are normally only formed on dead or dying host plants. Chlamydospores are the most significant survival structures of this pathogen.

The teleomorph or sexual reproductive stage of *F. oxysporum* is unknown.

Four races of this pathogen have been described which attack different banana cultivars:

- Race 1 attacks cultivars in the *Musa* (AAA group) 'Gros Michel' and caused the 20th century epidemic. It also attacks *Musa* (AAB group) 'Pome' and its subgroups, *Musa* (AAB group) 'Silk' and *Musa* (ABB group) 'Pisang Awak'.
- Race 2 attacks *Musa* (ABB group) 'Bluggoe' and its close relatives.
- Race 3 attacks *Heliconia* spp.
- Race 4 attacks *Musa* (AAA group) 'Dwarf Cavendish' as well as the hosts of races 1 and 2.

## **Symptoms**

Infection by *Fusarium oxysporum f.sp. cubense* triggers the self-defense mechanisms of the host plant causing the secretion of a gel. This is followed by the formation of tylose in the vascular vessels which blocks the movement of water and nutrients to the upper parts of the plant. The tips of the feeder roots are the initial sites of infection which then moves on to the rhizome. The signs of the disease are most noticeable as a dark stain where the stele joins the cortex. As the disease develops, large portions of the xylem turn a reddish-brown colour. Externally, the oldest leaves start turning yellow and there is often a longitudinal splitting of the lower part of the outer leaf sheaths on the pseudostem. The leaves begin to wilt and may buckle at the base of the petiole. As the disease progresses, younger leaves are affected, turn yellow and crumple and the whole canopy begins to consist of dead or dying leaves.

## **Management**

There are few effective options for managing Panama disease as fungicides are largely ineffective. Chemical sterilisation of the soil with methyl bromide significantly reduced incidence of the disease but was found to be effective for only three years after which the pathogen had recolonised the fumigated areas. Injecting the host plants with carbendazim and potassium phosphonate appears to provide some control but results have been inconclusive. Heat treatment of soil has also been tried in the Philippines but the pathogen is likely to reinvade the treated area. The greatest hope for managing this disease in infested soils is the development of genetic modifications that will provide resistant cultivars. Modified bananas developed in collaboration by Ugandan and Belgian scientists were reported in 2008 to be being grown experimentally in Uganda.

## **Research**

Much research is being undertaken because of the urgency in formulating effective control methods for Panama disease and breeding resistant banana cultivars. Researchers at University Sains, Malaysia are examining variability in the genome of the pathogen and its genetic variability is being studied, as are the evolutionary relationships within vegetative compatibility groups of the pathogen. The resistance of different banana cultivars to the pathogen is under scrutiny.

Research into the phylogenetic relationships among the different strains of *F. oxysporum* that cause wilt of banana has been undertaken to determine whether the strains that are specific to the banana have descended from a common ancestor or have developed independently. Results of this study show that it is not monophyletic and appears to have multiple evolutionary origins.

## **Panama disease**

**Panama disease**, a Fusarium wilt, is a banana plant disease caused by the fungus *Fusarium oxysporum*. The fungus attacks the roots of the banana plant. The disease is resistant to fungicide and cannot be controlled chemically.

## **History**

Gros Michel or 'Big Mike' was an early export cultivar of banana. This cultivar was wiped out by Panama disease in the 1950s. The disease first appeared in Suriname, then made its way to the Caribbean, and, by the 1920s, to Honduras, the world's largest producer of bananas at the time. Although there are many banana cultivars, Gros Michel was especially suitable for export to non-tropical nations. A search for a substitute located the Vietnamese Cavendish cultivar, which is resistant to the disease. However, more care is required for shipping the Cavendish banana, and its quality compared to Gros Michel is debated.

Recently, a new strain called 'tropical race four Panama disease' has begun to attack Cavendish banana plants in south Asia. Given the high volume of modern international trade, banana producers expect this strain to spread through Africa and into South America and the Caribbean.

Plant breeders and geneticists are trying to develop new cultivars that are resistant to this new strain of Panama disease. Unfortunately, such efforts are progressing slowly because the triploid banana cultivars selected for human consumption are seedless and reproduce asexually, which decreases genetic variation and makes breeding difficult.

## Chapter- 12

# Citrus Canker

### *Xanthomonas axonopodis*

#### Scientific classification

Kingdom: Bacteria  
Phylum: Proteobacteria  
Class: Gamma  
Proteobacteria  
Order: Xanthomonadales  
Family: Xanthomonadaceae  
Genus: *Xanthomonas*  
Species: *X. axonopodis*

#### Binomial name

*Xanthomonas axonopodis*  
(Hasse, 1915)

#### Synonyms

*Pseudomonas citrii*  
*Xanthomonas campestris* pv.  
*citri*  
*Xanthomonas citri*

**Citrus canker** is a disease affecting citrus species that is caused by the bacterium *Xanthomonas axonopodis*. Infection causes lesions on the leaves, stems, and fruit of citrus trees, including lime, oranges, and grapefruit. While not harmful to humans, canker significantly affects the vitality of citrus trees, causing leaves and fruit to drop prematurely; a fruit infected with canker is safe to eat but too unsightly to be sold.

The disease, which is believed to have originated in South East Asia, is extremely persistent when it becomes established in an area. Citrus groves have been destroyed in attempts to eradicate the disease. Australia, Brazil and the United States are currently suffering from canker outbreaks.

## **Biology**

*Xanthomonas axonopodis* is a rod shaped gram negative bacterium with polar flagella. The bacterium has a genome length approximately 5 Mbp. There are a number of types of citrus canker disease caused by different pathovars and variants of the bacterium:

- The Asiatic type of canker (Canker A), *X. axonopodis* pv. *citri*, caused by a group of strains originally found in Asia, is the most widespread and severe form of the disease.
- Cancrosis B, caused by a group of *X. axonopodis* pv. *aurantifolii* strains originally found in South America is a disease of lemons, key lime, bitter orange, and pomelo.
- Cancrosis C, also caused by strains within *X. axonopodis* pv. *aurantifolii*, only infects key lime and bitter orange.
- A\* strains, discovered in Oman, Saudi Arabia, Iran, and India, only infects key lime.

## **Pathology**



Lesions on leaves

Plants infected with citrus canker have characteristic lesions on leaves, stems, and fruit with raised, brown, water-soaked margins, usually with a yellow halo or ring effect around the lesion. Older lesions have a corky appearance, still in many cases retaining the halo effect. The bacterium propagates in lesions in leaves, stems, and fruit. The lesions ooze bacterial cells that, when dispersed by windblown rain, can spread to other plants in the area. Infection may spread further by hurricanes. The disease can also be spread by from contaminated equipment, and by transport of infected or apparently healthy plants. Due to latency of the disease, a plant may appear to be healthy, but actually be infected.

Citrus canker bacteria can enter through a plant's stomata or through wounds on leaves or other green parts. In most cases, younger leaves are considered to be the most susceptible. Also, damage caused by Citrus Leaf Miner larvae (*Phyllocnistis citrella*) can be sites for infection to occur. Within a controlled laboratory setting, symptoms can appear in 14 days following inoculation into a susceptible host. In the field environment, the time for symptoms to appear and be clearly discernible from other foliar diseases varies; it may be on the order of several months after infection. Lower temperature increases the latency of the disease. Citrus canker bacteria can stay viable in old lesions and other plant surfaces for several months.



Citrus canker lesions on fruit

## Detection

The disease can be detected in groves and on fruit by the appearance of lesions. Early detection is critical in quarantine situations. Bacteria can be tested for pathogenicity by inoculating multiple citrus species with the bacterium. Additional diagnostic tests

(antibody detection, fatty-acid profiling, and genetic procedures using polymerase chain reaction (PCR) can be conducted to confirm diagnosis and may help to identify the particular canker strain.

## Susceptibility

All species and varieties of citrus have not been tested for citrus canker. Most of the common species and varieties of citrus are susceptible to citrus canker. Some species are more susceptible than others, while a few species are resistant to infection.

Susceptibility	Variety
Highly susceptible	Grapefruit ( <i>Citrus x paradisi</i> ), Key lime ( <i>C. aurantiifolia</i> ), Pointed leaf Hystrich ( <i>C. hystrich</i> ), lemon ( <i>C. limon</i> )
Susceptible	Limes ( <i>C. latifolia</i> ) including Tahiti lime, Palestine sweet lime; Trifoliolate orange ( <i>Poncirus trifoliata</i> ); Citranges/Citrumelos ( <i>P. trifoliata</i> hybrids); Tangerines, Tangors, Tangelos ( <i>C. reticulata</i> hybrids); Sweet oranges ( <i>C. sinensis</i> ); Bitter oranges ( <i>C. aurantium</i> )
Resistant	Citron ( <i>C. medica</i> ), Mandarins ( <i>C. reticulata</i> )
Highly resistant	Calamondin ( <i>X Citrofortunella</i> ), Kumquat ( <i>Fortunella spp.</i> )

## Management

Citrus canker outbreaks are prevented and managed in a number of ways. In countries that do not have canker, the disease is prevented from entering the country by quarantine measures. In countries with new outbreaks, eradication programs that are started soon after the disease has been discovered have been successful; such programs rely on destruction of affected groves. When eradication has been unsuccessful and the disease has become established, management options include replacing susceptible citrus cultivars with resistant cultivars, applying preventive sprays of copper-based bactericides, and destroying infected trees and all surrounding trees within an appropriate radius.

## ***Distribution and economic impact***

Citrus canker is thought to originate in the area of Southeast Asia-India. It is now also present in Japan, South and Central Africa, the Middle East, Bangladesh, the Pacific Islands, some countries in South America, and Florida. Some areas of the world have eradicated citrus canker and others have ongoing eradication programs, but the disease remains endemic in most areas where it has appeared. Because of its rapid spread, high potential for damage, and impact on export sales and domestic trade, citrus canker is a significant threat to all citrus-growing regions.

### **Australia**

The citrus industry is the largest fresh-fruit exporting industry in Australia. Australia has had three outbreaks of citrus canker; two were successfully eradicated and one is ongoing. The disease was found twice during the 1900s in the Northern Territory and was eradicated each time. In 2004 an unexplained outbreak occurred in Central Queensland. The state and federal governments ordered that all commercial groves, all non-commercial citrus trees, and all native lime trees (*C. glauca*) in the vicinity of Emerald be destroyed rather than trying to isolate infected trees. Eradication was successful with permission to replant being granted to farmers by the biosecurity unit of the Queensland Department of Primary Industries in early 2009.

### **Brazil**

Citrus is an important domestic and export crop for Brazil. Citrus agriculture is the second most important agricultural activity in the Brazilian state of São Paulo, the largest sweet orange production area in the world. There are over 100,000 groves in São Paulo, and the area planted with citrus is increasing. Of the ~2 million trees, greater than 80% of the trees are a single variety of orange, and the remainder is made up of tangerine and lemon trees. Because of the uniformity in citrus variety the state has been adversely affected by canker, causing crop and monetary losses. In Brazil rather than destroying entire groves to eradicate the disease, contaminated trees and trees within a 30 m radius are destroyed, up to 1998 over half a million trees had been destroyed.

### **United States**

Citrus canker was first found in the United States in 1910 not far from the Georgia - Florida border. Subsequently, canker was discovered in 1912 in Dade County, more than 400 miles (600 km) away. Beyond Florida, the disease was discovered in the Gulf States and reached as far north as South Carolina. It took more than 20 years to eradicate that outbreak of citrus canker, from 1914 through 1931, \$2.5 million in state and private funds were spent to control it—a sum equivalent to \$28 million in 2000 dollars. In 26 counties, some 257,745 grove trees and 3,093,110 nursery trees were destroyed by burning. Citrus Canker was detected again on the Gulf Coast of Florida in 1986 and declared eradicated in 1994.

The most recent outbreak of citrus canker was discovered in Florida in 1995. Despite eradication attempts, by late 2005 the disease had been discovered in many places distant from the original discovery, for example, in Orange Park, FL, 315 miles (500 km) away. In January 2000, the Florida Department of Agriculture adopted a policy of removing all infected trees and all citrus trees within a 1900 ft radius of an infected tree in both residential areas and commercial groves. Previous to this eradication policy, the department eradicated all citrus trees within 125 ft of an infected one. The program ended in January 2006 following a statement from the USDA that eradication was infeasible.

## Chapter- 13

# Plant Disease Resistance

**Plant disease resistance** is crucial to the reliable production of food, and it provides significant reductions in agricultural use of fuel, land, water and other inputs. There are numerous examples of devastating plant disease impacts, as well as recurrent severe plant disease issues. However, disease control measures are reasonably successful for most crops. Across large regions and many crop species, it is estimated that diseases typically reduce plant yields by 10% every year in more developed settings, but yield loss to diseases often exceeds 20% in less developed settings.

Plant disease resistance derives both from **pre-formed defenses** and from **infection-induced responses** mediated by the plant immune system. Relative to a disease-susceptible plant, **disease resistance** is often defined as reduction of pathogen growth on or in the plant, while the term **disease tolerance** describes plants that exhibit less disease damage despite similar levels of pathogen growth. Disease outcome is determined by the three-way interaction of the pathogen, the plant, and the environmental conditions (an interaction known as the **disease triangle**). Defense-activating compounds can move cell-to-cell and systemically through the plant vascular system, but plants do not have circulating immune cells so most cell types in plants retain the capacity to express a broad suite of antimicrobial defenses. Although obvious *qualitative* differences in disease resistance can be observed when some plants are compared (allowing classification as “resistant” or “susceptible” after infection by the same pathogen strain at similar pathogen inoculum levels in similar environments), a gradation of *quantitative* differences in disease resistance is more typically observed between plant lines or genotypes. Plants are almost always resistant to certain pathogens but susceptible to other pathogens; resistance is usually pathogen species-specific or pathogen strain-specific.

Note that plant defense against herbivory (plant resistance to insect pests) exhibits some mechanistic similarities to, but also differences from, plant disease resistance (plant resistance to microscopic organisms).

## ***Common Mechanisms of Plant Disease Resistance***

### **Pre-formed structures and compounds that contribute to resistance**

- Plant cuticle/surface
- Plant cell walls
- Antimicrobial chemicals (for example: glucosides, saponins)
- Antimicrobial proteins
- Enzyme inhibitors
- Detoxifying enzymes that break down pathogen-derived toxins
- Receptors that perceive pathogen presence and activate inducible plant defenses

### **Inducible plant defenses that are generated after infection**

- Cell wall reinforcement (callose, lignin, suberin, cell wall proteins)
- Antimicrobial chemicals (including reactive oxygen species such as hydrogen peroxide, or peroxynitrite, or more complex phytoalexins such as genistein or camalexin)
- Antimicrobial proteins such as defensins, thionins, or PR-1
- Antimicrobial enzymes such as chitinases, beta-glucanases, or peroxidases
- Hypersensitive response - a rapid host cell death response associated with defense mediated by “Resistance genes.”

## ***Plant Immune Systems and Plant Defense Signal Transduction***

Plant immune systems show some mechanistic similarities and apparent common origin with the immune systems of insects and mammals, but also exhibit many plant-specific characteristics. As in most cellular responses to the environment, defenses are activated when receptor proteins directly or indirectly detect pathogen presence and trigger ion channel gating, oxidative burst, cellular redox changes, protein kinase cascades, and/or other responses that either directly activate cellular changes (such as cell wall reinforcement), or activate changes in gene expression that then elevate plant defense responses.

Plants, like animals, have a basal immune system that includes a small number of pattern recognition receptors that are specific for broadly conserved microbe-associated molecular patterns (MAMPs, also called pathogen-associated molecular patterns or PAMPs). Examples of these microbial compounds that elicit plant basal defense include bacterial flagellin or lipopolysaccharides, or fungal chitin. The defenses induced by MAMP perception are sufficient to repel most potentially pathogenic microorganisms. However, pathogens express effector proteins that are adapted to allow them to infect certain plant species; these effectors often enhance pathogen virulence by suppressing basal host defenses.

Importantly, plants have evolved R genes (resistance genes) whose products allow recognition of specific pathogen effectors, either through direct binding of the effector or

by recognition of the alteration that the effector has caused to a host protein. R gene products control a broad set of disease resistance responses whose induction is often sufficiently rapid and strong to stop adapted pathogens from further growth or spread. Plant genomes each contain a few hundred apparent R genes, and the R genes studied to date usually confer specificity for particular strains of a pathogen species. As first noted by Harold Flor in the mid-20th century in his formulation of the gene-for-gene relationship, the plant R gene and the pathogen “avirulence gene” (effector gene) must have matched specificity for that R gene to confer resistance. The presence of an R gene can place significant selective pressure on the pathogen to alter or delete the corresponding avirulence/effector gene. Some R genes show evidence of high stability over millions of years while other R genes, especially those that occur in small clusters of similar genes, can evolve new pathogen specificities over much shorter time periods.

The use of receptors carrying leucine-rich repeat (LRR) pathogen recognition specificity domains is common to plant, insect, jawless vertebrate and mammal immune systems, as is the presence of Toll/Interleukin receptor (TIR) domains in many of these receptors, and the expression of defensins, thionins, oxidative burst and other defense responses.

Some of the key endogenous chemical mediators of plant defense signal transduction include salicylic acid, jasmonic acid or jasmonate, ethylene, reactive oxygen species, and nitric oxide. Numerous genes and/or proteins have been identified that mediate plant defense signal transduction. Cytoskeleton and vesicle trafficking dynamics help to target plant defense responses asymmetrically within plant cells, toward the point of pathogen attack.

Plant immune systems can also respond to an initial infection in one part of the plant by physiologically elevating the capacity for a successful defense response in other parts of the plant. These responses include systemic acquired resistance, largely mediated by salicylic acid-dependent pathways, and induced systemic resistance, largely mediated by jasmonic acid-dependent pathways. Against viruses, plants often induce pathogen-specific gene silencing mechanisms mediated by RNA interference. These are primitive forms of adaptive immunity.

In a small number of cases, plant genes have been identified that are broadly effective against an entire pathogen species (against a microbial species that is pathogenic on other genotypes of that host species). Examples include barley MLO against powdery mildew, wheat Lr34 against leaf rust, and wheat Yr36 against stripe rust. An array of mechanisms for this type of resistance may exist depending on the particular gene and plant-pathogen combination. Other reasons for effective plant immunity can include a relatively complete lack of coadaptation (the pathogen and/or plant lack multiple mechanisms needed for colonization and growth within that host species), or a particularly effective suite of pre-formed defenses (see above).

## ***Plant Breeding for Disease Resistance***

Plant breeders focus a significant part of their effort on selection and development of disease-resistant plant lines. Plant diseases can also be partially controlled by use of pesticides, and by cultivation practices such as crop rotation, tillage, planting density, purchase of disease-free seeds and cleaning of equipment, but plant varieties with inherent (genetically determined) disease resistance are generally the first choice for disease control. Breeding for disease resistance has been underway since plants were first domesticated, but it requires continual effort. This is because pathogen populations are often under natural selection for increased virulence, new pathogens can be introduced to an area, cultivation methods can favor increased disease incidence over time, changes in cultivation practice can favor new diseases, and plant breeding for other traits can disrupt the disease resistance that was present in older plant varieties. A plant line with acceptable disease resistance against one pathogen may still lack resistance against other pathogens.

### **Plant breeding for disease resistance typically includes:**

- Identification of resistant breeding sources (plants that may be less desirable in other ways, but which carry a useful disease resistance trait). Ancient plant varieties and wild relatives are very important to preserve because they are the most common sources of enhanced plant disease resistance.
- Crossing of a desirable but disease-susceptible plant variety to another variety that is a source of resistance, to generate plant populations that mix and segregate for the traits of the parents.
- Growth of the breeding populations in a disease-conducive setting. This may require artificial inoculation of pathogen onto the plant population. Careful attention must be paid to the types of pathogen isolates that are present, as there can be significant variation in the effectiveness of resistance against different isolates of the same pathogen species.
- Selection of disease-resistant individuals. Breeders are trying to sustain or improve numerous other plant traits related to plant yield and quality, including other disease resistance traits, while they are breeding for improved resistance to any particular pathogen.

Each of the above steps can be difficult to successfully accomplish, and many highly refined methods in plant breeding and plant pathology are used to increase the effectiveness and reduce the cost of resistance breeding.

Resistance is termed *durable* if it continues to be effective over multiple years of widespread use, but some resistance “breaks down” as pathogen populations evolve to overcome or escape the resistance. Resistance that is specific to certain races or strains of a pathogen species is often controlled by single R genes and can be less durable; *broad-spectrum resistance* against an entire pathogen species is often quantitative and only incompletely effective, but more durable, and is often controlled by many genes that segregate in breeding populations. However, there are numerous exceptions to the above

generalized trends, which were given the names vertical resistance and horizontal resistance, respectively, by J.E. Vanderplank.

Crops such as potato, apple, banana and sugarcane are often propagated by vegetative reproduction to preserve highly desirable plant varieties, because for these species, outcrossing seriously disrupts the preferred plant varieties. Vegetatively propagated crops may be among the best targets for resistance improvement by the biotechnology method of plant transformation to add individual genes that improve disease resistance without causing large genetic disruption of the preferred plant varieties.

### ***Host Range***

There are thousands of species of plant pathogenic microorganisms, but only a small minority of these pathogens have the capacity to infect a broad range of plant species. Most pathogens instead exhibit a high degree of host-specificity. Non-host plant species are often said to express *non-host resistance*. The term *host resistance* is used when a pathogen species can be pathogenic on the host species but certain strains of that plant species resist certain strains of the pathogen species. There can be overlap in the causes of host resistance and non-host resistance. Pathogen host range can change quite suddenly if, for example, the capacity to synthesize a host-specific toxin or effector is gained by gene shuffling/mutation, or by horizontal gene transfer from a related or relatively unrelated organism.

### ***Epidemics and Population Biology***

Plants in native populations are often characterized by substantial genotype diversity and dispersed populations (growth in a mixture with many other plant species). They also have undergone millions of years of plant-pathogen coevolution. Hence as long as novel pathogens are not introduced from other parts of the globe, natural plant populations generally exhibit only a low incidence of severe disease epidemics. In agricultural systems, humans often cultivate single plant species at high density, with numerous fields of that species in a region, and with significantly reduced genetic diversity both within fields and between fields. In addition, rapid travel of people and cargo across large distances increases the risk of introducing pathogens against which the plant has not been selected for resistance. These factors make modern agriculture particularly prone to disease epidemics. Common solutions to this problem include constant breeding for disease resistance, use of pesticides to suppress recurrent potential epidemics, use of border inspections and plant import restrictions, maintenance of significant genetic diversity within the crop gene pool, and constant surveillance for disease problems to facilitate early initiation of appropriate responses. Some pathogen species are known to have a much greater capacity to overcome plant disease resistance than others, often because of their ability to evolve rapidly and to disperse broadly.

## Chapter- 14

# Crop Rotation



Satellite image of circular crop fields in Haskell County, Kansas in late June 2001. Healthy, growing crops are green. Corn would be growing into leafy stalks by then.

Sorghum, which resembles corn, grows more slowly and would be much smaller and therefore, (possibly) paler. Wheat is a brilliant gold as harvest occurs in June. Fields of brown have been recently harvested and plowed under or lie fallow for the year.

**Crop rotation** is the practice of growing a series of dissimilar types of crops in the same area in sequential seasons for various benefits such as to avoid the build up of pathogens and pests that often occurs when one species is continuously cropped. Crop rotation also seeks to balance the fertility demands of various crops to avoid excessive depletion of soil nutrients. A traditional element of crop rotation is the replenishment of nitrogen through the use of green manure in sequence with cereals and other crops. It is one component of polyculture. Crop rotation can also improve soil structure and fertility by alternating deep-rooted and shallow-rooted plants.

### ***Method and purpose***



Effects of crop rotation and monoculture: On the left field, the "Norfolk" crop rotation sequence (potatoes, oats, peas, rye) is being applied; on the right field, rye has been grown for 45 years in a row.

Crop rotation avoids a decrease in soil fertility, as growing the same crop in the same place for many years in a row disproportionately depletes the soil of certain nutrients. With rotation, a crop that leaches the soil of one kind of nutrient is followed during the next growing season by a dissimilar crop that returns that nutrient to the soil or draws a different ratio of nutrients, for example, rice followed by cotton. By crop rotation farmers can keep their fields under continuous production, without the need to let them lie fallow, and reducing the need for artificial fertilizers, both of which can be expensive. Rotating crops adds nutrients to the soil. Legumes, plants of the family Fabaceae, for instance,

have nodules on their roots which contain nitrogen-fixing bacteria. It therefore makes good sense agriculturally to alternate them with cereals (family Poaceae) and other plants that require nitrates. An extremely common modern crop rotation is alternating soybeans and maize (corn). In subsistence farming, it also makes good nutritional sense to grow beans and grain at the same time in different fields.

Crop rotation is a type of cultural control that is also used to control pests and diseases that can become established in the soil over time. The changing of crops in a sequence tends to decrease the population level of pests. Plants within the same taxonomic family tend to have similar pests and pathogens. By regularly changing the planting location, the pest cycles can be broken or limited. For example, root-knot nematode is a serious problem for some plants in warm climates and sandy soils, where it slowly builds up to high levels in the soil, and can severely damage plant productivity by cutting off circulation from the plant roots. Growing a crop that is not a host for root-knot nematode for one season greatly reduces the level of the nematode in the soil, thus making it possible to grow a susceptible crop the following season without needing soil fumigation.

It is also difficult to control weeds similar to the crop which may contaminate the final produce. For instance, ergot in weed grasses is difficult to separate from harvested grain. A different crop allows the weeds to be eliminated, breaking the ergot cycle.

This principle is of particular use in organic farming, where pest control may be achieved without synthetic pesticides.

A general effect of crop rotation is that there is a geographic mixing of crops, which can slow the spread of pests and diseases during the growing season. The different crops can also reduce the effects of adverse weather for the individual farmer and, by requiring planting and harvest at different times, allow more land to be farmed with the same amount of machinery and labor.

The choice and sequence of rotation crops depends on the nature of the soil, the climate, and precipitation which together determine the type of plants that may be cultivated. Other important aspects of farming such as crop marketing and economic variables must also be considered when crop rotation

## ***History***

Historic crop rotation methods are mentioned in Roman literature, and referred to by several civilizations in Asia and on three major elements: sophisticated systems of crop rotation, highly developed irrigation techniques and the introduction of a large variety of crops which were studied and catalogued according to the season, type of land and amount of water they require. Numerous farming encyclopaedias were produced.

In Europe, since the times Charlemagne, there was a transition from a two-field crop rotation to a three-field crop rotation. Under a two-field rotation, half the land was planted in a year while the other half lay fallow. Then, in the next year, the two fields

were reversed. Under three-field rotation, the land was divided into three parts. One section was planted in the Autumn with winter wheat or rye. The next Spring, the second field was planted with other crops such as peas, lentils, or beans and the third field was left fallow. The three fields were rotated in this manner so that every three years, a field would rest and be unplanted. Under the two field system, if one has a total of 600 fertile acres of land, one would only plant 300 acres. Under the new three-field rotation system, one would plant (and thereby harvest) 400 acres. But, the additional crops had a more significant effect than mere productivity. Since the Spring crops were mostly legumes, they increased the overall nutrition of the people of Northern Europe.

From the end of the Middle Ages until the 20th century, the three-year rotation was practiced by farmers in Europe with a rotation of rye or winter wheat, followed by spring oats or barley, then letting the soil rest (leaving it fallow) during the third stage. The fact that suitable rotations made it possible to restore or to maintain a productive soil has long been recognized by planting spring crops for livestock in place of grains for human consumption.

A four-field rotation was pioneered by farmers, namely in the region Waasland in the early 16th century and popularised by the British agriculturist Charles Townshend in the 18th century. The system (wheat, turnips, barley and clover), opened up a fodder crop and grazing crop allowing livestock to be bred year-round. The four-field crop rotation was a key development in the British Agricultural Revolution.

George Washington Carver pioneered crop rotation methods in the United States by teaching southern farmers to rotate soil depleting crops like cotton with soil enriching crops like peanuts and peas.

In the Green Revolution, the traditional practice of crop rotation gave way in some parts of the world to the practice of supplementing the chemical inputs to the soil through top dressing with fertilizers, e.g., adding ammonium nitrate or urea and restoring soil pH with lime in the search for increased yields, preparing soil for specialist crops, and seeking to reduce waste and inefficiency by simplifying planting and harvesting. Some disadvantages of this type of monoculture have since become apparent, notably from the perspective of sustainable agriculture and the risk of catastrophic crop failure.

### ***Effects on soil erosion***

Crop rotation can greatly affect the amount of soil lost from erosion by water. In areas that are highly susceptible to erosion, farm management practices such as zero and reduced tillage can be supplemented with specific crop rotation methods to reduce raindrop impact, sediment detachment, sediment transport, surface runoff, and soil loss.

Protection against soil loss is maximized with rotation methods that leave the greatest mass of crop stubble (plant residue left after harvest) on top of the soil. Stubble cover in contact with the soil minimizes erosion from water by reducing overland flow velocity, stream power, and thus the ability of the water to detach and transport sediment Soil

Erosion and Cill prevent the disruption and detachment of soil aggregates that cause macropores to block, infiltration to decline, and runoff to increase. This significantly improves the resilience of soils when subjected to periods of erosion and stress.

The effect of crop rotation on erosion control varies by climate. In regions under relatively consistent climate conditions, where annual rainfall and temperature levels are assumed, rigid crop rotations can produce sufficient plant growth and soil cover. In regions where climate conditions are less predictable, and unexpected periods of rain and drought may occur, a more flexible approach for soil cover by crop rotation is necessary. An opportunity cropping system promotes adequate soil cover under these erratic climate conditions. In an opportunity cropping system, crops are grown when soil water is adequate and there is a reliable sowing window. This form of cropping system is likely to produce better soil cover than a rigid crop rotation because crops are only sown under optimal conditions, whereas rigid systems are sown in the best conditions available.

Crop rotations also affect the timing and length of when a field is subject to fallow. This is very important because depending on a particular region's climate, a field could be the most vulnerable to erosion when it is under fallow. Efficient fallow management is an essential part of reducing erosion in a crop rotation system. Zero tillage is a fundamental management practice that promotes crop stubble retention under longer unplanned fallows when crops cannot be planted. Such management practices that succeed in retaining suitable soil cover in areas under fallow will ultimately reduce soil loss