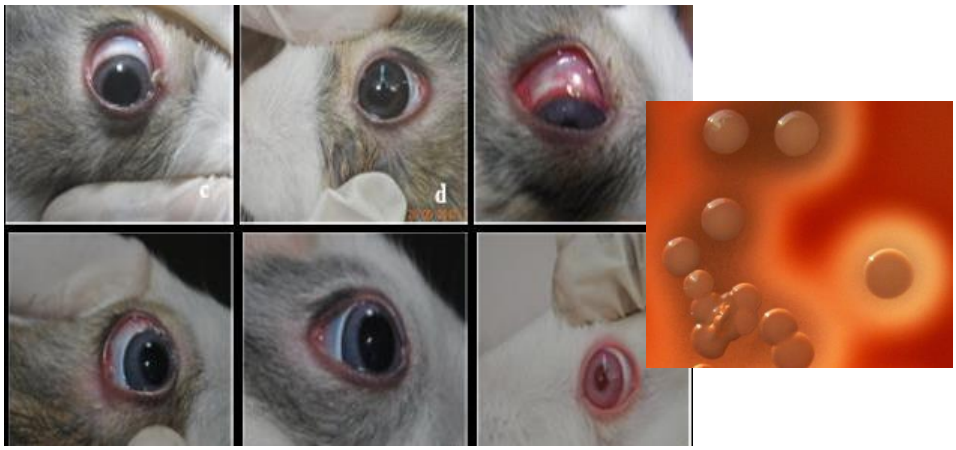
HOST- MICROBE INTERACTIONS

FOR 4TH YEAR STUDENTS

MICROBIOLOGY & CHEMISTERY

FACULTY OF SCIENCE



BY

PROF. DR. WESAM SALEM

2024

توصيف مقرر دراسي

١- بيانات المقرر

الرمز الكودي: ن	اسم المقرر: علاقة العائل بالميكروب	الفرقة / المستوي: رابعه ك ميكروبيولوجي
التخصص: نبـــات	عدد الوحدات الدراسية نظري (٢)	

٢- أهدف المقرر Aims	- إجاده التعرف على أنواع العلاقات المختلفه للكائنات الدقيقة وعوائلها. - فهم ودراسة كيفية تطور هذة التفاعلات و تأثيراتها على البيئه المحيطه.
٣- المستهدف من تدريس المقرر (المخرجات) : طلاب تمهيدي ماجستير Intended Learning Outcomes	
أ- المعلومات والمفاهيم Knowledge and Understanding	بنهاية تدريس المقرر يجب أن يكون الطالب قادر على: أ١-أن يحدد العلاقات المختلفه بين الميكروبات والعائل ومدى تأثيرها على العائل. أ٢-أن يصف الطالب نتائج العلاقات المختلفه للعائل والميكروب وتأثيراتها على البيئه المحيطة. أ٣-أن يذكر الطالب أمثله واضحة لطرق الدفاع للعائل ضد الميكروب.
ب- المهارات الذهنية Intellectual Skills	بنهاية تدريس المقرر يجب أن يكون الطالب قادر على: ب١-أن يقارن الطالب ما بين الميكروبات المفيده و الضاره للعائل. ب٣-أن يميز الطالب بين العلاقات المختلفه للميكروبات و العائل. ب٤-أن يوضح الطالب النتائج المتوقعه من علاقه الميكروب بالعائل. ب٥- أن يستنتج الطالب الفروق بين رد فعل البكتريا و الفطريات لدفاع العائل.
جـ- المهارات المهنية الخاصة بالمقرر Professional Skills	بنهاية تدريس المقرر يجب أن يكون الطالب قادر على: ج١ – أن يستخدم الميكروسكوب الضوئي و وسائل و أدوات التعقيم المختلفة. ج٢ - أن يجهز الطالب الأوساط الغذائيه و يعقمها. ج٣ -أن يعزل (يجمع) الطالب الميكروبات من النباتات المصابه و يعيد زراعتها على الأوساط الغذائيه المناسبه. ج٤ -أن يتناول الطالب النتائج المختلفة من علاقه العائل بالميكروب.
د- المهارات العامة General Skills	بنهاية تدريس المقرر يجب أن يكون الطالب قادر على: د١- التواصل الجيد من خلال المناقشات داخل المحاضرات. د٢-العمل في جماعة، تنظيم الوقت، يتعاون ويشارك مع الاخرين بايجابية.

٤- محتوي المقرر Course Content	- علاقه العائل بالميكروب: مقدمه عن الكائنات الدقيقه و علاقتها بالإنسان- العلاقات المختلفه بين العائل و الميكروب- تبادل المنفعه (في النبات و الإنسان)- commensalism – علاقه التطفل- نتائج العلاقات المختلفه للعائل و الميكروب- أمثله واضحه لهذه العلاقات-الفطريات المتطفلة- طرق الدفاع للعائل ضد الميكروب- رد فعل الميكروب على العائل- رد فعل العائل على الميكروب.

٥- أساليب التعليم والتعلم Teaching and Learning Methods	١- **المحاضرات النظرية باستخدام أجهزه Data show** ٢- **عرض إسطوانه مدمجه تحتوي على بعض الصور و العروض للكائنات الدقيقه.** ٣- **تكليف الطلاب بعمل مقالات في بعض الموضوعات.** ٤- **الدروس العملية.**
٦- أساليب التعليم والتعلم للطلاب ذوي القدرات المحدودة Teaching and Learning Methods of Disables	١- **التعلم التعاونى.** ٢- **المتابعة في الساعات المكتبية.** ٣- **تحديد الطلاب المتعثرين من خلال الإمتحانات الدورية.** ٤- **تبسيط المعلومة بإستخدام عروض الفيديو.**

٧- تقويم الطلاب Student Assessment :

أ- الأساليب المستخدمة Tools

Tool	Measured Skils
Mid-term Exam & Semester Work	Knowledge, Understanding, Professional and intellectual
Practical Exam	Intellectual; Professional , General Skills
Final Written Exam	Knowledge, understanding and intellectual Skills

ب- التوقيت Time Schedule

The Tool	Time	Weight
Final Written Exam	end of the semester	100%

جـ- توزيع الدرجات Grading System

The grade	Excellent	Very Good	Good	Pass	Fail
% of the total marks	> 90	80-89	70-79	60-69	<60

٨- قائمة الكتب الدراسية والمراجع List of Reference :

أ- مذكرات Course Note	- **مذكره علاقه العائل بالميكروب (دراسات عليا).**
ب- كتب ملزمة Required Books (Text Book)	- **General Microbiology** - **Microbes in action**
جـ- كتب مقترحة Recommended Books	**Introduction to microbiology** **Bacteriology**
د- دوريات علمية أو نشرات Periodicals, web .....إلخ sites... etc.	- **دورات في الميكروسكوب الإلكتروني – تحضير العينه و الفحص.**

أستاذ المادة: أ.د/ وسام محمد علي محمود سالم

(المصدر: دليل الهيئة القومية لضمان الجودة والاعتماد)

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GENERAL OUTLOOK

1. Microbes can grow on other living things

FOR the most part microorganisms do not exist in isolation, but instead are members of larger environmental communities. Each microbe has an effect on its environment, and its presence influences all the other residents of that community. Sometimes these effects can be damaging, as when a microbe grows in polluted water and uses up all the oxygen, making it unavailable for fish. In other cases, they improve an environment. Photosynthetic microbes in the ocean (plankton) are major primary producers that much of the rest of the organisms in the ocean are dependent upon. Such loose interconnections (a topic of environmental microbiology) are essential in the web of life.

In this course we will concern ourselves with the interaction of microbes with larger organisms. The presence of large eukaryotic organisms (from this point on we will refer to these as **hosts**) in an environment does not go unnoticed, and microbes will naturally take advantage of the niches they provide. Why do hosts make such great environments for microbes? Primarily, the hosts often provide a physically stable environment (constant temperature, pH, osmolarity, etc.) with a ready supply of nutrients. For example, the cow's rumen is teaming with microorganisms that flourish in this very stable environment, metabolizing the steady supply of plant material eaten by the cow. Another host environment is your

mouth. The soft tissues and teeth in the mouth provide readily available sites for attachment and a steady supply of food such as chips, pizza, etc. provides plenty for microbes to metabolize.

What defines a host-microbe interaction?

Before we begin, it is important to clearly define what is meant by a host-microbe interaction. One might define these interactions as any time a microbe and host are near each other, but the question then becomes how near do they have to be before it is considered an interaction? It is entirely possible for a host and a microbe to benefit one another yet be very far apart. For example, human waste is an excellent source of nutrients for many microbes to grow in and the accumulation of these wastes in sewage treatment facilities results in high populations of bacteria. The action of the bacteria helps to degrade the sewage so that the release of it into the environment does not damage the ecosystem. It is clear that both humans and microbes got some benefit, but this is not really an interaction. Therefore, for it to be considered an interaction there must be **contact for a significant period of time**. For this text we will define a host-microbe interaction as one that involves some sort of physical contact between a microorganism and a host where the microbe gains some benefit.

2. General Themes

While microbes can have numerous and diverse interactions with eukaryotic organisms, there are some properties that all host-microbe interactions share.

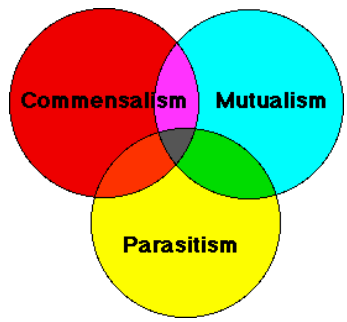
- These interactions are dynamic. Changes in the environment provided by the host (hormonal alterations, changes in diet, antibiotic therapy, etc.) affect the types and numbers of microorganisms present on the host.
- The main benefit to the microbe is always the same - **a good environment for growth and reproduction**. The goal of prokaryotes is simply to make more of themselves.
- Some microbes that grow on hosts are also capable of living freely (separate from their host) in the environment. In most cases the physiology required in these two lifestyles is vastly different. This has two implications. **First**, the microbe must have a way of detecting whether on not it is interacting with the host. One might detect a host by recognizing host surface molecules, by sensing the presence or absence of a signal molecule or even by the hosts temperature. **Second**, the microbe must react to the presence or absence of the host by changing the expression of appropriate genes.
- Tied up in this last point is that the microbe must be careful to recognize the correct host, which can be difficult in a

complex environment. There is often a complex molecular conversation going on between the microbe and its appropriate host.

- The microbe must be capable of colonizing the host. That is, it needs to set up a fairly stable interaction. This typically involves a specific recognition between surface molecules on both the host and the microbe. Also, the microbe must be able to obtain useful growth compounds from that interaction.
- The microbe must have mechanisms for dealing with the hosts microbial defenses so that it can maintain its colonization. Without these devices, many hosts will rapidly eliminate the microbe.

We are just now starting to decipher these interactions at the molecular level, and this analysis will be a central feature in microbiology for years to come.

3. **Types of interactions**



In classifying these interactions, we tend to look at them from the host's point of view, since we serve that role for a large number of microorganisms. The outcomes of these interactions fall into three categories: (1) **mutualistic**, where both the host and the microbe benefit from the association; (2) **commensal**, where the microbe benefits and the host is unharmed; and (3) **pathogenic** (or **parasitic**), where the microbe benefits and the host is harmed by the association. The differentiation among the types of interaction can be difficult in some cases as well as dynamic. For example, when living in our noses, *Staphyloccus aureus* causes little harm and this can be thought of as a commensal interaction, yet this same bacterium growing on sub-surface layers of the skin will result in a pathogenic interaction that causes boils.

1. ***Mutualistic interactions***

The host's role in a mutualistic interaction is often a very active one. The host provides space (room) for the microbe and transports nutrients to encourage its growth and metabolism. In many cases the host actively recruits the microbe by sending out cellular signals to which the target microbe reacts. Once contact is made between the microbe and its host, gene expression in the host results in the production of proteins important in initiating and maintaining the association. The host will not antagonize the growth of the microbe but will typically confine it to a desired area since unchecked microbial growth throughout a eukaryotic

organism is undesirable. In return, the microorganism will provide a benefit to the host through its metabolism.

The microbe in this interaction uses the nutrients and space provided by the host to multiply. It also actively seeks out a host and turns on a series of genes important for creating and continuing the association. Once the interaction is established, the microbe expresses gene products that are beneficial to the host. Often these genes are induced by an environmental condition that signals the presence of the host. It is becoming clear that the behavior of a mutualistic microbe and that of a pathogen when infecting a host can be very similar. The major difference is that the mutualistic microbe does not harm the host and provides some benefit. There are numerous examples of mutualistic interactions and we will discuss two in some depth later in this course.

2. ***Commensal interactions***

In commensal interactions, the microbe colonizes the host, but there is no effort by the host to support that interaction. In fact, the host often antagonizes colonization by the microbe, but since no harm is done, it is unclear how vigorous the host's defense usually is. Similarly, the interaction begins with the microbe actively seeking out the host, since the host makes no effort to attract it. Once a host is found, the microbe aggressively attaches to host surfaces, and prepares to deal with any host defenses present in the area. For its part, the microbe produces no toxins

nor does the growth of the microbe occur in critical areas that would impede the well being of the host.

The microbes that reside in your mouth are excellent examples of commensal interactions and this is described in some detail later in this course. Many of these microbes have extracellular polysaccharides or surface proteins that help them specifically attach to surfaces in your mouth where they grow to high numbers. For the most part these organisms do not produce toxins or any other harmful products and, provided you brush and floss to keep the numbers under control, they do not damage your teeth.

3. ***Parasitic and pathogenic interactions***

In these types of interactions, the presence of the microbe is harmful to the host, and the microbe is considered a **parasite**. If the interaction causes a diseased state in the host, then the parasite is considered a **pathogen**. Many of the ideas in the ensuing discussion apply to both parasites and pathogens, but we will restrict our terminology to pathogens for simplicity and because these interactions are of greater concern.

In addition to their ability to damage the host, pathogens have other traits that define them. Many have the ability to invade into areas where they are not wanted. This is in contrast to mutualistic and commensal organisms that avoid areas whose infection would cause a problem for the host. Pathogens also tend to have more

aggressive mechanisms for evading the host defenses than do mutualistic or commensal microbes, and these help them succeed in prohibited areas of the host.

A host being attacked by a pathogen is certainly an unwilling participant. The host actively avoids infection by trying to rid itself of the pathogens as quickly as possible. The defenses of hosts are focused on both preventing associations with pathogens and then getting rid of the microbes that succeed in associating. This process is literally a life-and-death struggle and generally, the host defenses are up to the task.

Streptococcus pneumoniae is one example of a bacterial pathogen that colonizes the lungs if given the chance. It is a common inhabitant of the upper respiratory tract where it is commensal, but under the right circumstances, it will penetrate into the lungs. To prevent removal, the microbe has specific proteins that help it attach to lung tissue and once it is settled, it will grow in the lungs and cause disease. This growth and the production of certain toxic molecules harm the host and elicit a strong host immune reaction that is responsible for the high fever often associated with streptococcal pneumonia.

Challenges faced by a colonizing microbe

Here we will look at some general concepts associated with host-microbe interactions. We will examine the host response to

pathogens at the end of this course, but now we will talk about the challenges faced by a microbe as it tries to colonize a host. We talk about these concepts here because they are faced by all microbes that interact with animal hosts, not just pathogens. These challenges can be broken into a series of steps that must be followed for a successful interaction: detecting a host, forming an interaction with a host, possibly invading a host, and dealing with hosts defenses. As we go through these different steps, we will describe specific examples of how microbes have overcome these challenges.

1. ***Detecting a host***

A microbe that has the ability to form an interaction with a host must first be able to find it in the environment. For the most part this involves detecting some feature of the environment that is strongly correlated with the presence of the host.

There are many different ways, by which different microbes can detect their respective host. Nodulating bacteria detect the presence of their host plant by the presence of specific signal molecules called flavonoids that the plant roots excrete into the soil. *Pseudomonas aeruginosa*, an opportunistic pathogen of humans, shows somewhat less specificity in host detection by recognizing the level of iron present in its environment. The body sequesters most iron it comes in contact with and the concentration of free iron in the body is very low. When *P.*

aeruginosa detects extremely low iron levels, it assumes it is in an appropriate host and turns on virulence genes. *Vibrio cholerae* uses temperature, pH, osmolarity, and certain amino acids as measures of the presence of the host. Outside the host, *V. cholerae* lives free in the ocean where the average temperature is 21 C or below and amino acids are at very low concentration. When ingested, the signals in the gastrointestinal tract turn on gene expression important in pathogenicity, including production of toxins and fimbriae. The fimbriae help the bacteria adhere to the intestine, while the toxin causes diarrhea.

Host specificity is often part of host detection. The microbe (and host in a mutualistic interaction) often send out signal molecules for reception by appropriate partners. Certain mutualistic interactions can be extremely selective as we described above. This type of specificity can also be found in pathogenic relationships. *Treponema pallidum* (the cause of syphilis) only infects humans, but other microbes are not so specific and infect a wide variety of hosts. Many commensal microbes such as some species of *Streptococcus* and *Staphylococcus* are found on a wide variety of mammals.

2. ***Attachment***

Once near a host, a microbe has to associate with it in some manner to allow more than a transient interaction. In many cases this involves a specific molecular interaction between the microbe

and the host (Table 1). There are numerous examples of this type of interaction. *E. coli* displays fimbriae on its surface that help it attach to the intestinal wall and uroepithelium. *Streptococcus mutans* uses an extracellular polysaccharide to bind tightly to teeth. *Neisseria meningitidis* produces pili that help it hold onto the nasopharyngeal epithelium cells. In each of these cases a product produced by the microbe interacts with a specific molecule on the host that helps it to stick. Often this reaction is important in determining the microbes tissue specificity since it can only bind to cells producing the recognized receptor.

Table (1) Examples of attachment mechanisms.

Bacterium	Adhesin	Receptor	Attachment site	Disease
Streptococcus pyogenes	Protein F	Amino terminus of fibronectin	Pharyngeal epithelium	Sore throat
Streptococcus mutans	Glycosyl transferase	Salivary glycoprotein	Pellicle of tooth	Dental caries
Streptococcus salivarius	Lipoteichoic acid	Unknown	Buccal epithelium of tongue	None
Streptococcus pneumoniae	Cell-bound protein	N-acetylhexosamine-galactose disaccharide	Mucosal epithelium	pneumonia
Staphylococcus aureus	Cell-bound protein	Amino terminus of fibronectin	Mucosal epithelium	Various
Neisseria gonorrhoeae	N-methylphenyl-alanine pili	Glucosamine-galactose carbohydrate	Urethral/cervical epithelium	Gonorrhea
Enterotoxigenic E. coli	Type-1 fimbriae	Species-specific carbohydrate(s)	Intestinal epithelium	Diarrhea
Uropathogenic *E. coli*	Type 1 fimbriae	Complex carbohydrate	Urethral epithelium	Urethritis
Uropathogenic *E. coli*	P-pili (pap)	Globobiose linked to ceramide lipid	Upper urinary tract	Pyelonephritis
Bordetella pertussis	Fimbriae ("filamentous hemagglutinin")	Galactose on sulfated glycolipids	Respiratory epithelium	Whooping cough
Vibrio cholerae	N-methylphenylalanine pili	Fucose and mannose carbohydrate	Intestinal epithelium	Cholera
Treponema pallidum	Peptide in outer membrane	Surface protein(fibronectin)	Mucosal epithelium	Syphilis

3. *Invasion*

Many associations get no farther than attachment, where the microbe remains at its initial site of infection and has its effect, either positive or negative. The normal flora are a good example of this in a mutualistic/commensal interaction, but even some pathogenic microbes such as *Corynebacterium diphtheriae*, the causative agent of diphtheria, fails to penetrate beyond the initial site of attachment in the throat (Fig. 1).

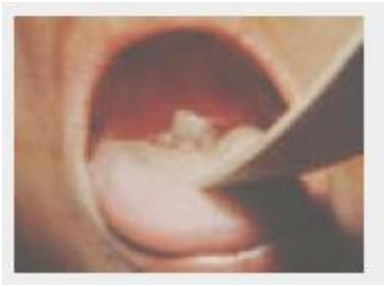


Fig. (1) Some stop here and go no further

In other cases, the microbe penetrates the host further. For microbes that *do* move beyond the initial interaction site, bacterial and sometimes host factors are necessary to allow further penetration.

In the case of mutualistic interactions host proteins help facilitate this movement, typically to a specific tissue in the host, and prevent further spread from there. In pathogenic interactions the host obviously tries to prevent penetration and as a result, pathogens have developed mechanisms to deal with the more aggressive host reaction.

Mammalian cells take up particles from the outside by a mechanism termed **endocytosis** and some pathogens have developed methods of exploiting this import mechanism to gain access to the inside of cells. Other defensive cells in the body, termed **phagocytes,** use engulfment (phagocytosis) to capture pathogens and kill them. Again, some pathogens are able to turn the tables on the phagocyte. They not only survive inside these cells but use them as a vehicle to spread throughout a host. In these cases, the microbe must have methods of surviving the harsh conditions faced when taken up by defensive host cells.

Mycobacterium tuberculosis is an example of a pathogen that can actually multiply inside a phagocyte and this immune cell then actually facilitates spread of the bacteria throughout the body. Table (2) lists some examples of microbes and factors involved in their invasion of a host.

Table (2) Invasion of a host by a microbe and some examples of factors that facilitate that invasion.

Microbe	Host	Factors involved in invasion	Activity
Rhizobia	Legumes	Nod factors	Induces nodulation reactions in plant root
S*treptococci, staphylococci and clostridia*	Humans	Hyaluronidase	Degrades hyaluronic of connective tissue

Staphylococci and streptococci	Mammals	Kinase	Converts plasminogen to plasmin, which digests fibrin
Staphylococcus aureus	Mammals	Leukocidin	Disrupts neutrophil membranes and causes discharge of lysosomal granules
Bacillus anthracis	Some mammals	Anthrax toxin	One component (EF or edema factor) is an adenylate cyclase, which causes increased levels of intracellular cyclic AMP. This in turn leads to edema.

Pathogens also have two other modes of entry besides penetration of healthy tissue that other symbionts do not seem to use. Many illnesses, including malaria, Lyme disease and the plague involve an insect vector that penetrates the initial outer defenses of the host. For example the bite of the deer tick allows *Borrelia burgdorferi*--the microbe that causes Lyme disease--to penetrate the skin and directly enter the blood stream. There are examples of other organisms serving as vectors, but insects are the most common. The second entry mode involves tissues that have already been compromised by an injury or by the action of a previous pathogen. An example is infection by *Clostridium tetani*. The organism itself cannot penetrate the skin, but if allowed entry by a puncture wound, will grow deep within muscular tissue, causing tetanus. Interestingly, *C. tetani* is a strict anaerobe and requires deep wounds with an anoxic environment to grow. This is an example of a microbes basic physiology restricting its tissue tropism.

At this point, the results of a symbiosis diverge depending upon the outcome of the interaction. In light of this fact, we will treat these outcomes in separate sections beginning first with mutualistic interactions and then looking at pathogenic interactions. Note that commensal interactions fall somewhere between these two, with the host neither mounting a strong defense nor helping the commensal microorganism.

Mutualistic outcomes

4. *Mutualistic outcomes*

Once the mutualistic microbe has arrived at its desired location, it is well provided for by the host. The environment is kept in a steady state with appropriate temperatures, levels of oxygen and necessary nutrients. **Leguminous plants** (Fig. 2) provide succinate, fumarate or malate as the carbon and energy sources and deliver oxygen using the protein leghemoglobin. Using a special protein to deliver oxygen prevents it from reacting with the oxygen-labile nitrogenase and is a good example of the lengths a host will go to make its microbial guest comfortable.

Fig. (2) Root hair legumes.

Inside the **rumen of a cow** (Fig. 3) the microbes are provided a steady flow of cellulose (grass) and other nutrients to metabolize. In this type of ideal environment, the microbial population grows to high densities. In other cases, the host is less accommodating, providing few nutrients and using its immune system to attack the microbe.

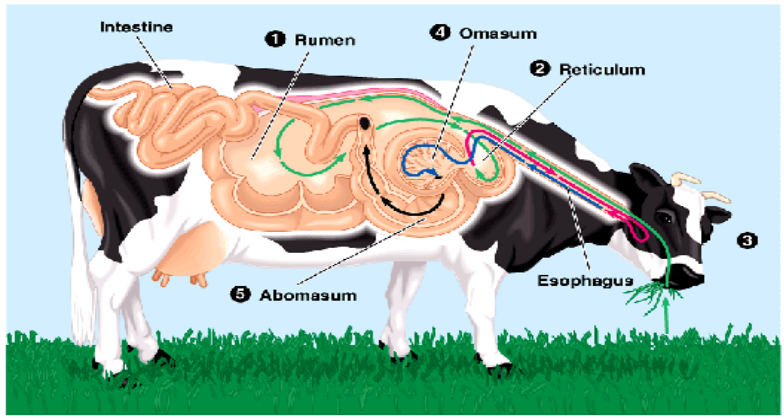


Fig. (3) Mutualistic interaction inside cow rumen. (The cow as an example of a ruminant animal)

In contrast to humans ruminant animals have complex stomachs that harbor large numbers of microorganisms. These microbes degrade the plant stuff eaten by the animal into usable nutrients. Without the assistance of the microbes, ruminant animals would not be able to digest the food they eat.

An interesting problem for the host in mutualistic interactions is identifying the correct bacteria out of the multitude in any environment and ushering that microbe to its proper host compartment. The host wants to form the association with just one or a few types of microbes, and this is achieved by the chemical conversation between the host and microbe noted above.

In many cases, this selection is absolute, so that a pure culture of a single bacterial type exists within the host.

5. ***Pathogenic outcomes***

After identifying and attaching to a host the pathogen typically spreads to various parts of the body and increases its population. This precipitates a vigorous host response and a pathogen has to be prepared to deal with it. This makes sense since the pathogen is invading places it ought not to be. There are many different **levels of defense**. Here are a few examples to give you an idea of the many different strategy's microbes have evolved. *Staphylococcus aureus* produces leukocidin, a protein that is lethal to phagocytes. *S. aureus* also produces Protein A, which inhibits phagocytic engulfment. These bacteria then use the phagocyte as a method to spread throughout the body. *Pseudomonas aeruginosa* produces a capsule (an outer layer of polysaccharide) that helps it hide from the host immune system. Finally, some microbes change their outer surface so that they are no longer recognized by previously generated immune responses. In all these cases the properties of the microbe help it to evade some part of the immune system and then survive and grow.

Direct damage to host

By definition, pathogenic microorganisms damage their hosts. The type and the extent of this damage depends upon the **pathogen**

and the **tissue**. For example, an infection that damages the muscle in the shoulder is a less devastating disease than an infection of the heart muscle. One pathogen may cause only a little damage while another causes severe damage and life-threatening disease. Some strains of *Bacillus cereus* can cause mild food poisoning that the body can deal with in a few days, whereas *Mycobacterium lepreae* (leprosy) causes many pathologies, including limb loss.

Damage may be directly caused by the pathogen or indirectly caused by the **hosts response to the pathogen**. Direct damage can be caused just by the mere growth of the microbe. *Streptococcus mutans* metabolizes sugars to lactic acid that then eats away at the enamel on the teeth, causing tooth decay. *Listeria monocytogenes* grows within host phagocytic cells and their high populations result in the death of the cell. However, pathogens very often make products that cause damage to the host and are important in the virulence of the microorganism. Many of these products are called toxins. Toxins come in two forms, **exotoxins** and **endotoxins**. Exotoxins are generally soluble proteins liberated from growing bacteria that then cause damage to the host. Endotoxins are lipopolysaccharides from the outer cell membrane of gram-negative bacteria that are normally released only after the death of the microbe.

Exotoxins

Each exotoxin-producing microorganism synthesizes a toxin that is typically unique to its species. For instance, *Bacillus anthracis* produces anthrax toxin and *Clostridium tetani* produces tetanus toxin. The exotoxin is often the major **virulence factor** and otherwise similar strains that do not produce the toxin often do not cause illness. Because they are proteins, exotoxins are commonly heat-sensitive; however, many are resistant to inactivation by gastric pH.

Exotoxins attack very specific targets in the cell and the mechanisms of action of many of them are well established. Table (3) lists some exotoxins and their mechanisms of action. Some toxins work only on certain tissues or cells. For example, tetanus and botulism toxins only act at the synapses of neurons. Other toxins such as phospholipases act systemically, destroying many types of cells all over the body.

Table (3) Examples of various exotoxins and their activities.

Name of toxin	Bacteria involved	Activity
Anthrax toxin (EF, LF)	*Bacillus anthracis*	Edema factor (EF) is an adenylate cyclase that causes increased levels of intracellular cyclic AMP in host cells, leading to swelling. Lethal factor kills primarily macrophages by disrupting its signal transduction pathway.
Adenylate cyclase toxin	*Bordetella pertussis*	This toxin acts locally to increase levels of cyclic AMP in mucosal cells leading to increase in respiratory secretions and mucus production.
Cholera enterotoxin	*Vibrio cholerae*	ADP-ribosylation of G proteins stimulates adenylate cyclase and increases cAMP in cells of the GI tract, causing secretion of water and electrolytes.
E. coli LT toxin	*Escherichia coli*	Similar to cholera toxin.

E. coli ST toxin	*Escherichia coli*	Stimulates guanylate cyclase and promotes secretion of water and electrolytes from intestinal epithelium.
Shiga toxin	*Shigella dysenteriae*	Enzymatically cleaves rRNA resulting in inhibition of protein synthesis in large intestinal cells.
Clostridium perfringens enterotoxin	*Clostridium perfringens*	Stimulates adenylate cyclase leading to increased cAMP in small intestinal cells.
Botulinum toxin	*Clostridium botulinum*	Zn^{++}-dependent protease that inhibits neurotransmission at neuromuscular synapses resulting in flaccid paralysis.
Tetanus toxin	*Clostridium tetani*	Zn^{++}-dependent protease that inhibits neurotransmission at inhibitory synapses resulting in spastic paralysis.
Diphtheria toxin	*Corynebacterium diphtheriae*	ADP-ribosylation of translation elongation factor 2, which leads to inhibition of protein synthesis in target cells.
Exotoxin A	*Pseudomonas aeruginosa*	Inhibits protein synthesis; similar to diphtheria toxin.
Pertussis toxin	*Bordeatella pertussis*	ADP-ribosylation of Gi proteins upregulates host cell adenylate cyclase, increasing concentration of cAMP in respiratory epithelium
Staphylococcus enterotoxins*	*Staphylococcus aureus*	Common cause of food poisoning. Certain serotypes have been associated with massive activation of the immune system resulting in toxic shock syndrome (TSS).
Toxic shock syndrome toxin (TSST-1)*	*Staphylococcus aureus*	Massive activation of the immune system resulting in TSS.
Exfoliative toxin*	*Staphylococcus aureus*	Cleavage of epidermis from dermis.
Pyrogenic toxin (formerly known as erythrogenic or scarlet fever toxin)*	*Streptococcus pyogenes*	Causes localized erythematous reactions.

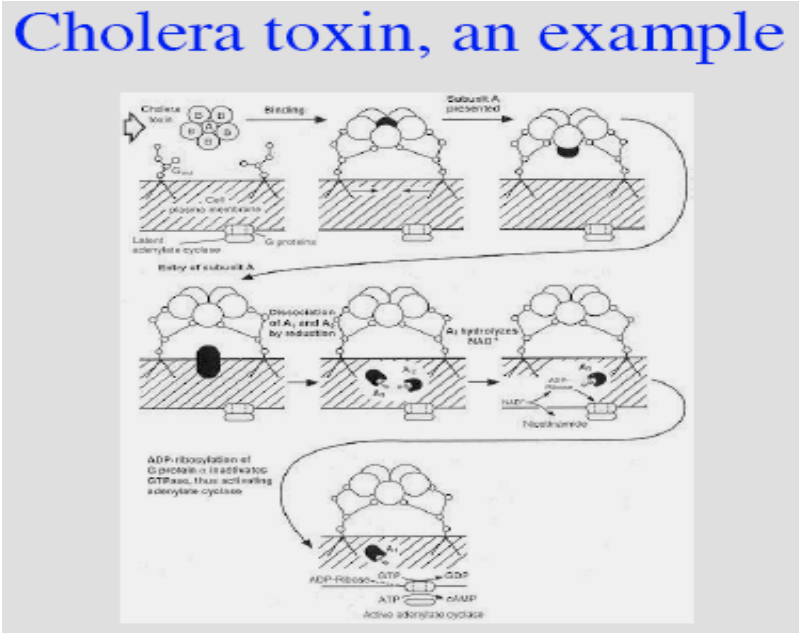
*These toxins are called superantigens. They represent a family of molecules capable of massive activation of the immune system by interaction with T cells. The important feature is the production of lymphokines that appear to be the principal mediators of disease processes associated with these toxins.

Exotoxins are some of the most powerful human poisons known and are effective at very low concentrations, because most of the proteins are catalytic enzymes. These toxins can sometimes lead to death of the host, which in most cases is not a desirable outcome for the pathogen.

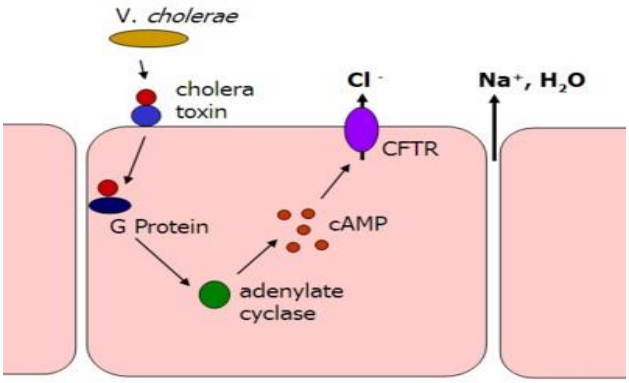
Structure and entry of exotoxins

Many of the protein toxins consist of two components. One component **(subunit A)** is responsible for the toxic activity, while the second component **(subunit B)** is necessary for attachment and transport of subunit A across the membrane of the target cells. Subunit A is not active until it is released from subunit B. There are two mechanisms of entry of toxins into target cells (Fig. 4). In direct entry, subunit B binds to a specific receptor on the membrane of the target cell, creates a pore and transports subunit A across the membrane. Subunit A then has its toxic effect. In the second method the A/B toxin binds to the target cell and is taken into the cell by receptor-mediated endocytosis. Inside the endosome the influx of protons triggers an unknown mechanism that results in the release of the A subunit. The B subunit remains in the endosome, eventually exiting the cell by exocytosis. By either method, a toxic effect reflects the transfer of the A subunit into the cell cytoplasm.

Fig. (4) Entry of Toxins.



The cholera toxin forces intestinal endothelia cells (cells that line the intestine) to purge Cl^- into the lumen of the intestine (hollow tube). Water follows. The end result, diarrhea as in the following figure.



Curiously, the genes for many toxins (e.g., diphtheria, botulism and some streptoccocal toxins) are located on temperate phages, while the genes for others (*E. coli* and *Staphylococcus aureus*) are on plasmids. This association with phage and plasmids is not important for the expression of the genes or for the function of the gene products. We therefore assume that these locations reflect the fact that these genes have been moving among different hosts, since phage and plasmids share this property. Genetic exchange by transduction and conjugation can therefore result in the transfer of toxin production from strain to strain, which must play a role in the virulence of particular bacteria in nature. It is still somewhat odd that such critical players in pathogenesis would be located on such potentially transient DNA as plasmids and temperate phages.

Endotoxins

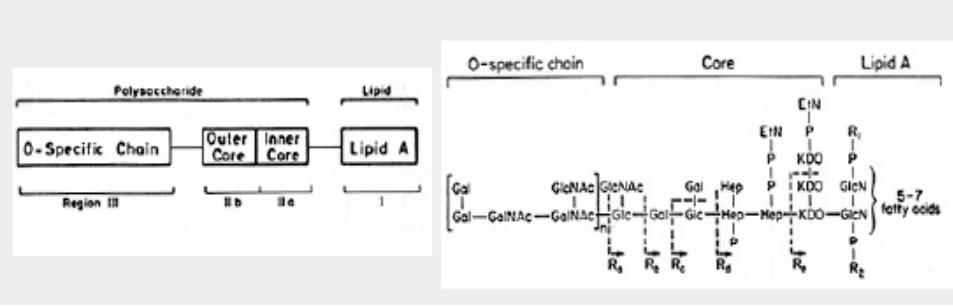
A second type of toxin is produced by Gram-negative pathogens. The outer layer of all gram-negative bacteria consists of an outer membrane that contains lipids, proteins and lipopolysaccharide (LPS). The LPS in the outer membrane has toxic systemic effects in mammals and has been given the name endotoxin. Endotoxins are less potent than exotoxins, since they do not act enzymatically; they are also less specific. They are not destroyed by heat, i.e., boiling for 30 minutes has no effect on LPS. But powerful oxidizing agents such as hypochlorite can destroy them. Since LPS is part of

the living microbial cell, it is not released in large quantities until the microbe dies, which is in contrast to exotoxins, which are released from growing cells. The levels of toxicity of endotoxins vary depending upon the producing microbe. Endotoxin from *E. coli* is more toxic than that from *Pseudomonas aeruginosa*, which is in turn more toxic than that from *Brucella sp.*

Structure

The LPS molecules from all known sources have roughly similar structures and are composed of three regions (Fig. 5).

Fig. (5) The stucture of LPS



Lipid A is a highly conserved region consisting of a phosphorylated N-acetylglucosamine (NAG) dimer with six or seven fatty acids attached. The toxicity of LPS is associated with Lipid A. Lipid A is absolutely required by the microbial cell, as it has been impossible to isolate mutants unable to synthesize lipid A. Lipid A is likely essential for the assembly of the outer membrane.

Core polysaccharide or **R antigen** consists of a short chain of sugars. For example in *E. coli* the sugars are 2-keto-3-deoxyoctonoic acid (KDO), heptose, glucose, galactose and N-acetyl glucosamine. KDO is unique to LPS and invariably present. This core polysaccharide is conserved within all strains in a species but will vary somewhat between different species. For example, the core polysaccharide of *Salmonella enterica* subsp. Typhimurium contains ethanolamine, while that from *E. coli* does not.

The core polysaccharide seems to be important in general cell function and specifically for outer membrane integrity. Loss of the core polysaccharide by mutation causes the microbe to be more sensitive to a range of hydrophobic compounds, including antibiotics, detergents, bile salts and mutagens. The sugars of the core polysaccharide contain a number of charged groups, and these are thought to be important in maintaining the permeability of the outer membrane. Loss of the core probably weakens the outer membrane, allowing many normally excluded molecules to come into contact with the cytoplasmic membrane.

O antigen is attached to the core antigen and consists of repeating units of three to five sugars. It varies in length and can contain up to 40 repeating units. At least 20 different sugars are known to occur in O antigens and many of these sugars are only found in Gram-negative cell walls. The sugars present in the O

antigen vary greatly between species and even within them. Sugars in the structure, especially the terminal ones, are what interact with the microbe's environment.

LPS is a major determinant of virulence for Gram-negative pathogens. Small changes in the O-polysaccharide can make large changes in the virulence of a strain. Strains that lose the ability to make O-polysaccharide are called rough strains due to their colony appearance, and these are much less pathogenic than regular strains (termed smooth). O antigen is important for interacting with the host structures, and mutants lacking the O antigen are also more easily taken up and destroyed by phagocytes.

Pathologies of endotoxin

Endotoxin from all pathogenic species causes the same range of biological activities affecting almost every organ in the body. When LPS is released into the blood stream it is bound by LPS-binding serum proteins. This is then presented to immune system cells, causing several important effects.

- The release of **cytokines**, a group of small peptides that are powerful mediators of inflammation, is stimulated. One important effect of this is the induction of vasodilation causing drop in blood pressure and setting the stage for septic shock.

- Endotoxins are pyrogenic (fever-inducing), because they cause the release of interleukin 1 (a type of cytokine produced by macrophages), which acts on the hypothalamus, inducing fever.
- Clotting cascade is activated, causing intravascular coagulation.
- Endotoxins induce various parts of the immune system, causing the production of antibodies and activation of immune cells. Table 4 summarizes the properties of endotoxins and exotoxins.

Table 4. A comparison of the properties of endotoxins and exotoxins.

Property	Endotoxin	Exotoxin
Chemical nature	Lipopolysaccharide (mw = 10kDa)	Protein (mw = 50-1000 kDa)
Relationship to cell	Part of outer membrane and has major non-toxin use for the molecule	Specifically synthesized protein that causes a toxic effect; extracellular and diffusible.
Denatured by boiling	No	Usually
Antigenic	Yes	Yes
Relative potency	Low (>100 g)	High (1 g)

Specificity	Low degree	High degree
Enzymatic action	No	Usually
Pyrogenicity	Yes	Occasionally

Indirect damage to host

The host causes indirect damage to itself by its very reaction to the presence of the microbe. Generally, this involves the inflammatory response to products from the pathogen. For example, lung infection by *Mycobacterium tuberculosis* causes very little direct damage. However, the host mounts a vigorous response to the presence of the organisms and forms large granulomas, which are grainy growths of host mononuclear cells. These wall off the bacteria, but also damage the lung tissue, impairing its function. In weakened hosts, *M. tuberculosis* can escape these granulomas, causing lysis of the cells. Cell contents, including lysosomal enzymes, spill out and damage lung tissue. Over many months, the infection destroys the lung tissue with most of the damage being caused by the immune system.

Indirect damage can also be caused by a variety of other host responses to infection. Infection by a microorganism precipitates the stress response, which involves the release of the hormones epinephrine and cortisol. This causes high blood pressure and high blood sugar, which is detrimental to the body. Inflammation

causes blood vessels to become leaky, which then allows the immune system to reach the tissue. During this process, the blood vessels and the tissue, into which the blood leaks, are damaged, and platelets are depleted. Finally, infection with one microorganism can weaken the local defenses enough to allow invasion by a second microorganism that causes the actual damage. The classic example of this is an ear infection, where a viral infection causes buildup of fluid in the ears, creating an environment for growth of bacteria such as *Streptococcus pneumoniae* or *Haemophilus influenzae*. Another example is infection with HIV (human immunodeficiency virus). Growth of the viruses causes the destruction of the immune system cells, thus allowing an uncontrolled proliferation of organisms such as *Mycobacterium tuberculosis* that can kill the host.

Examples of host-microbe interactions

Every animal or plant examined has been found to maintain stable relationships with an array of microorganisms. These associations most commonly involve colonization of the surface or the digestive canal (in the case of animals). Most hosts contain a consortium of different microbial types and understanding the numerous and complex interactions that are occurring is extremely difficult. However, there are examples of simpler interactions between two partners, a host and a single microbial species, that are easier to address scientifically and these have become model systems for

understanding host-microbe interactions. We finish this part by examining some of these model systems. Finally, we will look at the consortia of microorganisms that are living on our bodies. In the next part we will describes, in detail, the consequences of infection with specific pathogens.

I- MUTUALISTIC INTERACTIONS

1- Microbes on plants

Plants live simultaneously in two very different environments. Their stems and leaves are exposed to the air and any weather that comes their way, while their roots are dug into the soil. The air contains a surprising number of microorganisms, but this pales in comparison to the situation in the soil, which is teeming with a huge number and range of prokaryotic and eukaryotic microbes. As the root grows, it excretes carbon-rich compounds, products of plant photosynthesis, on which soil bacteria can grow. Because of the secreted nutrients, the number of soil bacteria is relatively high near a root. Every type of plant favors certain types of microbes over others because of the compounds it excretes. These attract both mutualistic and pathogenic microbes.

One example of mutualistic microbes is the set of microbes capable of forming mutualistic interactions, primarily with legumes. These microbes do not fall into a single phylogenetic group, but rather belong to several distinct groups based upon 16S rRNA

sequencing. These include members of the *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium, Allorhizobium,* and *Azorhizobium* genera. This diverse collection of microbe is commonly referred to as the **rhizobia** since they inhabit the rhizosphere, which is the region around plant roots. These microbes provide a variety of chemicals to the plants, including plant growth hormones, the most important of which is fixed nitrogen, since these bacteria are all nitrogen-fixers. Legumes include soybean, alfalfa, clover, pea, peanut, mesquite, mimosa and acacia, and because of their microbial symbionts, they can grow in nitrogen-poor soils. Leguminous plants are very diverse in morphology and habitat, ranging from arctic annuals to tropical trees.

The host range of the rhizobia can be narrow or broad depending upon the genetic make-up of the microorganisms. For example, *Rhizobium leguminosarum* bv. trifolii will only associate with clover, while *Rhizobium* species NGR234 has a broadover 230 host range. As you will read below, successful interaction between plant and microbe involves the exchange of chemical signals and part of the broad host-range of some rhizobia reflects the ability to sense and produce a range of such signals.

The formation of a rhizobia-plant association begins in the rhizosphere and ultimately results in the formation of a tumor-like growth on the roots of the plant called a **nodule**. Nodulation is a

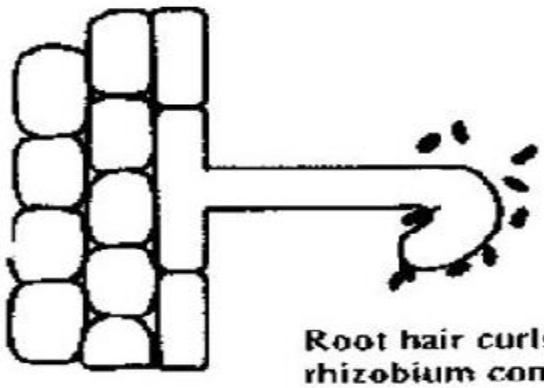
multi-step process with the exchange of a number of chemical signals between the plant and the microbe. The initial step involves the synthesis of specific compounds by the plant called flavonoids that are secreted as part of the root exudate (Fig. 6).

I. Chemical recognition of root and *Rhizobium*

Flavonoids, along with amino acids and dicarboxylic acids, serve as chemoattractants for the motile rhizobia, which migrate toward the plant root. Rhizobia then attach at target sites near young growing root hairs. Attachment is not specific to a particular legume and in most cases, appears to be mediated by outer surface macromolecules that vary between rhizobia. Single cells of *R. leguminosarum* bv. *viciae* bind loosely to plant lectins on the root using a Ca^{++}-dependent protein. This is followed by a strong binding step, where additional bacteria accumulate at the attachment site. Once bound the specific interactions between plant and microbe begin.

The other half of the conversation between plant and microbe involves what are called **nod factors,** which are oligosaccharides consisting of 3-5 sugars. These are the main molecular signal produced by the rhizobia that indicate to the plant that a suitable symbiotic microbe is nearby. Nod factors are hormone-like molecules, and their synthesis and excretion stimulate cortical cell division and root hair curling in the plant (Fig. 7).

2. Root hairs curl

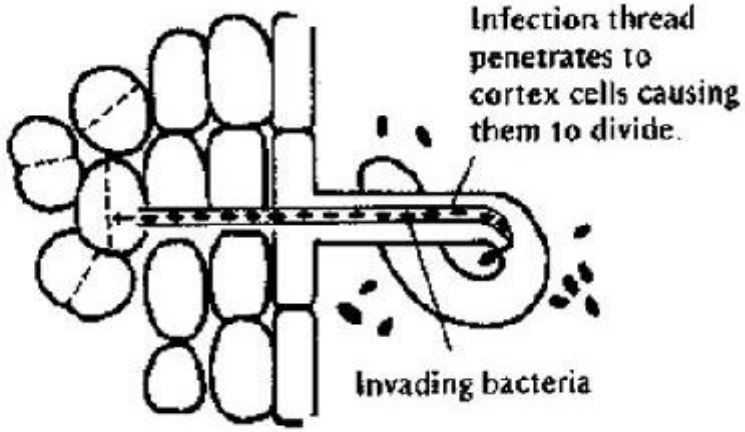


Root hair curls after rhizobium contact stops cell growth.

Curling of the root hair then causes entrapment of the microbes, followed by the formation of a local lesion in the plant cell wall. The rhizobia then begin to invaginate the plant plasma membrane and the plant lays new cell wall material around the growing invasion creating an infection thread (Fig. 8).

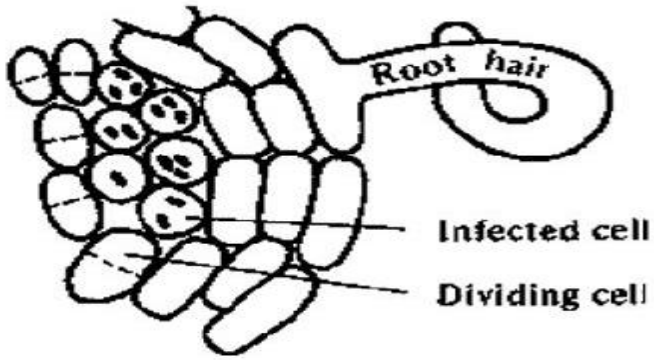
3. Formation of infection threads

4. Invasion of the roots by *Rhizobia*



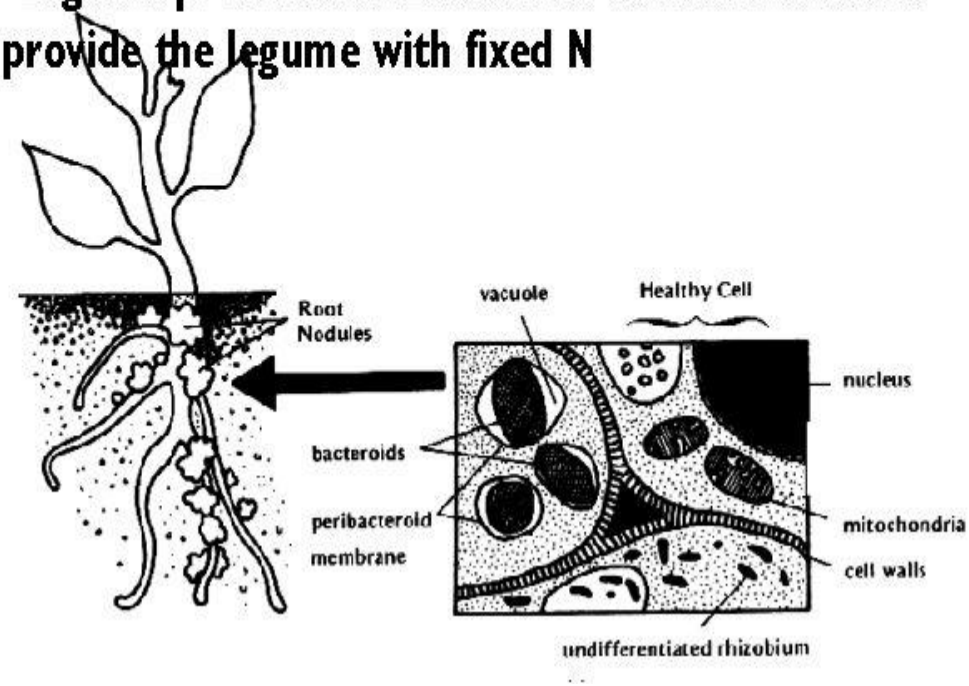
The thread of entering microbes is guided toward the multiplying cortical stem cells, which form a waiting habitat. Eventually, the rhizobia are deposited inside the cortical stem cells and these enlarge and create the mature nodule (Fig. 9- 10).

5. Nodule tissue forms



6. **Bacteria convert to bacteriods and begin to form nitrogenase enzyme**

7. **Legume provides *Rhizobia* with carbon. *Rhizobia* provide the legume with fixed N**



Once inside the nodule, rhizobia differentiate into amorphous cell shapes and begin expressing nitrogenase. The plant provides carbon and energy to the microbes and in return they fix nitrogen gas into ammonia, which is taken up for use by the host.

Rhizobia are not the only microbes capable of forming these associations. Other examples include the actinomycete *Frankia*, which forms nodules on actinorhizal plants such as Casuarinas tree.

The benefits for plants in such associations are fairly clear. They get exclusive rights to fixed nitrogen and other useful chemicals, at least when they support the formation of nodules. There is a bit of cost to this, of course, since the plant has to create the structures necessary for the nodules, as well as supply carbon and energy, but this is a very small price to pay for the return.

So what is the benefit to the rhizobia to enter into this very close relationship with plants? It is true that bacteria do increase their numbers *inside* the nodule, but this doesn't do the bacteria any good if they can never get out. In other words, this association only makes evolutionary sense for the bacteria if it is a way to increase their numbers in the environment. It happens that this issue has not been studied much, but it is assumed that as the plants senesce at the end of the growing season, the nodules deteriorate and release the bacteria.

So here is a bit of a curiosity. Presumably, this mutualistic interaction arose because of the benefits described for each party. There is a bit of a complication, however, in that, while the plant can select for bacteria that produce the right factors, it cannot demand good nitrogen fixation. One finds that many of the rhizobia in fields are very good at infecting plants and forming nodules, but are rather poor nitrogen-fixers. When infected by such rhizobia, the plant gets very little benefit. Interestingly, plants infected with such poor nitrogen-fixers allow more nodules to form

on their roots, suggesting that they are making an effort to create at least some productive associations. The rhizobia that don't fix nitrogen well don't provide much assistance for the plant. This failure probably does not hurt the bacteria, because the health of the plant, perhaps, doesn't affect the number of bacteria released at the end of the growing season, though this assumption might be wrong. We, therefore, have a highly complex relationship that has evolved, but which appears to lack some checks and balances on the participants. This is one of the reasons why some scientists have hypothesized that this relationship actually evolved from a pathogenic one. The theory is that rhizobia were initially pathogens, and plants found a way to control the infection and turn it toward their own well-being. In any event, it is an interesting and thought-provoking situation.

2- The Bacterial Flora of Humans

The Normal Flora

In a healthy animal, the internal tissues, e.g. blood, brain, muscle, etc., are normally free of microorganisms. On the other hand, the surface tissues, e.g. skin and mucous membranes, are constantly in contact with environmental organisms and become readily colonized by certain microbial species. The mixture of organisms regularly found at any anatomical site is referred to as the **normal flora**.

The normal flora of humans is exceedingly complex and consists of more than 200 species of bacteria. The makeup of the normal flora depends upon various factors, including genetics, age, sex, stress, nutrition and diet of the individual. The normal flora of humans consists of a few eukaryotic fungi and protists, and some methanogenic **Archaea** that colonize the lower intestinal tract, but the **Bacteria** are the most numerous and obvious microbial components of the normal flora. The distribution of the bacterial flora of humans is shown in Table 1. This table lists only a fraction of the total bacterial species that occur as normal flora of humans, and it does not express the total number or concentration of bacteria at any site.

TABLE 1. BACTERIA COMMONLY FOUND ON THE SURFACES OF THE HUMAN BODY.

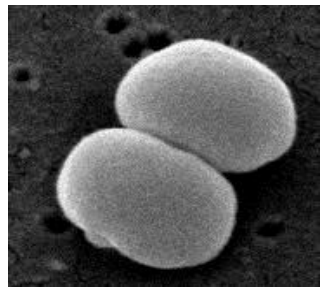
BACTERIUM	Skin	Conjunctiva	Nose	Pharynx	Mouth	Lower Intestine	Anterior urethra	Vagina
Staphylococcus epidermidis (1)	++	+	++	++	++	+	++	++
Staphylococcus aureus* (2)	+	+/-	+	+	+	++	+/-	+
Streptococcus mitis				+	++	+/-	+	+
Streptococcus salivarius				++	++			
Streptococcus mutans* (3)				+	++			
Enterococcus faecalis* (4)				+/-	+	++	+	+
Streptococcus		+/-	+/-	+	+			+/-

pneumoniae* (5)								
Streptococcus pyogenes* (6)	+/-	+/-		+	+	+/-		+/-
Neisseria sp. (7)		+	+	++	+		+	+
Neisseria meningitidis* (7)			+	++	+			+
Veillonellae sp.					+	+/-		
***Enterobacteriaceae** (Escherichia coli)* (8)		+/-	+/-	+/-	+	++	+	+
Proteus sp.		+/-	+	+	+	+	+	+
Pseudomonas aeruginosa* (9)				+/-	+/-	+	+/-	
Haemophilus influenzae* (10)		+/-	+	+	+			
Bacteroides sp.* (11)						++	+	+/-
Bifidobacterium bifidum (12)						++		
Lactobacillus sp. (13)				+	++	++		++
Clostridium sp.* (14)					+/-	++		
Clostridium tetani (15)						+/-		
Corynebacteria (16)	++	+	++	+	+	+	+	+
Mycobacteria	+		+/-	+/-		+	+	
Actinomycetes				+	+			
Spirochetes				+	++	++		
Mycoplasmas				+	+	+	+/-	+

++ = nearly 100 percent + = common +/- = rare * = potential pathogen

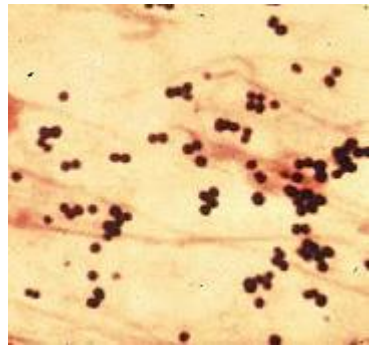
Table 1 Notes

(1) The staphylococci and corynebacteria occur at every site listed. *Staphylococcus epidermidis* is highly adapted to the diverse environments of its human host. *S. aureus* is a potential pathogen. It is a leading cause of bacterial disease in humans. It can be transmitted from the nasal membranes of an asymptomatic carrier to a susceptible host.



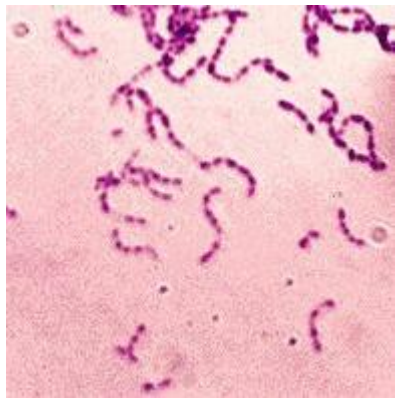
S. epidermidis. Scanning EM.

(2) Many of the normal flora are either pathogens or opportunistic pathogens, The asterisks indicate members of the normal flora a that may be considered major pathogens of humans.



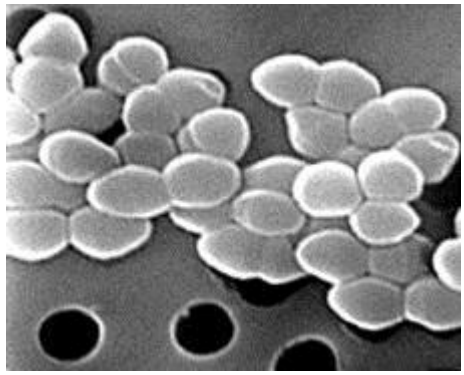
S. aureus. Gram stain.

(3) *Streptococcus mutans* is the primary bacterium involved in plaque formation and initiation of dental caries. Viewed as an opportunistic infection, dental disease is one of the most prevalent and costly infectious diseases in the United States.



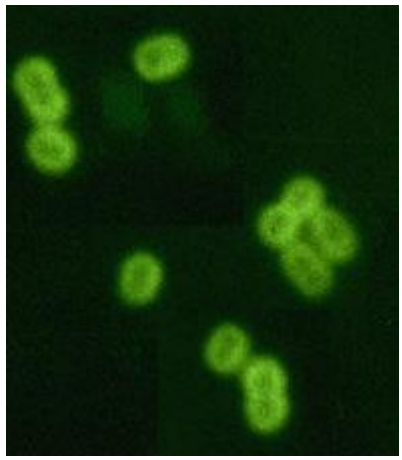
Streptococcus mutans. Gram stain.

(4) *Enterococcus faecalis* was formerly classified as *Streptococcus faecalis.* The bacterium is such a regular a component of the intestinal flora, that many European countries use it as the standard indicator of fecal pollution, in the same way we use *E. coli* in the U.S. In recent years, *Enterococcus faecalis* has emerged as a significant, antibiotic-resistant, nosocomial pathogen.



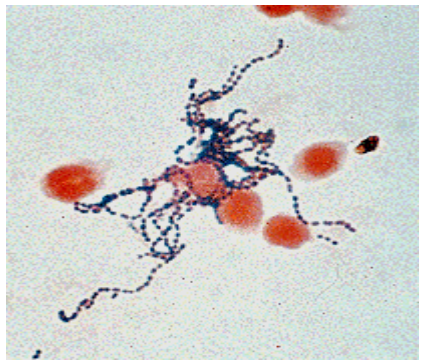
Vancomycin Resistant *Enterococcus faecalis.* Scanning E.M.

(5) *Streptococcus pneumoniae* is present in the upper respiratory tract of about half the population. If it invades the lower respiratory tract it can cause pneumonia. *Streptococcus pneumoniae* causes 95 percent of all bacterial pneumonia.



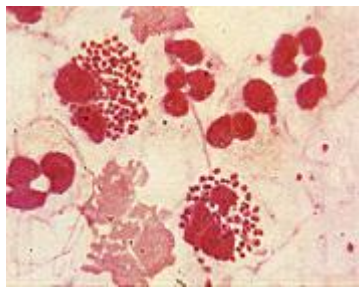
Streptococcus pneumoniae. Direct fluorescent antibody stain.

(6) *Streptococcus pyogenes* refers to the Group A, Beta-hemolytic streptococci.



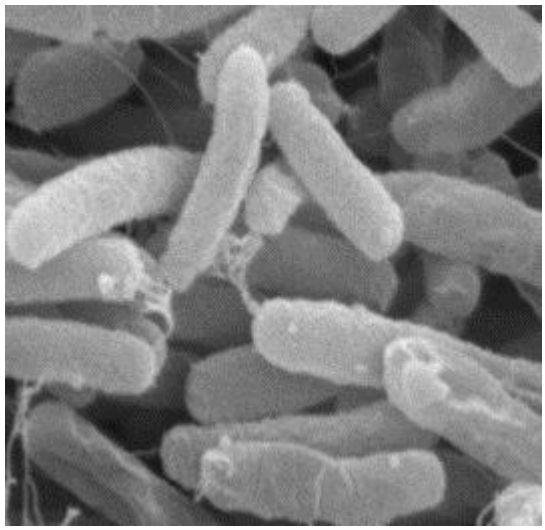
Streptococcus pyogenes. Gram stain.

(7) Gram-negative cocci, represented by various *Neisseria,* are frequent inhabitants of the upper respiratory tract, mainly the pharynx. *Neisseria meningitidis,* an important cause of bacterial meningitis, can colonize as well, until the host can develop active immunity against the pathogen.



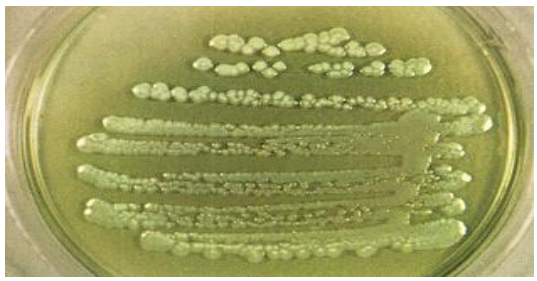
*Neisseria meningitidis.*Gram stain.

(8) While *E. coli* is a consistent resident of the small intestine, many other enteric bacteria may reside here as well, including *Klebsiella, Enterobacter* and *Citrobacter.* Some strains of *E. coli* are pathogens that cause intestinal infections, urinary tract infections and neonatal meningitis.



E. coli. Scanning E.M.

(9) *Pseudomonas aeruginosa* is the quintessential opportunistic pathogen of humans that can invade virtually any tissue. It is a leading cause of hospital-acquired (nosocomial) Gram-negative infections, but its source is often exogenous (from outside the host).

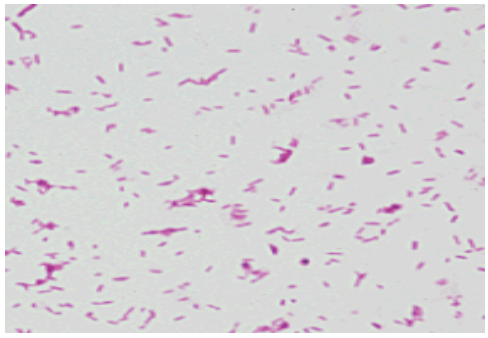


Colonies of *Pseudomonas aeruginosa* growing on an agar plate.

(10) *Haemophilus influenzae* is a frequent secondary invader to viral influenza, and was named accordingly. The bacterium was the leading cause of meningitis in infants and children until the recent development of the Hflu type B vaccine.

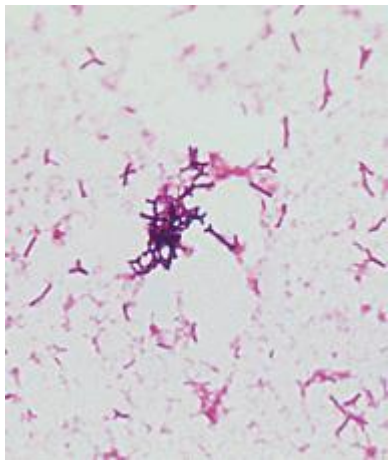
Haemophilus influenzae. Gram stain.

(11) The greatest number of bacteria are found in the lower intestinal tract, specifically the colon and the most prevalent bacteria are the *Bacteroides,* a group of Gram-negative, anaerobic, non-sporeforming bacteria. They have been implicated in the initiation colitis and colon cancer.



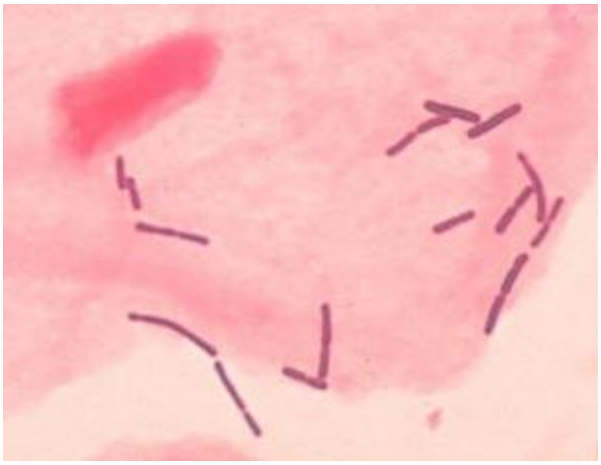
Bacteroides fragilis. Gram stain.

(12) Bifidobacteria are Gram-positive, non-sporeforming, lactic acid bacteria. They have been described as "friendly" bacteria in the intestine of humans. *Bifidobacterium bifidum* is the predominant bacterial species in the intestine of breast-fed infants, where it presumably prevents colonization by potential pathogens. These bacteria are sometimes used in the manufacture of yogurts and are frequently incorporated into probiotics.



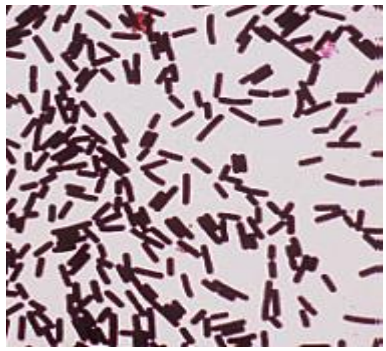
Bifidobacterium bifidum. Gram stain

(13) Lactobacilli in the oral cavity probably contribute to acid formation that leads to dental caries. *Lactobacillus acidophilus* colonizes the vaginal epithelium during child-bearing years and establishes the low pH that inhibits the growth of pathogens.



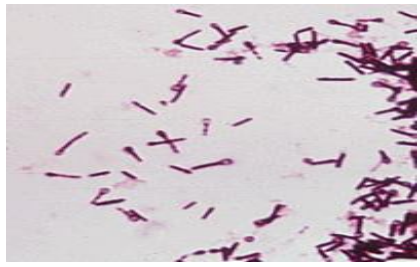
Lactobacillus species and a vaginal squaemous epithelial cell.

(14) There are numerous species of *Clostridium* that colonize the bowel. *Clostridium perfringens* is commonly isolated from feces. *Clostridium difficile* may colonize the bowel and cause "antibiotic-induced diarrhea" or pseudomembranous colitis.



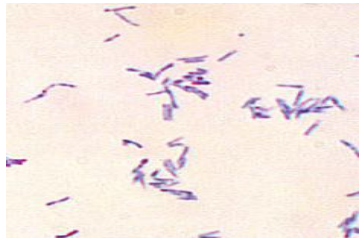
Clostridium perfringens. Gram stain.

(15) *Clostridium tetani* is included in the table as an example of a bacterium that is "transiently associated" with humans as a component of the normal flora. The bacterium can be isolated from feces of (up to) 25 percent of the population. The endospores are probably ingested with food and water, and the bacterium does not Colonize the intestine.



Clostridium tetani. Gram stain.

(16) The corynebacteria, and certain related propionic acid bacteria, are consistent skin flora. Some have been implicated as a cause of acne. *Corynebacterium diphtheriae, the* agent of diphtheria, was considered a member of the normal flora before the widespread use of the diphtheria toxoid, which is used to immunize against the disease.



Corynebacterium diphtheriae. Methylene blue stain.

Very little is known about the nature of the associations between humans and their normal flora, but they are thought to be dynamic interactions rather than associations of mutual indifference. Both host and bacteria are thought to derive benefit from each other, and the associations are, for the most part, **mutualistic**. The normal flora derives from the host a supply of nutrients, a stable environment and constant temperature, protection, and transport. The host obtains from the normal flora certain nutritional benefits, stimulation of the immune system, and colonization strategies that exclude potential pathogens at the site.

The normal flora is obviously adapted to their host (tissues), most probably by biochemical interactions between bacterial surface components (**ligands** or **adhesins**) and host cell molecular **receptors**. A great deal of information is available on the nature of adhesion of bacterial pathogens to animal cells and tissues, and reasonably similar mechanisms should apply to the normal flora.

In general, there are three explanations for why the normal bacterial flora are located at particular anatomical sites.

1. The normal flora exhibit a tissue preference or predilection for colonization.

2. Many, perhaps most, of the normal flora are able to **specifically colonize a particular tissue** or surface using their own surface components (e.g. capsules, fimbriae, cell wall

components, etc.) as specific ligands for attachment to specific receptors located at the colonization site.

3. Some of the indigenous bacteria are able to **construct bacterial biofilms** on a tissue surface, or they are able to colonize a biofilm built by another bacterial species. Many biofilms are a mixture of microbes, although one member is responsible for maintaining the biofilm and may predominate.

THE COMPOSITION OF THE NORMAL FLORA

The normal flora of corresponding anatomical sites in different animal species varies widely. Within a single species (e.g. humans) there is additional variation in the normal flora that is related to factors such as age, sex, diet and nutrition. Some bacteria are found regularly at particular anatomical locales; others are present only occasionally, or at certain times during life. Developmental changes in humans such as weaning, the eruption of the teeth, and the onset and cessation of ovarian functions, invariably affect the composition of the normal flora in the intestinal tract, the oral cavity, and the vagina, respectively. However, within the limits of these fluctuations, the bacterial flora of humans is sufficiently constant to a give general description of the situation.

It has been calculated that the normal human houses about 10^{12} bacteria on the skin, 10^{10} in the mouth, and 10^{14} in the gastrointestinal tract. The latter number is far in excess of the

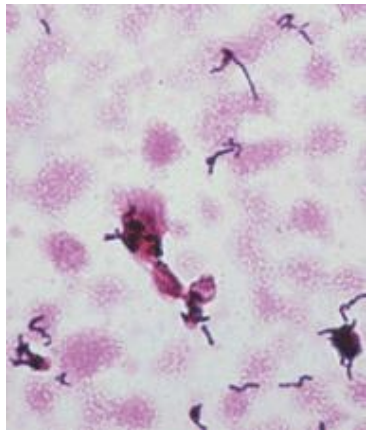
number of eukaryotic cells in all organs which comprise the human host.

Normal Flora of the Skin. The adult human is covered with approximately 2 square meters of skin. The density and composition of the normal flora of the skin vary with anatomical locale. The high moisture content of the axilla, and areas between the toes supports the activity and growth of relatively high densities of bacterial cells, but the density of bacterial populations at most other sites is fairly low, generally in 100s or 1000s per square cm. Qualitatively, the bacteria on the skin near any body orifice (e.g. mouth, nose and anus) may be similar to those in the orifice.

The majority of skin microorganisms are found in the most superficial layers of the epidermis and the upper parts of the hair follicles. They consist largely of micrococci (*Staphylococcus epidermidis* and *Micrococcus* sp.) and corynebacteria. These are generally non-pathogenic and considered to be commensal, although mutualistic and parasitic roles have been assigned to them. Sometimes potentially pathogenic *Staphylococcus aureus* is found on the face and hands.

Normal Flora of the Cunjunctiva. A variety of bacteria may be cultivated from the normal conjunctiva, but the number of organisms is usually small. *Staphylococcus epidermidis* and certain coryneforms (Propoinibacterium acnes) are dominant.

Staphylococcus aureus, some streptococci, *Haemophilus* sp. and *Neisseria* sp. are occasionally found. The conjunctiva is kept moist and healthy by the continuous secretions from the lachrymal glands. Blinking wipes the conjunctiva every few seconds mechanically washing away foreign objects including bacteria. Lachrymal secretions (tears) also contain bactericidal substances including lysozyme. There is little or no opportunity for microorganisms to colonize the conjunctiva without special mechanisms to attach to the epithelial surfaces and some ability to withstand attack by lysozyme. Pathogens which do infect the conjunctiva (e.g. *Neisseria gonorrhoeae* and *Chlamydia trachomatis*) are thought to be able to specifically attach to the conjunctival epithelium by means of sialic acid receptors on epithelial cells, but this is not certain.



Propionibacterium acnes

Normal Flora of the Respiratory Tract. The nares (nostrils) are always heavily colonized, predominantly with *Staphylococcus*

epidermidis and corynebacteria, and often (about 20% of the general population) with *Staphylococcus aureus,* this being the main carrier site of this important pathogen. The healthy sinuses, in contrast are sterile. A large number of bacterial species colonize the upper respiratory tract (nasopharynx). The predominant species are non-hemolytic and alpha-hemolytic streptococci and *Neisseria,* but sometimes pathogens such as *Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae* and *Neisseria meningitidis* colonize the pharynx.

The lower respiratory tract (trachea, bronchi, and pulmonary tissues) are virtually free of microorganisms, mainly because of the efficient cleansing action of the ciliated epithelium which lines the tract. Any bacteria reaching the lower respiratory tract are swept upward by the action of the mucociliary blanket that lines the bronchi, to be removed subsequently by coughing, sneezing, swallowing, etc. If the respiratory tract epithelium becomes damaged, as in bronchitis or viral pneumonia, the individual may become susceptible to infection by pathogens descending from the nasopharynx (e.g. *H. influenzae* or *S. pneumoniae*). The pathogen *Bordetella pertussis* is specifically able to colonize the tracheal epithelium of humans, allowing it to produce the disease, pertussis (whooping cough)

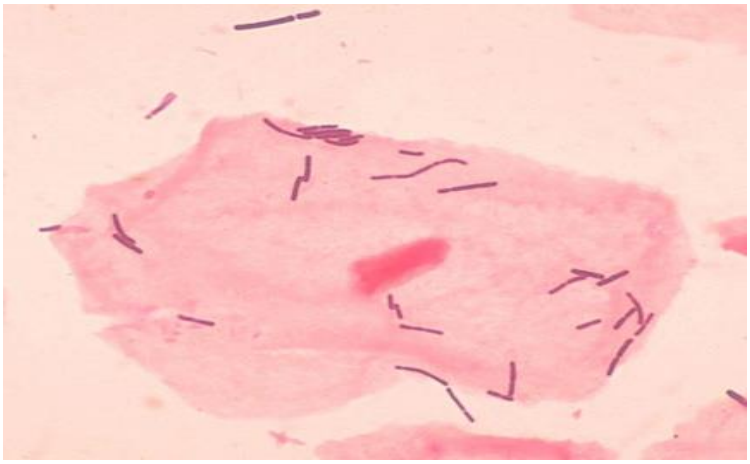
Normal flora of the Urogenital Tract. The urogenital tract consists of the **bladder**, the **ureter**, the **urethra** and the

genitalia. The bladder and internal regions of the ureter and urethra are typically devoid of microorganisms. The few found in urine cultures are probably contaminants from end of the urethra and the genitals, which have bacteria that are common on the skin and the colon. These include *S. epidermidis, Enterococcus faecalis, E. coli, Proteus spp.*, corynebacteria and streptococci.

The vagina of the healthy female is colonized soon after birth by typical microbes found on the skin and rectum, including corynebacteria, staphylococci, non-pyogenic streptococci, *E. coli*, and a lactic acid bacterium named Dderleins bacilli (most likely, *Lactobacillus acidophilus*). After the onset of puberty and throughout reproductive life, circulating estrogen causes the secretion of glycogen in the vagina. Metabolism of glycogen to lactic acid by Dderleins bacilli drops the pH and inhibits colonization by all except this microbe and a few others. Significantly, this helps prevent colonization by *Candida albicans,* the cause of yeast infections.

Urine is normally sterile, and since the urinary tract is flushed with urine every few hours, microorganisms have problems gaining access and becoming established. The flora of the anterior urethra, as indicated principally by urine cultures, suggests that the area may be inhabited by a relatively consistent normal flora consisting of S*taphylococcus epidermidis, Enterococcus faecalis* and some alpha-hemolytic streptococci. Their numbers are not plentiful,

however. In addition, some enteric bacteria (e.g. *E. coli, Proteus*) and corynebacteria, which are probably contaminants from the skin, vulva or rectum, may occasionally be found at the anterior urethra.



Lactic acid bacteria, possibly Doderlein's bacillus, in ascociation with a vaginal epithelial cell.

Normal Flora of the Human Oral Cavity

The presence of nutrients, epithelial debris, and secretions makes the mouth a favorable habitat for a great variety of bacteria. Oral bacteria include streptococci, lactobacilli, staphylococci and corynebacteria, with a great number of anaerobes, especially bacteroides.

The mouth presents a succession of different ecological situations with age, and this corresponds with changes in the composition of the normal flora. At birth the oral cavity is composed solely of the

soft tissues of the lips, cheeks, tongue and palate, which are kept moist by the secretions of the salivary glands. At birth the oral cavity is sterile but rapidly becomes colonized from the environment, particularly from the mother in the first feeding. *Streptococcus salivarius* is dominant and may make up 98% of the total oral flora until the appearance of the teeth (6 - 9 months in humans). The eruption of the teeth during the first year leads to colonization by *S. mutans* and *S. sanguis*. These bacteria require a nondesquamating (nonepithelial) surface in order to colonize. They will persist as long as teeth remain. Other strains of streptococci adhere strongly to the gums and cheeks but not to the teeth. The creation of the gingival crevice area (supporting structures of the teeth) increases the habitat for the variety of anaerobic species found. The complexity of the oral flora continues to increase with time, and bacteroides and spirochetes colonize around puberty.

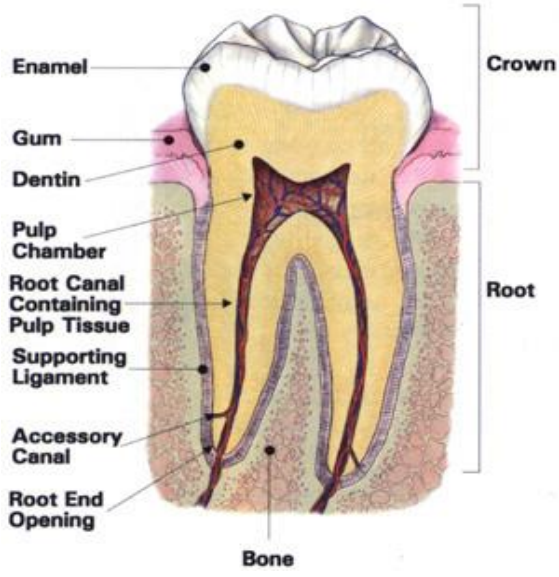
Several oral bacteria have been implicated in diseases of the oral cavity, including dental caries and gum diseases such as gingivitis and periodontitis.

The oral bacteria can invade compromised tissues in their hosts and produce disease outside the oral cavity. Oral bacteria invade deeper tissues they may cause abscesses of alveolar bone, lung, brain, or the extremities. Such infections usually contain mixtures of bacteria with *Bacteroides melaninogenicus* often playing a dominant role. If oral streptococci are introduced into wounds

created by dental manipulation or treatment, they may adhere to heart valves and initiate subacute bacterial endocarditis.

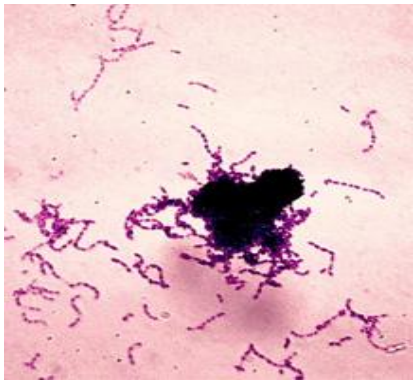
Dental Plaque, Caries, and Gingivitis

Dental plaque, which is material adhering to the teeth, consists of bacterial cells (60-70% the volume of the plaque), salivary polymers, and bacterial extracellular products. Plaque is a naturally-constructed biofilm, in which the consortia of bacteria may reach a thickness of 300-500 cells on the surfaces of the teeth. These accumulations subject the teeth and gingival tissues to high concentrations of bacterial metabolites, which result in dental disease.



Cross section of a tooth illustrating the various structural regions susceptible to colonization or attack by microbes.

In relationship dental caries and periodontitis oral bacteria produce three forms of toxins: soluble protein toxins (exotoxins) which work as extracellular enzymes; endotoxins which are lippolysaccharides in the Gran-negative cell wall; products of their metabolism (metabolites) which may be toxic. The latter include volatile sulfur compounds such as hydroghen sulfide and methylmercaptan; polyamines with names like putrescine, cadaverine and spermidine; and fatty acids such as propionic acid and butyric acid. By far the dominant bacterial species in dental plaque are *Streptococcus sanguis* and *Streptococcus mutans,* both of which are considered responsible for plaque.

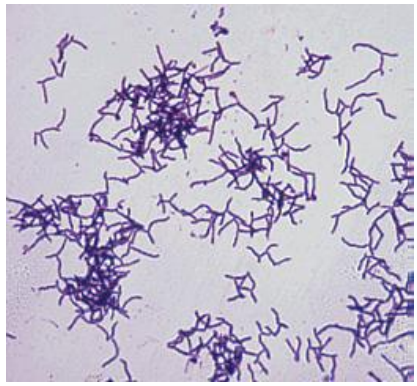


Streptococcus mutans. **Gram stain.**

Plaque formation is initiated by a weak attachment of the streptococcal cells to salivary glycoproteins forming a pellicle on the surface of the teeth. This is followed by a stronger attachment by means of extracellular sticky polymers of glucose (glucans) which are synthesized by the bacteria from dietary sugars (principally sucrose). An enzyme on the cell surface of

Streptococcus mutans, glycosyl transferase, is involved in initial attachment of the bacterial cells to the tooth surface and in the conversion of sucrose to dextran polymers (glucans) which form plaque.

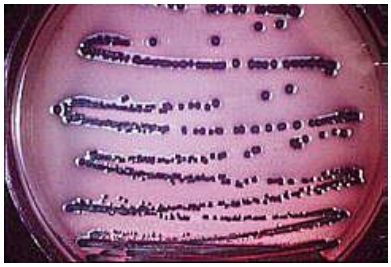
Dental Caries is the destruction of the enamel, dentin or cementum of teeth due to bacterial activities. Caries are initiated by direct demineralization of the enamel of teeth due to lactic acid and other organic acids which accumulate in dental plaque. Lactic acid bacteria in the plaque produce lactic acid from the fermentation of sugars and other carbohydrates in the diet of the host. Streptococcus mutans has most consistently been associated with the initiation of dental caries, but other lactic acid bacteria are probably involved as well. These organisms normally colonize the occlusal fissures and contact points between the teeth, and this correlates with the incidence of decay on these surfaces.



Actinomyces israelii

Lactobacilli, *Actinomyces,* and various proteolytic bacteria are commonly found in human carious dentin and cementum, which suggests that they are secondary invaders that contribute to the progression of the lesions.

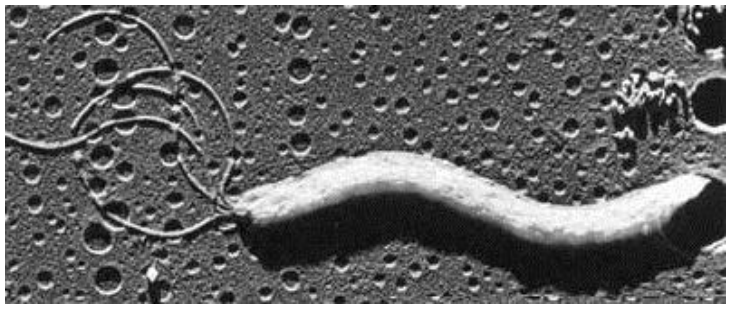
Normal Flora of the Gastrointestinal Tract.



Colonies of *E. coli* growing on EMB agar.

The bacterial flora of the GI tract of animals has been studied more extensively than that of any other site. The composition differs between various animal species. In humans, there are differences in the composition of the flora which are influenced by age, diet, cultural conditions, and the use of antibiotics. The latter greatly perturbs the composition of the intestinal flora.

Because of the high acidity of the gastric juice very few bacteria (mainly acid-tolerant lactobacilli) can be cultured from the normal stomach. However, at least half the population in our country is colonized by a pathogenic bacterium, *Helicobacter pylori.* Since the 1980s, this bacterium has been known to be the cause of gastric ulcers, and it is probably a cause of gastric and duodenal cancer as well.



Helicobacter pylori

The proximal small intestine has a relatively sparse Gram-positive flora, consisting mainly of lactobacilli and *Enterococcus faecalis.* This region has about 10^5 - 10^7 bacteria per ml of fluid. The distal part of the small intestine contains greater numbers of bacteria (10^8/ml) and additional species including coliforms and *Bacteroides,* in addition to lactobacilli and enterococci. The flora of the large intestine (colon) is qualitatively similar to that found in feces. Populations of bacteria in the colon reach levels of 10^{11}/ml feces. Coliforms become more prominent, and enterococci, clostridia and lactobacilli can be regularly found, but the predominant species are anaerobic *Bacteroides* and anaerobic lactic acid bacteria in the genus *Bifidobacterium* (*Bifidobacterium bifidum*). These organisms may outnumber *E. coli* by 1,000:1 to 10,000:1. It is now known that significant numbers of anaerobic methanogenic bacteria (up to 10^{10}/gm) also reside in the colon of humans. The range of incidence of certain bacteria in the large intestine of humans is shown below.

At birth the entire intestinal tract is sterile, but bacteria enter with the first feed. The initial colonizing bacteria vary with the food source of the infant. In breast-fed infants bifidobacteria account for more than 90% of the total intestinal bacteria. *Enterobacteriaceae* and enterococci are regularly present, but in low proportions, while bacteroides, staphylococci, lactobacilli and clostridia are practically absent. In bottle-fed infants, bifidobacteria are not predominant. When breast-fed infants are switched to a diet of cow's milk or solid food, bifidobacteria are progressively joined by enterics, bacteroides, enterococci lactobacilli and clostridia. Apparently, human milk contains a growth factor that enriches for growth of bifidobacteria, and these bacteria play an important role in preventing colonization of the infant intestinal tract by non indigenous or pathogenic species.

Clostridium difficile. Gram stain.

THE BENEFITS OF THE NORMAL FLORA

Many microorganisms enjoy the lush environment our bodies provide for them, but what are the benefits for the host?

1. The normal flora synthesize and excrete vitamins in excess of their own needs, which can be absorbed as nutrients by the host. For example, enteric bacteria secrete Vitamin K and Vitamin B12, and lactic acid bacteria produce certain B-vitamins. Germ-free animals may be deficient in Vitamin K to the extent that it is necessary to supplement their diets.

2. The normal flora prevent colonization by pathogens by competing for attachment sites or for essential nutrients. This is thought to be their most important beneficial effect, which has been demonstrated in the oral cavity, the intestine, the skin, and the vaginal epithelium. In some experiments, germ-free animals can be infected by 10 *Salmonella* bacteria, while the infectious dose for conventional animals is near 10^6 cells.

3. The normal flora may antagonize other bacteria through the production of substances which inhibit or kill nonindigenous species. The intestinal bacteria produce a variety of substances ranging from relatively nonspecific fatty acids and peroxides to highly specific bacteriocins, which inhibit or kill other bacteria.

4. The normal flora stimulate the development of certain tissues, i.e., the caecum and certain lymphatic tissues (Peyer's

patches) in the GI tract. The caecum of germ-free animals is enlarged, thin-walled, and fluid-filled, compared to that organ in conventional animals.

5. The normal flora stimulate the production of cross-reactive antibodies. Since the normal flora behave as antigens in an animal, they induce an immunological response, in particular, an antibody-mediated immune (AMI) response. Low levels of antibodies produced against components of the normal flora are known to cross react with certain related pathogens, and thereby prevent infection or invasion. Antibodies produced against antigenic components of the normal flora are sometimes referred to as "natural" antibodies, and such antibodies are lacking in germ-free animals.

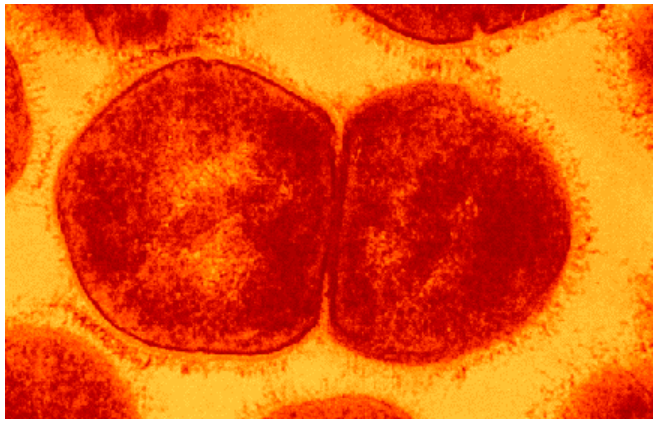
II- PATHOGENIC INTERACTION

BACTERIAL STRUCTURE IN RELATIONSHIP TO PATHOGENICITY

The Importance of the Bacterial Surface

All of the various surface components of a bacterial cell are important in its ecology since they mediate the contact of the bacterium with its environment. The only "senses" that a bacterium has result from its immediate contact with its environment. It must use its surface components to assess the environment and respond in a way that supports its own existence and survival in that environment. The surface properties of a

bacterium are determined by the exact molecular composition of its membrane and cell wall, including LPS, and the other surface structures such as flagella, fimbriae and capsules.

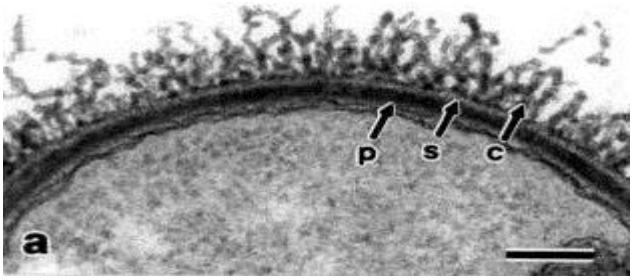


The surface of *Streptococcus pyogenes*. High magnification electron micrograph of an ultra-thin section. At this magnification, especially in the cell on the left, the cell wall and cell surface fibrils, consisting mainly of M protein, are well defined. The interdigitaion of these fibrils between neighboring cells of different chains can also be seen.

Bacterial **surface components** may have a primary biological function that has nothing to do with pathogenicity. Thus, the function of the LPS in the outer membrane of Gram-negative bacteria has to do with its permeability characteristics, rather than its toxicity for animals. However, there are endless examples wherein a bacterial surface component plays an indispensable role in the pathogenesis of infectious disease. Bacterial surface structures may act as (1) **permeability barriers** that allow selective passage of nutrients and exclusion of harmful substances (e.g. antimicrobial agents); (2) **adhesins** used to attach or adhere to specific surfaces or tissues; (3) **enzymes** to mediate specific

reactions on the cell surface important in the survival of the organism; (4) **protective structures against phagocytic engulfment** or killing; (5) **antigenic disguises**; (6) "**sensing proteins**" that can respond to temperature, osmolarity, salinity, light, oxygen, nutrients, etc., resulting in a **molecular signal** to the genome of the cell that will cause expression of some determinant of virulence (e.g. an exotoxin).

In medical situations, the surface components of bacterial cells are major determinants of virulence for many pathogens. Pathogens can colonize tissues, resist phagocytosis and the immune response, and induce inflammation, complement activation and immune responses in animals by means of various structural components.



The surface of *Bacillus anthracis*.

The bacterial membrane is evident as the innermost layer surrounding the cytoplasm. P denotes the peptidoglycan cell wall. S refers to the S-layer which consists of two proteins including the major antigen. C denotes the poly-D-glutamic acid capsule that is exterior to and completely covers the S-layer proteins.

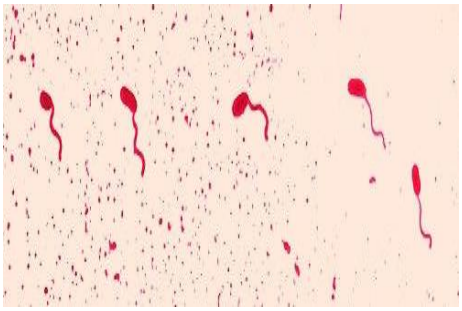
The Structure of the Bacterial Surface

Structurally, a bacterial cell has **three architectural regions: appendages** (proteins attached to the cell surface) in the form of flagella and fimbriae; a **cell envelope** consisting of a capsule, cell wall and plasma membrane; and a **cytoplasmic region** that contains the cell genome (DNA) and ribosomes and various sorts of inclusions. The surface components of a bacterium are the constituents of its cell envelope and appendages.

Flagella are filamentous protein structures attached to the cell surface that provide swimming movement for most motile bacterial cells. The diameter of a bacterial flagellum is about 20 nanometers, well-below the resolving power of the light microscope. The flagellar filament is rotated by a motor apparatus in the plasma membrane allowing the cell to swim in fluid environments. Bacterial flagella are powered by proton motive force (chemiosmotic potential) established on the bacterial membrane.

Bacteria are known to exhibit a variety of types of **tactic behavior**, i.e., the ability to move (swim) in response to environmental stimuli. For example, during **chemotaxis** a bacterium can sense the quality and quantity of certain chemicals in its environment and swim towards them (if they are useful nutrients) or away from them (if they are harmful substances). During **aerotaxis**, bacteria swim toward or away from O_2.

For a few pathogens motility is known to be a determinant of virulence. In the case of *Vibrio cholerae*, the vibrios apparently swim (laterally) into the intestinal mucosa to avoid being flushed out by the peristaltic action of the gut. Flagella are antigenic, and therefore, vulnerable to attack by host antibody molecules. Antibody molecules directed against flagellar antigens can agglutinate and/or immobilize bacterial cells, or possibly opsonize them from phagocytosis, which presumably would aid in host defense.



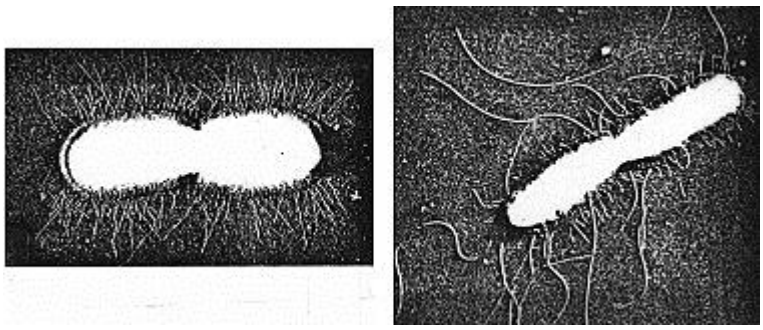
***Vibrio cholerae*. Liefson's flagellar stain. Bacterial flagella are below the resolving power of the light microscope. In order to be visualized, the bacteria must be reacted with a stain that precipitates along the flagellar filaments, which increases their effective diameter to the point of resolution. *Vibrio cholerae* is motile by means of a single polar flagellum inserted into one pole of the cell.**

Fimbriae

Fimbriae and **pili** are interchangeable terms used to designate short, hair-like structures on the surfaces of procaryotic cells. Like flagella, they are composed of protein. Fimbriae are shorter and stiffer than flagella, and slightly smaller in diameter. Generally, fimbriae have nothing to do with bacterial movement (there are exceptions, e.g twitching movement on *Pseudomonas*). Fimbriae are very common in Gram-negative bacteria, but occur in some archaea and Gram-positive bacteria as well. Fimbriae are most often involved in adherence of bacteria to surfaces, substrates and

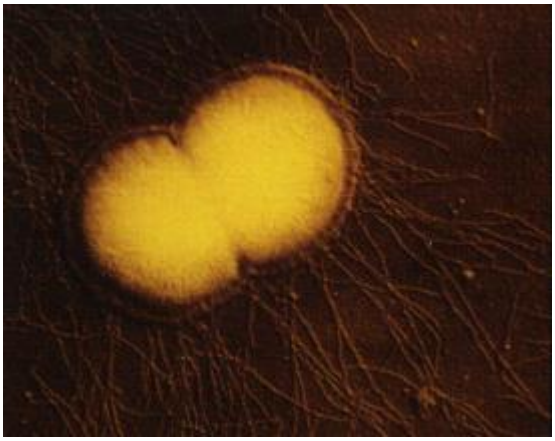
other cells or tissues in nature. In *E. coli*, a specialized type of pilus, the **F or sex pilus**, mediates the transfer of DNA between mating bacteria during the process of **conjugation**, but the function of the smaller, more numerous common pili is quite different.

Common pili (almost always called **fimbriae**) are usually involved in specific adherence (attachment) of procaryotes to surfaces in nature. In medical situations, they are major determinants of bacterial virulence because they allow pathogens to attach to (colonize) tissues and/or to resist attack by phagocytic white blood cells. For example, pathogenic *Neisseria gonorrhoeae* adheres specifically to the human cervical or urethral epithelium by means of its fimbriae; enterotoxigenic strains of *E. coli* adhere to the mucosal epithelium of the intestine by means of specific fimbriae; the M-protein and associated fimbriae of *Streptococcus pyogenes* are involved in adherence and to resistance to engulfment by phagocytes.



Fimbriae (common pili) and flagella on the surface of bacterial cells.

Left: dividing *Shigella* enclosed in fimbriae. The structures are probably involved in the bacterium's ability to adhere to the intestinal surface. Right: dividing pair of *Salmonella* displaying both its peritrichous flagella and its fimbriae. The fimbriae are much shorter and slightly smaller in diameter than flagella. Both *Shigella* and *Salmonella* are enteric bacteria that cause different types of intestinal diarrheas.The bacteria can be differentiated by a motility test. *Salmonella* is motile; *Shigella* is nonmotile.



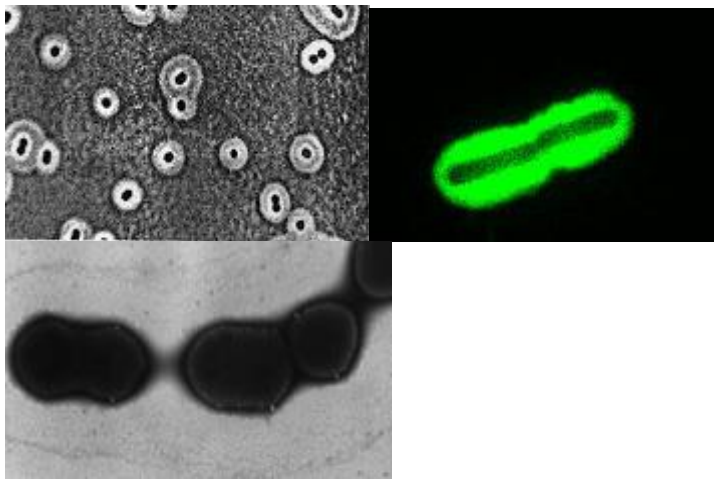
***Neisseria gonorrhoeae*. Electron micrograph. This pathogen utilizes its fimbriae in order to initially colonize the urethral or cervical epithelium.**

Most bacteria contain some sort of a polysaccharide layer outside of the cell wall or outer membrane. In a general sense, this layer is called a **capsule**. A true capsule is a discrete detectable layer of polysaccharides deposited outside the cell wall. A less discrete structure or matrix which embeds the cells is a called a **slime layer**. A type of capsule found in bacteria called a **glycocalyx** is a thin layer of tangled polysaccharide fibers which is almost always observed on the surface of cells growing in nature (as opposed to the laboratory). Capsules, slime layers, and glycocalyx are known to mediate specific or non specific adherence of bacteria to

particular surfaces. Capsules are known to protect bacteria from engulfment by predatory phagocytes and from attack by antimicrobial agents.

In nature, and in many medical situations, colonies of bacteria construct and live in a **biofilm**, made up principally of capsule material. A biofilm usually consists of a consortium (mixture) of bacteria living in a matrix of slime which is secreted by one of the bacterial members. Dental plaque is an example of a natural biofilm, as is a slimy mass of bacteria attached to a rock in a mountain stream. In medical situations, bacteria in a biofilm may have certain advantages over planktonic counterparts. For example, biofilm bacteria may be less susceptible to phagocytes, drugs, or neutralizing antibodies.

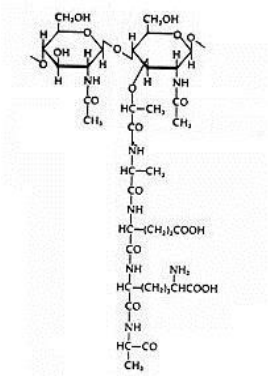
Many polysaccharide capsules possess an antigenic epitope so they will induce and react with host antibodies. Where the capsule is a main determinant of virulence of a pathogen (e.g. *Streptococcus pneumoniae*) antibodies against the bacterium neutralize its virulence.



Bacterial capsules visualized by various techniques. Left. *Streptococcus pneumoniae* -India ink capsule outline; Middle. *Bacillus anthracis* -fluorescent-tagged antibody; Right. *Streptococcus pyogenes* -transmission electron micrograph, *S. pneumoniae* capsular material is composed of polysaccharide. The capsule is the pathogen's most important determinant of virulence because it allows the bacterial cells to escape phagocytes in the lung. The *B. anthracis* capsule is composed of poly-D-glutamic acid. Its capsule is antiphagocytic, and it protects the bacteria from complement- mediated lysis in serum or blood. The capsule of *S. pyogenes* is composed of hyaluronic acid, the same polymer as found in human connective tissue. The capsule is an antigenic disguise that prevents recognition of the streptococci by phagocytes or the immune system.

The **cell wall** of a bacterium is an essential structure that protects the delicate cell protoplast from osmotic lysis. The cell wall of Bacteria consists of a polymer of disaccharides cross-linked by short chains of amino acids (peptides). This molecule is a type of **peptidoglycan** called **murein**. Murein is unique to Bacteria. In the **Gram-positive bacteria,** the cell wall is thick (15-80 nanometers), consisting of several layers of peptidoglycan complexed with molecules called **teichoic acids**. In the Gram-

negative bacteria, the cell wall is relatively thin (10 nanometers) and is composed of a single layer of peptidoglycan surrounded by a membranous structure called the **outer membrane**. Murein is a substance unique in nature to bacterial cell walls. Also, the outer membrane of Gram-negative bacteria invariably contains a unique component, **lipopolysaccharide (LPS or endotoxin)**, which is toxic to animals.

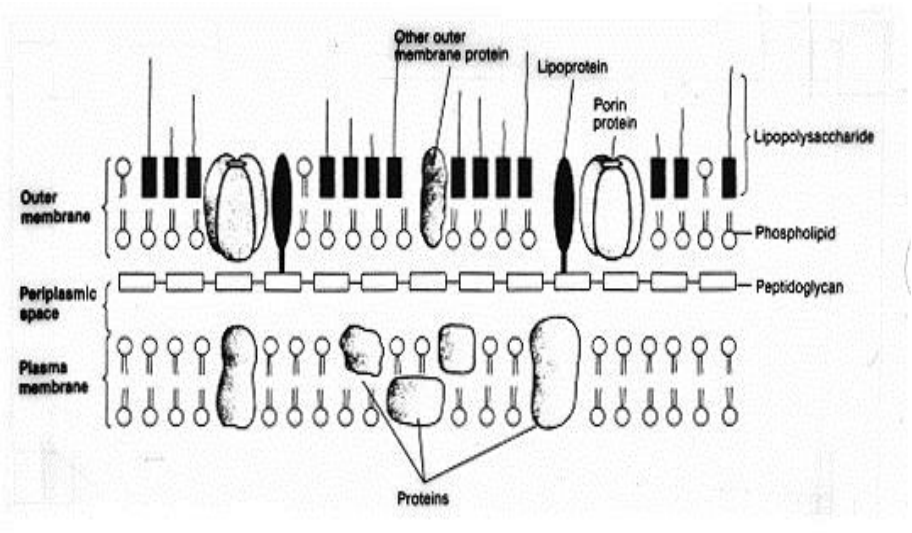


The structure of the muramic acid subunit in the peptidoglycan *Escherichia. coli.* The molecule consists of N-acetyl glucoasamine (NAG) attached (via a beta 1,4 link) to N-acetyl-muramic acid (NAM). Attached to the NAM is a peptide chain, which (in the case of *E. coli,* as illustrated) consists of L-alanine, D-glutamate, diaminopimelic acid and D-alanine. Some antibiotics, including bacitracin, act by blocking the synthesis of the muramic acid subunit. Penicillin and related antibiotics (beta lactams), as well as vancomycin, block the assembly of the muropeptide subunits into the peptidoglycan polymer.

The cell wall is a complicated structure, fundamentally different in Gram-positive and Gram-negative bacteria. Cell wall components are major determinants of virulence in both groups of bacteria. **Endotoxin**, inherent to all Gram-negative bacteria, is toxic to animals in a variety of ways. Peptidoglycan and LPS, as well as some teichoic acids, induce the alternate complement pathway

leading to inflammation. Teichoic acids and **O-specific polysaccharides** may be used as adhesins by Gram-positive and Gram-negative bacteria, respectively. Some cell wall components protect against phagocytic engulfment or digestion. Variations in the macromolecular structure of cell wall components may be at the basis of **antigenic variation** as well as specific host resistance to pathogens.

The essential outer membrane of Gram-negative bacteria is the target for attack by complement, hydrophobic agents and certain antibiotics. Murein (peptidoglycan) is dismantled by a host enzyme, lysozyme, found in most body fluids. Several antibiotics, mainly the beta lactams, exert their antimicrobial effect by blocking the synthesis and assembly of peptidoglycan.

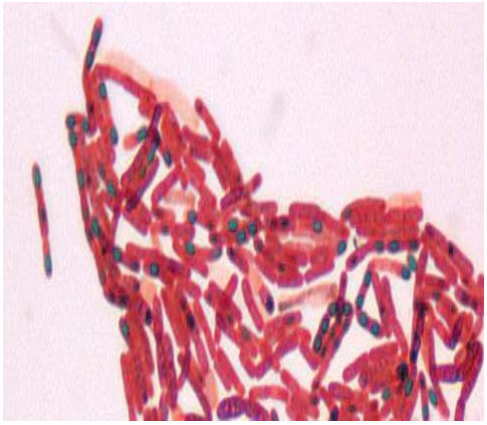


Schematic drawing the outer membrane of a Gram-negative bacterium

The **membranes** of Bacteria are structurally similar to the cell membranes of eukaryotes, except that bacterial membranes consist of saturated or monounsaturated fatty acids (never polyunsaturated fatty acids) and do not normally contain sterols. The plasma membrane is an exceptionally dynamic structure in bacteria which mediates permeability, transport, secretion and energy generation. In terms of pathogenesis of a bacterium, it is absolutely dependent upon the integrity and function of its plasma membrane. The membrane might be responsible for secretion of toxins, resistance to antimicrobial agents, tactic responses or sensing other environmental signals to turn on or off genes for virulence.

Endospores are bacterial structures (resting cells) formed by a few groups of bacteria as intracellular structures, but ultimately, they are released as free endospores. Biologically, endospores are a fascinating type of cell. Endospores exhibit no signs of life, being described as cryptobiotic. They are highly resistant to environmental stresses such as high temperature (some endospores can be boiled for hours and retain their viability), irradiation, strong acids, disinfectants, etc. They are probably the most durable cell produced in nature. Although cryptobiotic, they retain viability indefinitely such that under appropriate environmental conditions, they germinate back into vegetative cells.

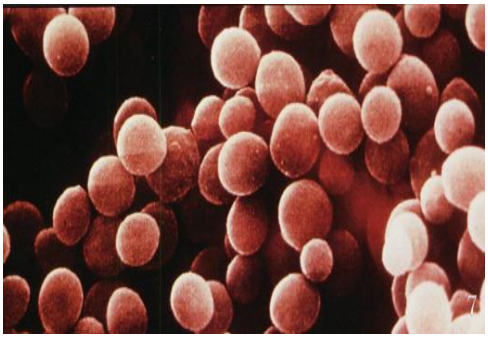
Endospores are formed by two genera of Gram-positive bacteria: *Bacillus,* the aerobic sporeformers, and *Clostridium,* the anaerobic sporeformers. Both genera contain pathogens, and the endospores produced by these bacteria invariably play some role in the toxicity, transmission or survival of the pathogen.



Spore stain of a *Bacillus* species. Mature spores stain green whether free or still inside the vegetative sporangium. Vegetative cells and sporangia stain red. The Schaeffer-Fulton stain technique was applied. The primary stain, malachite green, is forced into the spores by heating the prepared slide to boiling for 4-5 minutes. After washing, the vegetative cells are counterstained with safranine.

NOW IT IS OUR TIME TO DESCRIBE, IN DETAIL, THE CONSEQUENCES OF INFECTION WITH SPECIFIC PATHOGENS.

Ex., *Staphylococcus*



Staphylococcus aureus. E.M.

The Staphylococci

Staphylococci are Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes. Bacteriological culture of the nose and skin of normal humans invariably yields staphylococci. In 1884, Rosenbach described the two pigmented colony types of staphylococci and proposed the appropriate nomenclature: *Staphylococcus aureus* (yellow) and *Staphylococcus albus* (white). The latter species is now named *Staphylococcus epidermidis.* Although more than 20 species of *Staphylococcus* are described in Bergey's Manual (2001), only *Staphylococcus aureus* and *Staphylococcus epidermidis* are significant in their interactions with humans. *S. aureus* colonizes mainly the nasal passages, but it may be found regularly in most other anatomical locales. *S epidermidis* is an inhabitant of the skin.

Staphylococcus aureus forms a fairly large yellow colony on rich medium, *S. epidermidis* has a relatively small white colony. *S. aureus* is often hemolytic on blood agar; *S. epidermidis* is non hemolytic. Staphylococci are facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid. The bacteria are catalase-positive and oxidase-negative. *S. aureus* can grow at a temperature range of 15 to 45 degrees and at NaCl concentrations as high as 15 percent. Nearly all strains of *S. aureus* produce the enzyme coagulase: nearly all strains of *S. epidermidis* lack this enzyme. *S. aureus* should always be considered a potential pathogen; most strains of *S. epidermidis* are nonpathogenic and may even play a protective role in their host as normal flora. *Staphylococcus epidermidis* may be a pathogen in the hospital environment.

Staphylococci are perfectly spherical cells about 1 micrometer in diameter. They grow in clusters because staphylococci divide in two planes. The configuration of the cocci helps to distinguish staphylococci from streptococci, which are slightly oblong cells that usually grow in chains (because they divide in one plane only). The catalase test is important in distinguishing streptococci (catalase-negative) from staphylococci, which are vigorous catalase-producers. The test is performed by adding 3% hydrogen peroxide to a colony on an agar plate or slant. Catalase-positive cultures produce O_2 and bubble at once. The test should not be done on blood agar because blood itself contains catalase.

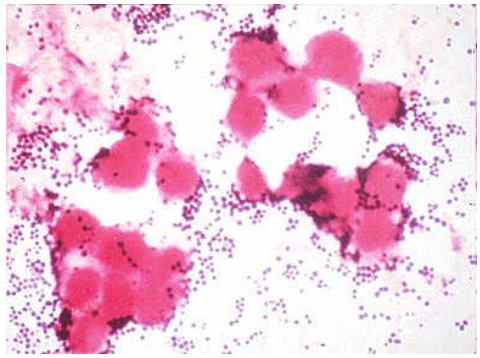


FIGURE 1. Gram stain of *Staphylococcus aureus* in pustular exudates

The important phenotypic characteristics of *Staphylococcus aureus*

Gram-positive, cluster-forming coccus
nonmotile, nonsporeforming facultative anaerobe
fermentation of glucose produces mainly lactic acid
ferments mannitol (distinguishes from *S. epidermidis)*
catalase positive
coagulase positive
golden yellow colony on agar
normal flora of humans found on nasal passages, skin and mucous membranes
pathogen of humans, causes a wide range of suppurative infections, as well as food poisoning and toxic shock syndrome

Pathogenesis of *S. aureus* infections

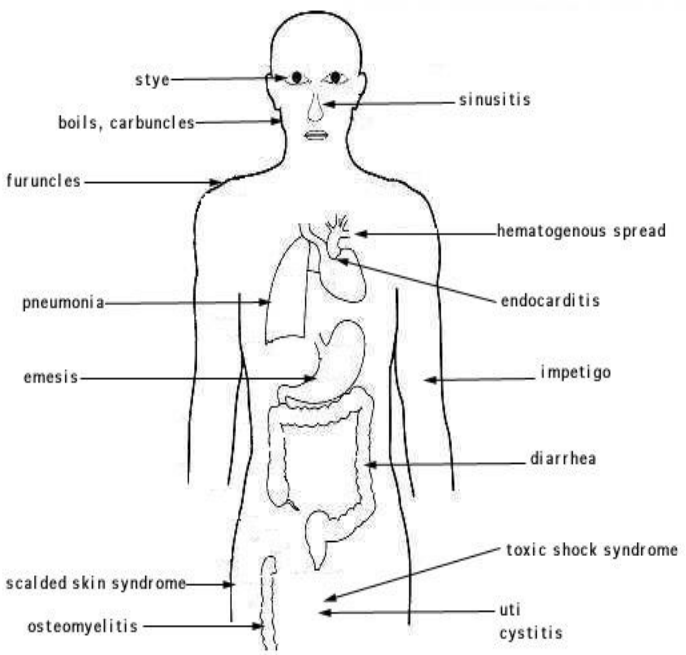
Staphylococcus aureus causes a variety of suppurative (pus-forming) infections and toxinoses in humans. It causes superficial skin lesions such as **boils**, **styes** and **furunculosis**; more serious infections such as **pneumonia**, **mastitis**, **phlebitis**, **meningitis**, and **urinary tract infections**; and deep-seated infections, such as **osteomyelitis** and **endocarditis**. *S. aureus* is a major cause of **hospital acquired (nosocomial) infection** of surgical

wounds and infections associated with indwelling medical devices. *S. aureus* causes **food poisoning** by releasing enterotoxins into food, and **toxic shock syndrome** by release of superantigens into the blood stream.

S. aureus expresses many potential **virulence factors**: (1) **surface proteins** that promote colonization of host tissues; (2) invasins that promote bacterial spread in tissues (**leukocidin**, **kinases**, **hyaluronidase**); (3) surface factors that inhibit phagocytic engulfment (**capsule**, **Protein A**); (4) biochemical properties that enhance their survival in phagocytes (**carotenoids**, **catalase** production); (5) immunological disguises (**Protein A**, **coagulase**, **clotting factor**); and (6) membrane-damaging toxins that lyse eukaryotic cell membranes (**hemolysins**, **leukotoxin**, **leukocidin**; (7) exotoxins that damage host tissues or otherwise provoke symptoms of disease (**SEA-G**, **TSST**, **ET** (8) inherent and acquired **resistance to antimicrobial agents**.

Human staphylococcal infections are frequent, but usually remain localized at the portal of entry by the normal host defenses. The portal may be a hair follicle, but usually it is a break in the skin which may be a minute needle-stick or a surgical wound. Another portal of entry is the respiratory tract. Staphylococcal pneumonia is a frequent complication of influenza. The localized host response to staphylococcal infection is inflammation, characterized by an

elevated temperature at the site, swelling, the accumulation of pus, and necrosis of tissue. Around the inflamed area, a fibrin clot may form, walling off the bacteria and leukocytes as a characteristic pus-filled boil or abscess. More serious infections of the skin may occur, such as furuncles or impetigo. Localized infection of the bone is called osteomyelitis. Serious consequences of staphylococcal infections occur when the bacteria invade the blood stream. A resulting septicemia may be rapidly fatal; a bacteremia may result in seeding other internal abscesses, other skin lesions, or infections in the lung, kidney, heart, skeletal muscle or meninges.



Sites of infection and diseases caused by *Staphylococcus aureus*

Adherence to Host Cell Proteins

S. aureus cells express on their **surface proteins** that promote attachment to host proteins such as laminin and fibronectin that form the extracellular matrix of epithelial and endothelial surfaces. In addition, most strains express a fibrin/fibrinogen binding protein (clumping factor) which promotes attachment to blood clots and traumatized tissue. Most strains of *S. aureus* express both fibronectin and fibrinogen-binding proteins. In addition, an adhesin that promotes attachment to collagen has been found in strains that cause osteomyelitis and septic arthritis. Interaction with collagen may also be important in promoting bacterial attachment to damaged tissue where the underlying layers have been exposed.

Invasion

The invasion of host tissues by staphylococci apparently involves the production of a huge array of extracellular proteins, some of which may occur also as cell-associated proteins. These proteins are described below with some possible explanations for their role in invasive process.

Membrane-damaging toxins

a-toxin (a-hemolysin) The best characterized and most potent membrane-damaging toxin of *S. aureus* is a-toxin. It is expressed as a monomer that binds to the membrane of susceptible cells.

Subunits then oligomerize to form heptameric rings with a central pore through which cellular contents leak.

In humans, platelets and monocytes are particularly sensitive to a-toxin. Susceptible cells have a specific receptor for a-toxin which allows the toxin to bind causing small pores through which monovalent cations can pass. The mode of action of alpha hemolysin is likely by osmotic lysis.

ß-toxin is a sphingomyelinase which damages membranes rich in this lipid.

d-toxin is a very small peptide toxin produced by most strains of *S. aureus*. It is also produced by *S. epidermidis*. The role of d-toxin in disease is unknown.

Leukocidin is a multicomponent protein toxin produced as separate components which act together to damage membranes. Leukocidin is hemolytic, but less so than alpha hemolysin.

Only 2% of all of *S. aureus* isolates express leukocidin, but nearly 90% of the strains isolated from severe dermonecrotic lesions express this toxin, which suggests that it is an important factor in necrotizing skin infections.

Coagulase and clumping factor

Coagulase is an extracellular protein which binds to prothrombin in the host to form a complex called staphylothrombin. The protease activity characteristic of thrombin is activated in the complex, resulting in the conversion of fibrinogen to fibrin. Coagulase is a traditional marker for identifying *S aureus* in the clinical microbiology laboratory. However, there is no overwhelming evidence that it is a virulence factor, although it is reasonable to speculate that the bacteria could protect themselves from phagocytic and immune defenses by causing localized clotting.

There is some confusion in the literature concerning coagulase and clumping factor, the fibrinogen-binding determinant on the *S. aureus* cell surface. Partly the confusion results from the fact that a small amount of coagulase is tightly bound on the bacterial cell surface where it can react with prothrombin leading to fibrin clotting. However, genetic studies have shown unequivocally that coagulase and clumping factor are distinct entities. Specific mutants lacking coagulase retain clumping factor activity, while clumping factor mutants express coagulase normally.

Staphylokinase

Many strains of *S aureus* express a plasminogen activator called staphylokinase. This factor lyses fibrin. The genetic determinant is associated with lysogenic bacteriophages. A complex formed between staphylokinase and plasminogen activates plasmin-like

proteolytic activity which causes dissolution of fibrin clots. The mechanism is identical to streptokinase, which is used in medicine to treat patients suffering from coronary thrombosis. As with coagulase, there is no strong evidence that staphylokinase is a virulence factor, although it seems reasonable to imagine that localized fibrinolysis might aid in bacterial spreading.

Other extracellular enzymes

S. aureus can express proteases, a lipase, a deoxyribonuclease (DNase) and a fatty acid modifying enzyme (FAME). The first three probably provide nutrients for the bacteria, and it is unlikely that they have anything but a minor role in pathogenesis. However, the FAME enzyme may be important in abscesses, where it could modify anti-bacterial lipids and prolong bacterial survival.

Avoidance of Host Defenses

S. aureus expresses a number of factors that have the potential to interfere with host defense mechanisms. This includes both structural and soluble elements of the bacterium.

Capsular Polysaccharide

The majority of clinical isolates of *S aureus* express a surface polysaccharide of either serotype 5 or 8. This has been called a microcapsule because it can be visualized only by electron microscopy unlike the true capsules of some bacteria which are readily visualized by light microscopy. *S. aureus* strains isolated

from infections express high levels of the polysaccharide but rapidly lose the ability when cultured in the laboratory. The function of the capsule in virulence is not entirely clear. Although it does impede phagocytosis in the absence of complement, it also impedes colonization of damaged heart valves, perhaps by masking adhesins.

Protein A

Protein A is a surface protein of *S. aureus* which binds IgG molecules by their Fc region. In serum, the bacteria will bind IgG molecules in the wrong orientation on their surface which disrupts opsonization and phagocytosis. Mutants of *S. aureus* lacking protein A are more efficiently phagocytosed in vitro, and mutants in infection models have diminished virulence.

Leukocidin

S. aureus can express a toxin that specifically acts on polymorphonuclear leukocytes. Phagocytosis is an important defense against staphylococcal infection so leukocidin should be a virulence factor.

Exotoxins

S. aureus can express several different types of protein toxins which are probably responsible for symptoms during infections. Those which damage the membranes of cells were discussed above under **Invasion**. Some will lyse erythrocytes, causing hemolysis, but it is unlikely that hemolysis is a relevant

determinant of virulence in vivo. Leukocidin causes membrane damage to leukocytes but is not hemolytic.

Systemic release of a-toxin causes septic shock, while enterotoxins and TSST-1 are superantigens that may cause toxic shock. Staphylococcal enterotoxins cause emesis (vomiting) when ingested and the bacterium is a leading cause of food poisoning.

The exfoliatin toxin causes the scalded skin syndrome in neonates, which results in widespread blistering and loss of the epidermis. There are two antigenically distinct forms of the toxin, ETA and ETB. The toxins have esterase and protease activity and apparently target a protein which is involved in maintaining the integrity of the epidermis.

Host Defense against Staphylococcal Infections

Phagocytosis is the major mechanism for combatting staphylococcal infection. Antibodies are produced which neutralize toxins and promote opsonization. However, the bacterial capsule and protein A may interfere with phagocytosis. Biofilm growth on implants is also impervious to phagocytosis. Staphylococci may be difficult to kill after phagocytic engulfment because they produce carotenoids and catalase which neutralize singlet oxygen and superoxide which are primary phagocytic killing mechanisms within the phagolysosome.

All the best wishes

Prof. Dr. Wesam Salem

Plant Diseases

For 4 th year students

Prof.Dr.

Ahmed Hussein Mohamed

Botany & Microbiology Dept., Faculty of Science

Faculty: Science

Specialization: Microbiology & Chemistry

Band: Four

Page No.: 42

The affiliated department: Botany & Microbiology Dept.

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INTRODUCTION

Plant pathology has four objectives:

1. To study the structure and life cycle of the etiologic organism.
2. To study the pathogenesis and the disease symptoms.
3. To study the epidemiology and the conditions that favor spread of the pathogen.
4. To know the methods of disease control or management to reduce losses in crop yield.

Terminology of Plant Pathology

1-Disease: pathological process involving harmful physiological changes in the living plant after infection by a living organism.

2-Disorders: Physiological changes due to non parasitic agents.

3-Pathogens: living organisms (fungi, bacteria, etc.) which cause damage to the host plants

4- Parasites: pathogens deriving nutrients for growth from a living plant. They are:

* Obligate (biotrophs), restricted to living tissues.
* Facultative colonize living or dead tissues.
* Necrotrophs: grow on dead tissue; they kill in advance, thus more dangerous than biotrophs.

5- Pathogenicity: the ability to cause disease.

6- Virulence: degree of pathogenicity in a qualitative sense. Some strains of a pathogen may be avirulent

7- Aggressiveness: capacity of a parasite to invade and grow in its host plant and to reproduce on or in it.

8- Inoculum: portion of a pathogen capable of infecting a host.

9- Inoculum potential: a measure of the biological energy available for the colonization of a host. It is a function of: i - inoculum density,
ii - nutrients available to the infectious units for germination or growth,
iii - virulence of the pathogen, iv - susceptibility of the host.

10 - Immune: exempt from infection.

11 – Resistance& susceptibility: the extent to which the plant is able to prevent the entry or subsequent growth of the pathogen within it. High resistance means low susceptibility that approaches immunity. Low resistance means high susceptibility.

12 – Hypersensitivity: development of necrotic spots resulting from rapid death of cells in the vicinity of invading pathogen (confers high resistance to host plant).

13 – Entry (Penetration): direct or indirect.

14 – Infection: Establishment of nutritional relationship between the pathogen and the host.

15– Colonization: the pathogen advances through the tissues of host to varying extent.

16 – Symptoms: visible external alterations on the host by which a disease can be recognized.

General categories of symptoms are:

a- Necrosis: (death of infected tissue).

b- Hyperplasia: (increased cell division) and / or hypertrophy (increase in cell size) leading to galls, tumors, and witches ' brooms

c- Hypoplasia (reduced growth or stunting of infected plant).

Significance of Plant Diseases

1. Reduction of quality and quantity of plant products (flowers, fruits, fibers, wood, latex, etc.).
2. Limitation the kinds of plants and industries in an area.
3. Contamination of plant products with poisonous substances.
4. Responsible for direct/indirect financial losses (costs of control).

Stages in the Development of a Disease (Disease Cycle)

1- Inoculation: pathogen in contact with plant.

2- Prepenetration: germination of spores, attachment to host and recognition // host &pathogen

3- Penetration: a- Direct through cuticle.
b- Indirect through natural openings (stomata, hydathodes)
c- Indirect through wounds caused by nematodes or farming tools.

4- Infection (includes invasion): pathogen establishes contact with host cells & tissues, absorbs nutrients.

5- Colonization: growth and reproduction of the pathogen on host surface, within the plant or its vascular elements.

6- Dissemination of the pathogen: transfer of inoculum from the site of its production to the susceptible host surface either actively or passively by air, water, human, animal, insects, agricultural practice, seeds transplants etc.

7- Seasonal carryover (overwintering or over summering): survival of the pathogen in the form of hyphae, resting spores, sclerotia, chlamydospores, etc.

Classification of Plant Diseases

A- According to mode of primary infection:

1- Soil-borne diseases: due to soil-borne pathogens e.g. damping-off of seedlings, vascular wilt, root rots etc.

2- Air-borne disease: fungal spores are disseminated by wind and infect the shoot of plant e.g. rusts, downy mildews, powdery mildews, etc.

3- Seed-borne diseases: some pathogens survive as dormant mycelium in the seeds or other propagative structures of host plants e.g. many smuts.

B- <u>According to extent of occurrence and geographic distribution:</u>

1- Endemic diseases: constantly present in a particular country or part of the earth.

2- Epidemic (epiphytotic) diseases: occur periodically but in a severe form under favorable environmental conditions

3- Sporadic: occur at very irregular intervals and locations in few instances A disease may be endemic in one region and epidemic in another.

C- <u>According to disease symptoms:</u>

I- Necrosis (death of cells & tissues)

1- Rusts 2- Smuts 3- Mildews 4- Root-rots 5- Blights

6- Leaf spots 7- Wilts 8- Cankers 9- Fruit rots 10- Dieback

11- Chlorosis 12- Bloch 13- Damping-off 14 - Scab

15- Streaks or stripes 16- Burn, scald or scorch 17- White blisters or pustules

II- Hypertrophy and hyperplasia

1- Elongated internodes: rice infected with *Gibberella fujikuroi*; *Euphorbia* with *Uromyces pisi*; sugarcane with *Sclerospora sacchari.*

2- Galls and tumors: globose, elongated or irregular large sized outgrowths formed on attacked part e.g. Club root of Crucifers;

3- Witche's broom: upright cluster of small shoots contrasting with horizontal growth habit of normal shoot.

4- Curls: leaves are arched, twisted and distorted eg. peach leaf curl,

5- Floral abnormalities: enlargement of infected inflorescence which become green and fleshy with stamens converted into leafy structures.

III- Hypoplasia

1- Chlorosis: reduced development of chlorophyll (mosaic, vein clearing yellowing).

2- Reduction of individual organ: e.g. leaves, flowers, internodes as in dwarf bunt of wheat by *Tilletia contraversa.*

3-Floral abnormalities: in anther smut of Caryophyllaceae caused by *Ustilago violacea,* stamens become sterile.

D- <u>According to major Phyla of fungi:</u>

1- Diseases caused by Myxomycota
2- Diseases caused by Oomycota
3- Diseases caused by Chytridiomycota
4- Diseases caused by Ascomycota
5- Diseases caused by Basidiomycota
6- Diseases caused by Deuteromycota

I- Diseases caused by Myxomycota

Class: Plasmodiophoromycetes
Order: Plasmodiophorales
Family: Plasmodiophoraceae
Plasmodiophora brassicae

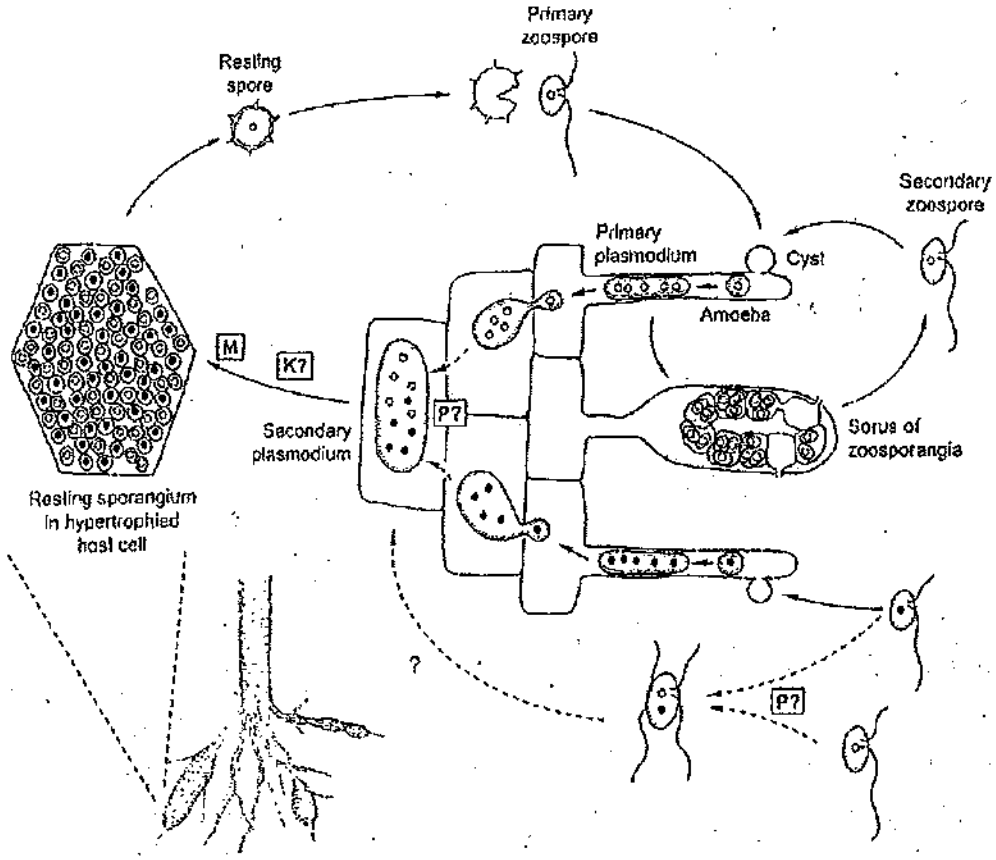
A- Club Root of Crucifers (finger and toe disease)

- Causal agent: *Plasmodiophora brassicae*
- Host plants: Cruciferous vegetables such as cabbage, cauliflower, radishes, and turnips; and field crops such as mustard.
- Symptoms:

Roots show malformation and enlargement due to spindle or club shaped swellings resulting from hypertrophy and hyperplasia of infected cells. Inside root cells, plasmodia followed by resting spores are formed. Leaves show yellowing and wilting.

- Disease cycle:

Plasmodiophora brassicae infects susceptible host plants through root hairs. It stimulates abnormal growth of affected parts, resulting in a swollen clubs. Infection is favored by excess soil moisture and low pH. Numerous resistant spores of the fungus are produced in the "clubbed" tissues. As tissues decay, spores are released into the soil where they can remain infectious for at least 10 years.



- Disease management:
  1. Eradication of cruciferous weeds.
  2. Use of well drained, pathogen free pots.
  3. Use of seedlings raised in pathogen free soil.
  4. Very long crop rotation with non cruciferous crops.
  5. Soil fumigation with volatile chemicals such as vapam, methyl dibromide etc.
  6. Alteration of soil pH to 7 or above by adding lime.
  7- Soil treatment with fungicides (e.g. PCNB)

B- Powdery scab of potatoes

- Causal agent: *Spongospora subterranea*

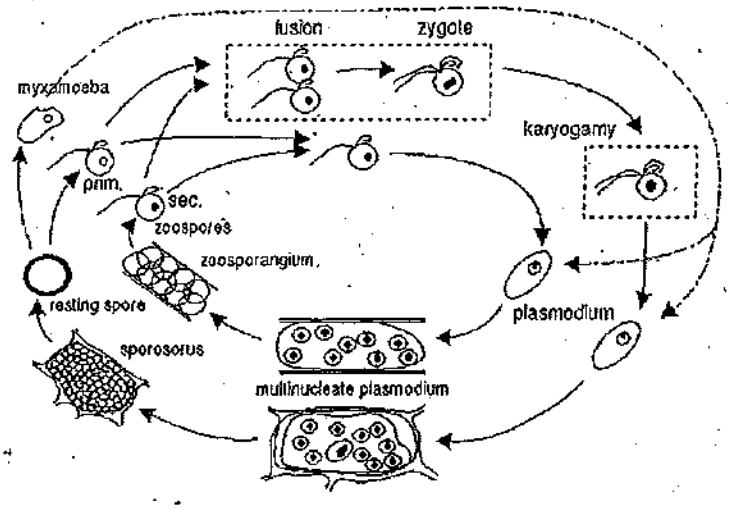
  It is generally found in wet, badly drained soils. The spores remain in the soil for several years.

Class: Plasmodiophoromycetes
Order: Plasmodiophorales
Family: Plasmodiophoraceae
Spongospora subterranea

- Symptoms:

  On tubers, irregular brown depressions with raised papery margins (scabs) are formed. These scabs are filled with dusty brown spongy masses of spore balls. Infected young tubers show distortion and swollen outgrowths.

- Control:
  1. Healthy, powdery scab-free seeds are only planted.
  2. Infected tubers should be disposed correctly not composted.
  3. Crop rotation is useful where replanting potatoes in the same position is avoided for three years.
  4. Improved soil aeration.
  5. There are no fungicides that can be used.



II- Diseases caused by Chytridiomycota

Class: Chytridiomycetes
Order: Chytridiales
Family: *Synchytriaceae*
Synchytrium endobioticum

1- Black Wart of Potatoes

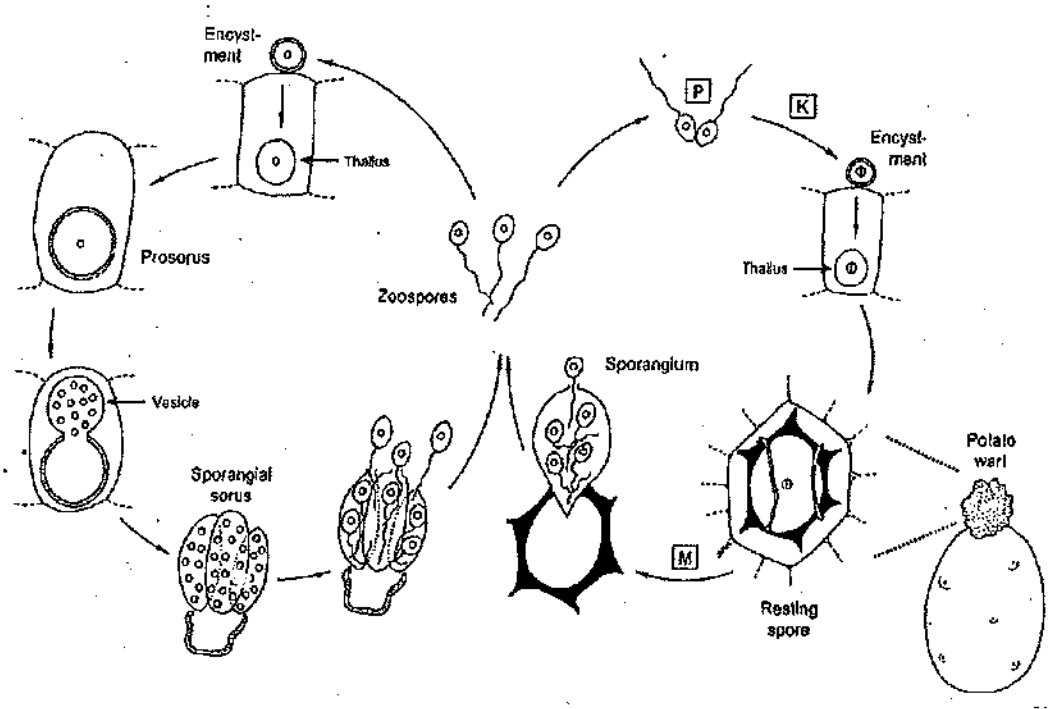
Caused by: *Synchytrium endobioticum*. It is a non mycelial, unicellular, holocarpic biotrophic chytrid fungus.

Disease Cycle:

- Infected host cells contain spherical (2n) resting sporangia (RS) of dark brown walls
- RS are released by the decay of warts and they may remain viable in soil for up to 40 years. They germinate producing prosporangia (vesicles) in which uniflagellate zoospores (n) are produced.
- Meiosis occurs during germination.
- Zoospores encyst on host epidermis before infection
- Inside the host cell the small fungal cell enlarges and the host is stimulated to enlarge.
- Zoosporangia (n) are formed producing up to 600 zoospores per spoangium.
- At later stages zoospores behave as gametes to give resting sporangia

Symptoms:

- Large irregular cauliflower-like warts or galls develop on all underground parts except roots.
- Warts at first greenish-white, becoming dark or black
- Warts develop due to hyperplasia and hypertrophy.
- The disease causes losses by reducing the quantity and quality of tubers.



III- Diseases caused by Oomycota

Class: Oomycetes
Order: Saprolegniales
Family: *Saprolegniaceae*
Aphanomyces euteiches

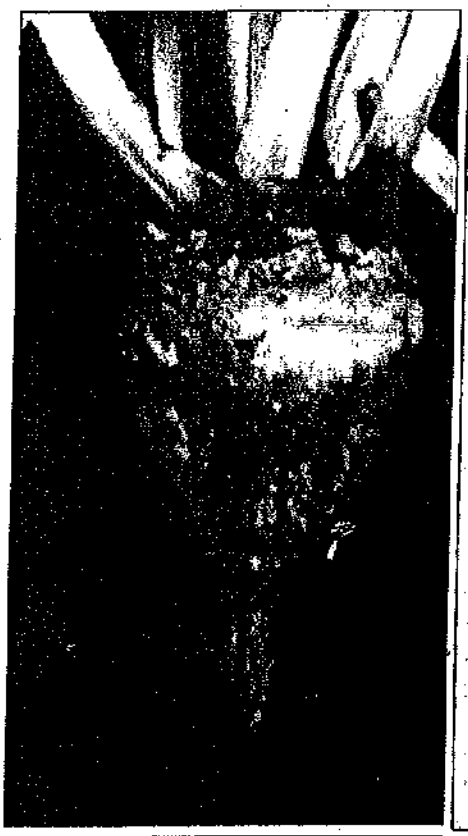
1- Aphanomyces root rot

Caused by *Aphanomyces* one of the zoosporic fungi belonging to the Family Saprolegniaceae

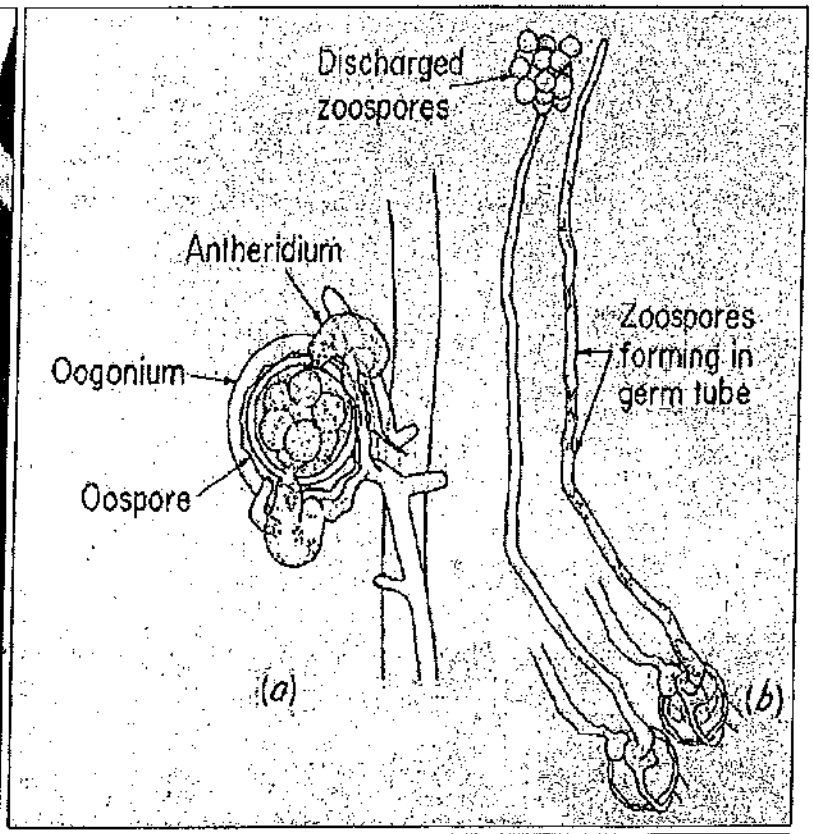
Hosts: Sugar beet, wheat, pea, etc

Symptoms:

- Soft decay of the root cortex.
- Vascular core of root tends to come out when roots are pulled up.
- Progressive death of the leaves from the base of the stem.
- General check in growth.
- Infected plants may survive but produce poorly filled pods.
- Oospores of the fungus are present in decaying tissues.
- Disease is most serious in soils with high moisture content and at 15° – 30° c.



Aphanomyces **root rot of Sugar beet**



Aphanomyces: **asexual and sexual reproductive units**

2- Damping off and seedling blight

Caused mainly by *Pythium* species, Family Pythiaceae

- Other fungal species belonging to *Phytophthora, Rhizoctonia, Fusarium, Helminthosporium and Botrytis* could be associated with damping off and seedling blight

Class: Oomycetes
Order: Peronosporales
Family: *Pythiaceae*
Pythium aphanidermatum
Pythium oligandrum

Symptoms:

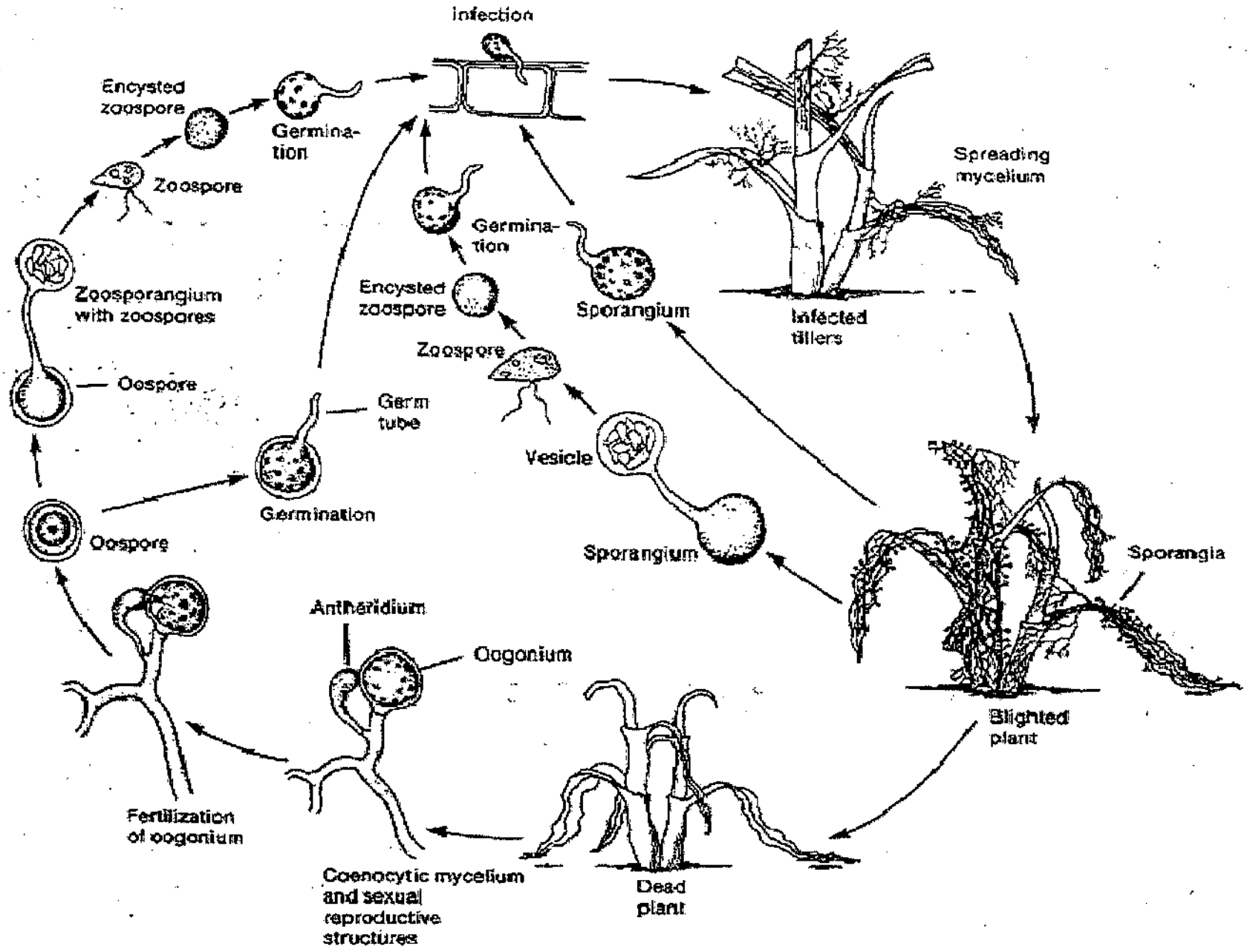
- Pre-emergence: Emergence of seedlings is poor even with seeds of high germinative capacity
- There are patches with no seedlings at all.
- Post- emergence: Seedlings that have emerged often show water soaking, browning or shriveling of the stem tissues at soil level and die.
- When plants are pulled up they show browning and rotting of the smaller roots or stem, and stem lesions at soil level.
- Plants are stunted.
- Plants wilt at midday and may recover at night.
- Plants show yellowing and die.
- Brown tissue on the outer portion of the root easily pulls off leaving a bare strand of vascular tissue exposed.
- Root tips are brown and dead
- The cells of roots contain round, microscopic, thick-walled oospores of *Pythium*

Control:

The most favorable environments for *Pythium* disease are soil treated with high-nitrogen fertilizers, alkaline soil and soil with low calcium levels.

To prevent or minimize contamination it is recommended to:

a- Utilize balanced fertilizers and keep soil pH neutral or slightly acid.
b- Prune trees and shrubs to improve air circulation.
c- Correct drainage problems, avoid over-watering,
d- Preventive fungicide treatment programs using metalaxyl, terrazole, coban, etc


Infection
Encysted zoospore
Germination
Zoospore
Spreading mycelium
Germination
Zoosporangium with zoospores
Encysted zoospore
Sporangium
Infected tillers
Oospore
Zoospore
Germ tube
Vesicle
Germination
Oospore
Sporangium
Sporangia
Antheridium
Oogonium
Blighted plant
Fertilization of oogonium
Coenocytic mycelium and sexual reproductive structures
Dead plant

3- Late blight of potato and tomato

Caused by: *Phytophthora Infestans*

Class: Oomycetes
Order: Peronosporales
Family: *Pythiaceae*
Phytophthora infestans

- Late blight epidemics in the 1840s led to the Irish Potato Famine, in which over a million people died and a million emigrated to other countries.
- Even today, *Phytophthora Infestans* poses a major threat to potato agriculture.

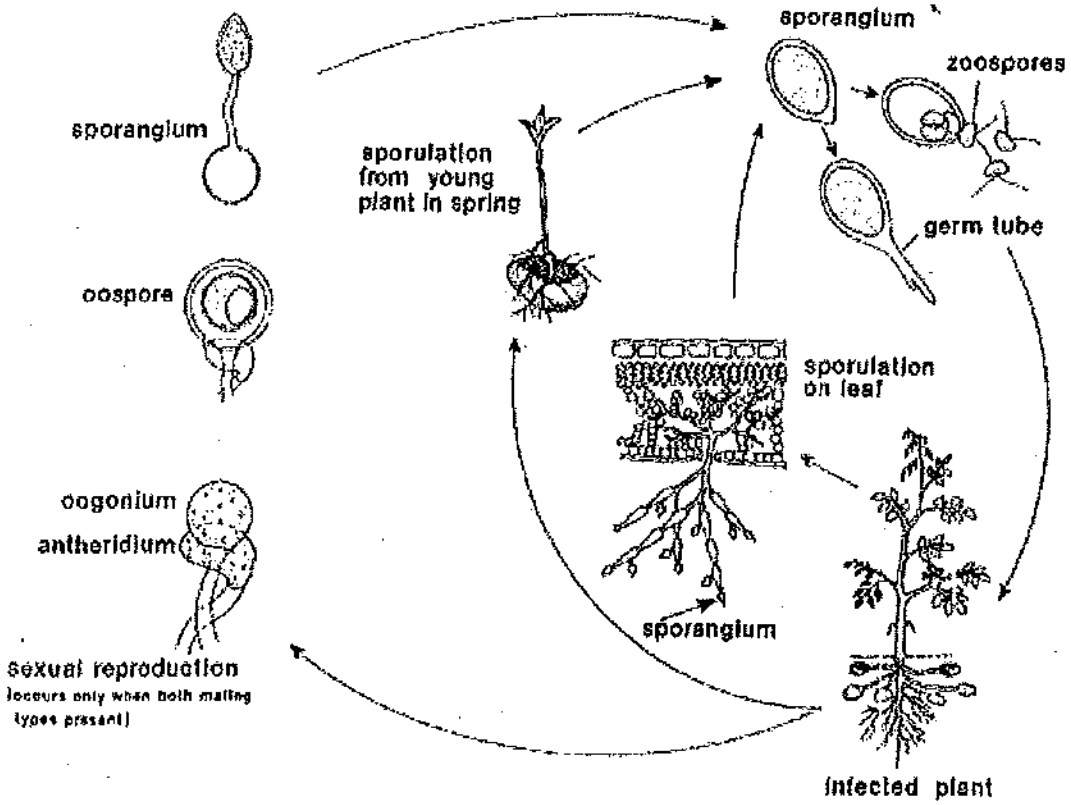
Symptoms:

On leaves and stems:

- Dark brown lesions of varying sizes and shapes.
- Under moist conditions a mass of sporangiophores
- (White bloom) develop on the lower leaf surfaces.
- Lesions increase rapidly and coalesce.
- Potato shoots are killed within 3-4 weeks.

On tubers:

- Tubers show irregular dark and sunken areas associated with brownish rot.
- Rotting often increase during storage due to further invasion by bacteria.



This is a simplified disease cycle for late blight of potato.

4- White rust Diseases

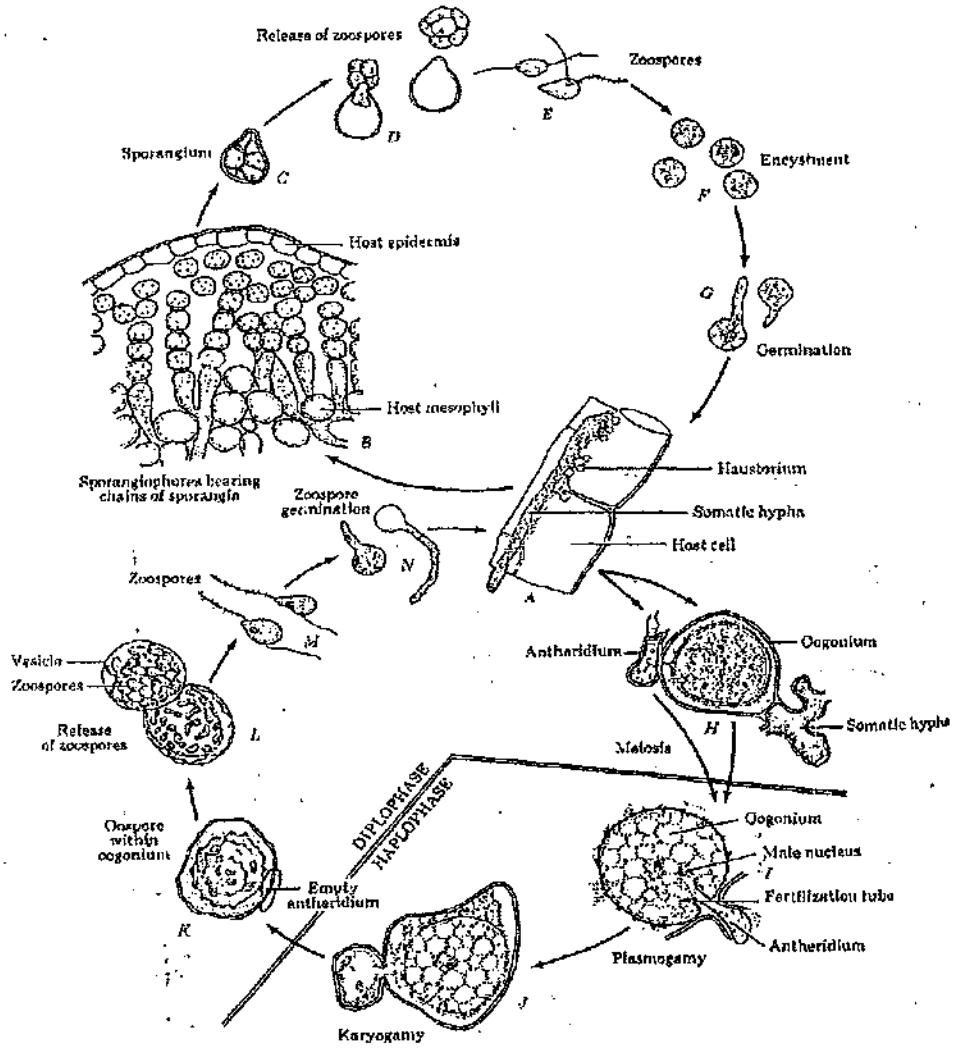
A- White rust of Crucifers by *Albugo candida* of the Hosts include radish, turnip, cabbage, cauliflower, mustard,

B- White rust of *Portulaca* by *Albugo portulacae*

Class: Oomycetes
Order: Peronosporales
Family: Albuginaceae
Albugo candida
Albugo portulacae

Symptoms:

- White pustules or sori develop on leaves and stems.
- Host epidermis ruptures exposing a white powdery mass of spores (chains of sporangia on clavate sporangiophores).
- The fungus grows inside the whole plant tissue stimulating various types of deformities.
- Inflorescences and flowers become thickened due to hypertophy and hyperplasia of affected cells.
- The swollen parts are full of oospores



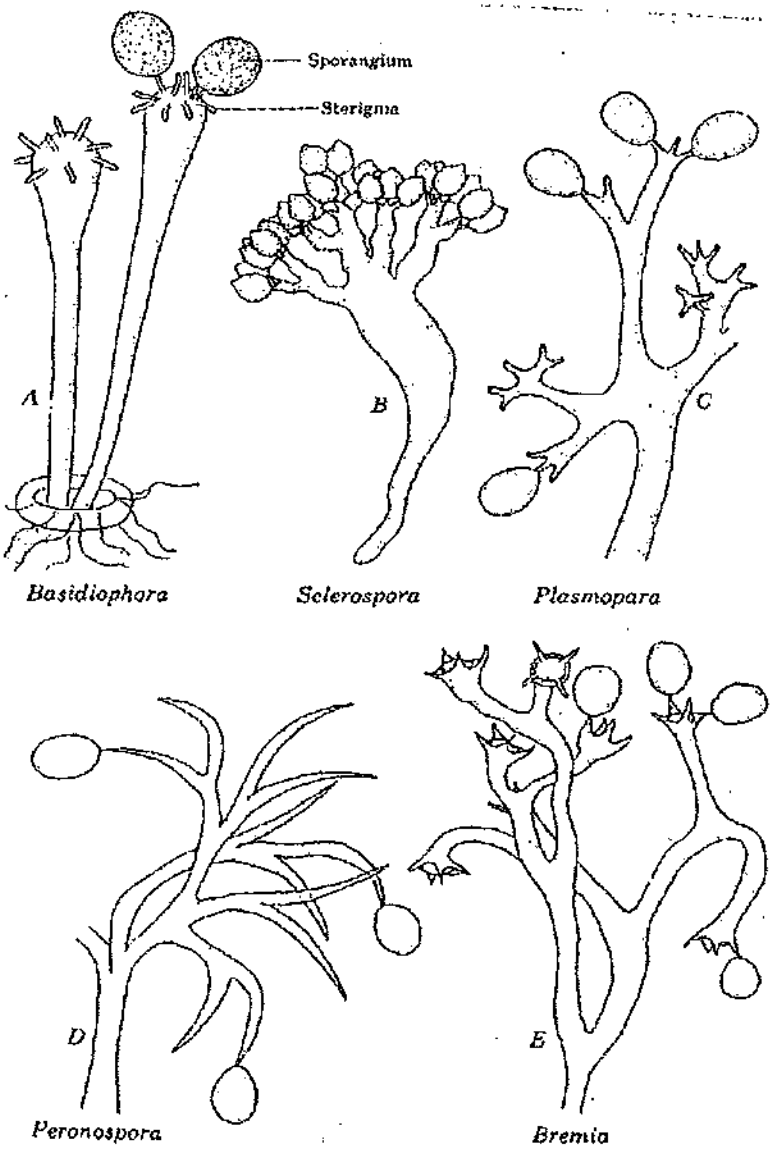
Life cycle of *Albugo candida*

5- Downy mildew diseases

Caused by members of the Family Peronosporaceae, Order Peronosporales, Class Oomycetes. Fungal species, their hosts are shown in the following table.

Pathogens	Hosts
Plasmopara viticola	grapevine
Bremia lactucae	lettuce
Peronospora destructor	onion
Peronospora pisi	peas
Peronospora parasitica	Cauliflower, Cabbage
Pseudoperonospora cubensis	Cucurbits
Sclerospora graminicola	cereals (wheat, sorghum, corn)
Basidiophora sp	Compositae (*Sonchus, Helianthus*)

The sporangiophores of downy mildew fungi are illustrated in the following figure



A- Downy Mildew of Grapevine by *Plasmopara viticola*

Class: Oomycetes
Order: Peronosporales
Family: *Peronosporaceae*
Plasmopara viticola

Signifcance of disease:

- The fungus causes direct yield losses by rotting inflorescences, clusters and shoots.
- Indirect losses can result from premature defoliation of infected vines.
- Premature defoliation predisposes the vine to winter injury.

Symptoms:

a- On leaves:

- Aappearance on the upper leaf surface of irregular pale-yellow to greenish-yellow spots up to 1/4 inch or more in diameter.
- On the underside of the leaf, the fungus mycelium (the "downy mildew") can be seen within the border of the lesion as a delicate, dense, and white to grayish, cotton-like growth.
- Infected tissue gradually becomes dark brown, irregular, and brittle.
- Severely infected leaves eventually turn brown, wither, curl, and drop.

b- On fruits:

- young berries turn light brown and soft, and under humid conditions are often covered with the downy-like growth of the fungus .
- Berries infected at late summer do not turn soft or become covered with the downy growth. Instead, they turn dull green, then dark brown to brownish-purple.
- They may wrinkle and will never mature normally

c- On shoots and tendrils:

- Early symptoms appear as water-soaked, shiny depressions on which the dense downy mildew growth appears.
- Young shoots usually are stunted and become thickened and distorted.
- Severely infected shoots and tendrils usually die.

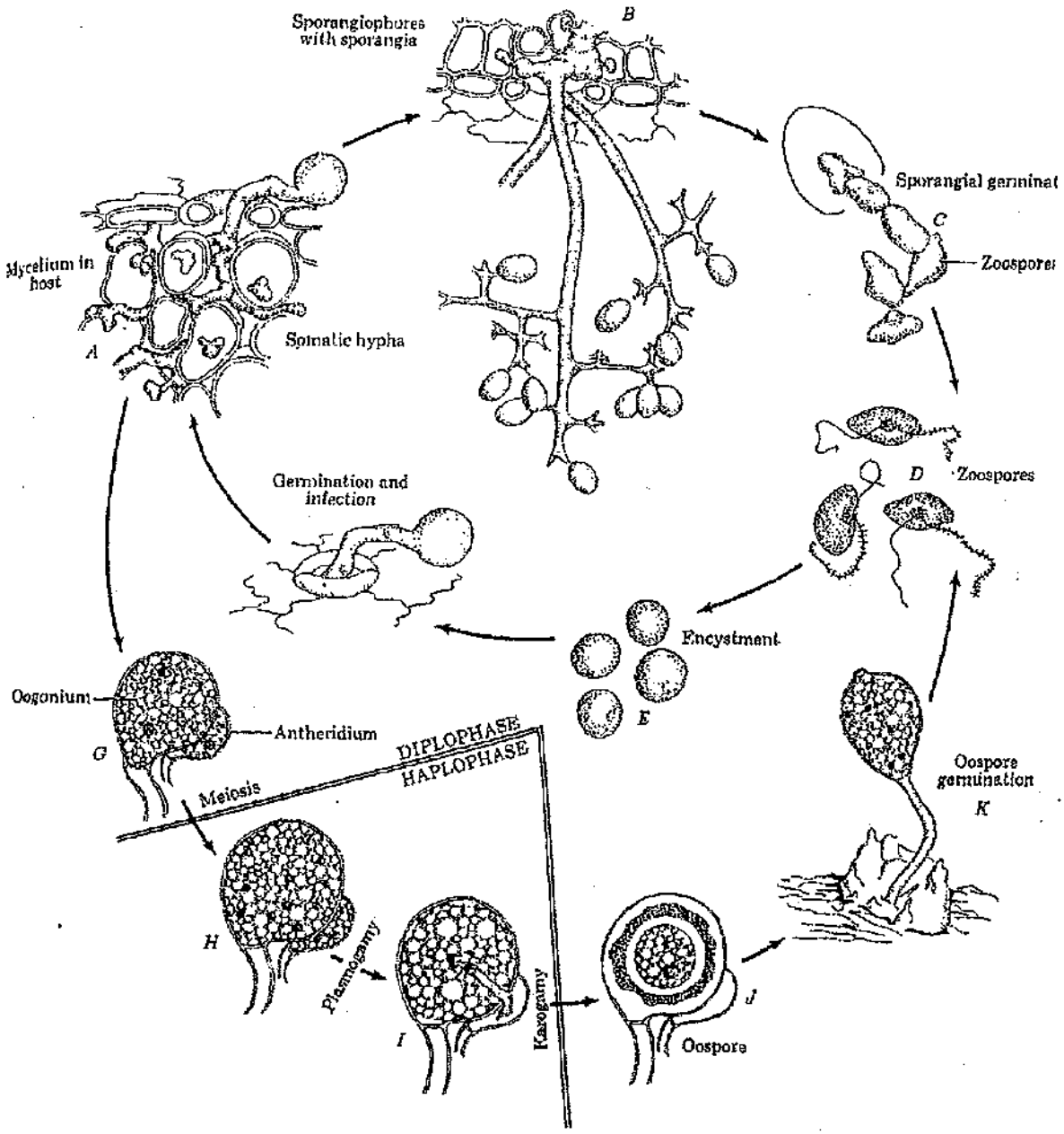
Disease cycle:

- The overwintering oospore germinates in the spring and produces a sporangium. Sporangia are spread by wind and splashing rain.
- When plant parts are covered with a film of moisture, the sporangia release small swimming spores, called zoospores.
- Zoospores, germinate by producing a germ tube that enters the leaf through stomata on the lower leaf surface.
- The optimum temperature for disease development is 18 to 25 C.
- Once inside the plant, the fungus grows and spreads through tissues.
- Infections are usually visible as lesions in about 7-12 days.
- At night during periods of high humidity and temperatures above 13 degrees C, the fungus grows out through the stomata of infected tissue and produces microscopic, branched, tree-like sporangiophores on the lower leaf surface.

- The small sporangiophores and sporangia make up the downy mildew growth.
- Sporangia cause secondary infections and are spread by rain

Control:

- Select a planting site where vines are exposed to all-day sun, with good air circulation and soil drainage.
- Proper spacing and orientation of vines in the rows to maximize air movement
- Removal of dead leaves and berries from vines and the ground after leaf drop.
- Downy mildew can be effectively controlled by properly timed and effective fungicides.



Life cycle of *Plasmopara viticola*

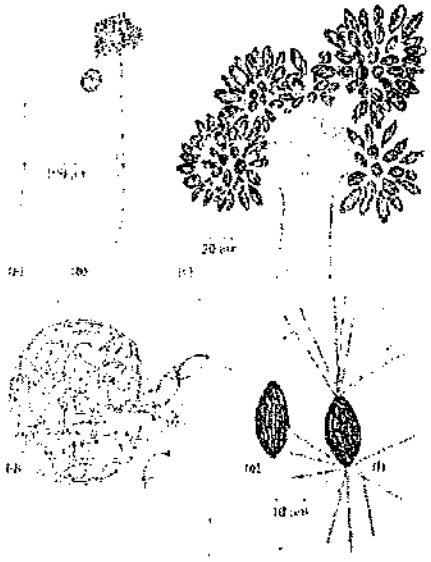
IV- Diseases caused by Zygomycota

Class: Zygomycetes
Order: Mucorales
Family: *Choanephoraceae*
Choanephora cucurbitarum

A- Choanephora rot

Symptoms: Fruits rot rapidly and white fungal mold appears on the infected area. With time, fruit look like a pin cushion with numerous small, black-headed pins stuck in it. Initially, the heads are white to brown but turn purplish-black within a few days. Affected flowers, pedicels, and immature fruit become water-soaked, and a soft, wet-rot develops. An entire fruit can rot in a 24 to 48 hour period. Symptoms usually begin on the blossom end of the fruit.

PERSISTENCE AND TRANSMISSION. The fungus overwinters as a saprophyte (living on dead plant tissue) and/or in a dormant spore form (such as a chlamydospore or zygospore). In spring, fungal spores are spread to squash flowers by wind and by insects such as bees and cucumber beetles. Infection occurs through the blossom, into the fruit and stem. Development of wet rot is favored by high relative humidity and excessive rainfall.



Choanephora cucurbitarum

Choanephora rot of squash by *Choanephora cucurbitarum*.

Other diseases by Zygomycetes:

a- Mucor rot of vegetables and fruits e.g. Guava, cucumber, grape, etc.
b- Rhizopus rot of vegetables and fruits e.g. Tomatoes, cantaloupe, mandarine, sweet potato, strawberry, etc.

V- Diseases caused by Ascomycota

Disease	Causal agent
Peach leaf curl	*Taphrina deformans*
Powdery mildews	Members of the Family Erysiphaceae
Apple scab	*Venturia Inaequalis*
Duch elm disease	*Ophiostoma ulmi*
Ergot disease	*Claviceps purpurea*
Canker of trees	*Nectria galligena*
Sclerotinia soft rot of vegetables	*Sclerotinia sclrotiorum*

1- Peach Leaf Curl

Caused by *Taphrina (Exoascus) deformans*

Class: Ascomycetes
Order: Exoascales
Family: Exoascaceae
Taphrina deformans

Taphrina has four unique features:

(A) The assimilative mycelium is dikaryotic.

(B) It produces an exposed layer of asci on the surface of the host leaf .

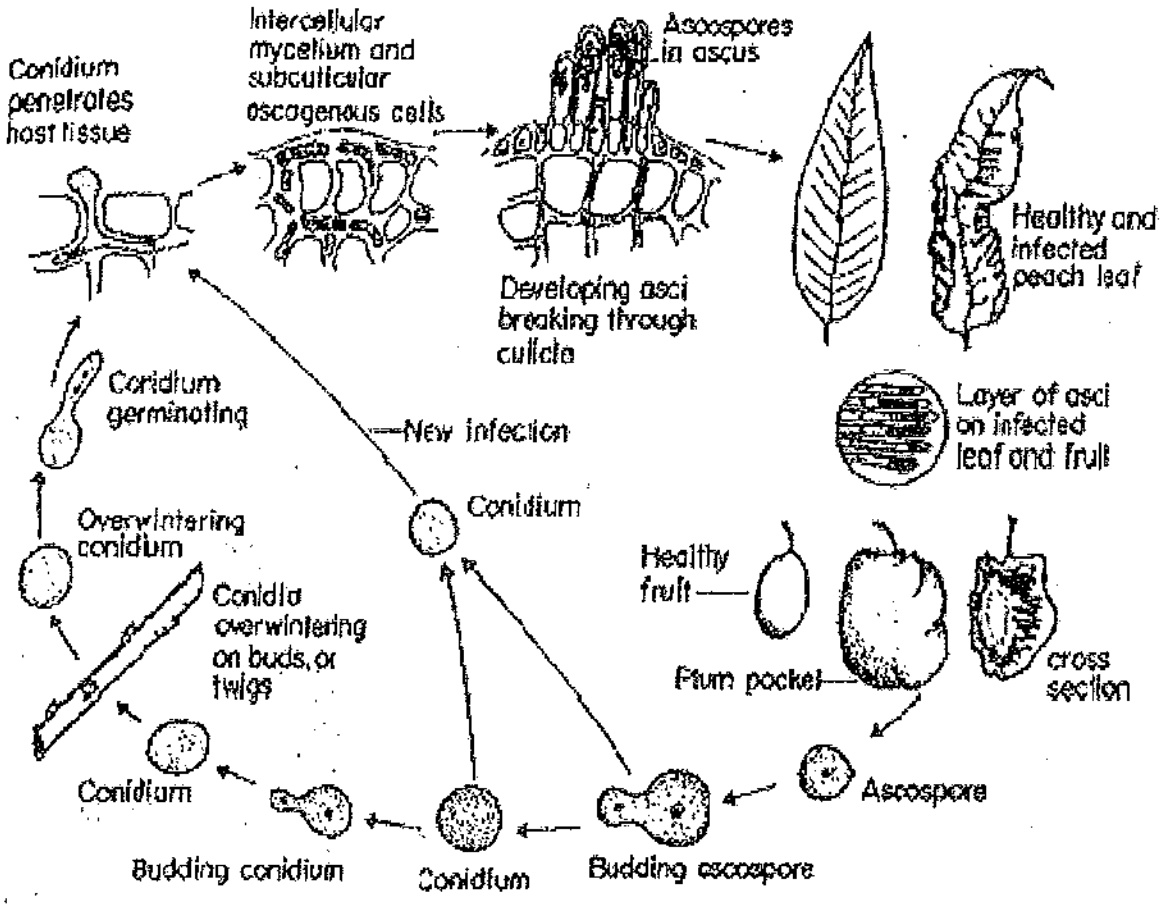
(C) Ascospores often bud in a yeast-like manner, even while still inside asci.

(D) When the asci open to release their spores, they tend to split across the tip.

(E) The anamorph of *Taphrina*, the phase in which it grows in culture, is single- celled budding yeast named *Lalaria*.

Symptoms:.

- Infected leaves are severely deformed and often display a variety of colors ranging from light green and yellow to shades of red and purple.
- The fungus causes the meristematic cells at leaf margins to proliferate quickly and randomly, which results in the leaves becoming variously wrinkled, puckered, and curled.
- As infected leaves mature, naked asci containing ascospores of the pathogen are produced on the surface giving them a dusty appearance, after which the leaves turn brown, shrivel, and drop from the tree.
- Many infected fruits drop early while others develop reddish to purple, wart-like deformities on the fruit surface.
- Infections on young peach leave occur at temperatures of 10-21 C.



Disease cycle of Peach leaf curl caused by *Taphrina deformans*

Control of peach leaf curl

1- Treat trees with a fungicide in late fall using:
   a- Copper ammonium complex
   b- 90 % tribasic copper sulfate
   c- Potassium resinate and potassium oleate
   d- Bordeaux Mixture
   e- Lime Sulfur
2- Select resistant Varieties.

2- Powdery mildews

- Caused by members of the Family Erysiphaceae of the Order Erysiphales, Class Pyrenomycetes, Phylum Ascomycota
- General characteristics of Powdery mildew fungi:

1- They are obligate, biotrophic parasites

2- They tend to grow superficially, or epiphytically, on plant surfaces producing whitish, powdery asexual structures (hyphae and conidia) on upper and lower leaf surfaces.

3- Few genera produce endophytic hyphae.

4- Infections can also occur on stems, flowers, or fruit.

5- Specialized absorption cells (haustoria) extend into the plant epidermal cells to obtain nutrients.

6- Conidia develop either singly or in chains on specialized conidiophores

7- Conidiophores arise from the epiphytic hyphae, or in the case of endophytic hyphae, the conidiophores emerge through stomata.

8- Tiny, dark sexual structures (ascomata) are produced later on infected shoots

9- Infection by these fungus is favored by high humidity but not by free water.

10- Individual species typically have a very narrow host range.

Powdery mildew fungi and their hosts

Fungal species	Hosts
Erysiphe graminis	Wheat, barley
E. cichoracearum	Cucurbits, *Sonchus,*
E. polygoni	Beans, peas
Leveillula taurica	artichoke
Sphaerotheca macularis	strawberry
Sphaerotheca pannosa	Rose, peach
Uncinula necator	Grape vine
Microsphaera alphitoides	Oak, lilac
Podsphaera leucotricha	apple
Phyllactinea corylea	Oak, elm, tulip,
Oidium mangiferae	mango

Types of conidiophores of Powdery mildew fungi

1- The oidium type: short stipe of one or more cells, conidiogenous cell and a chain of maturing conidia, e.g. ***Erysiphe, Uncinula, Microsphaera, Podosphaera and Sphaerotheca***

2- The ovulariopsis type: with clavate conidia e.g. ***Phyllactinia.***

3- The oidiopsis type: conidiophores branched arising from stomata. e.g. ***Leveillula***

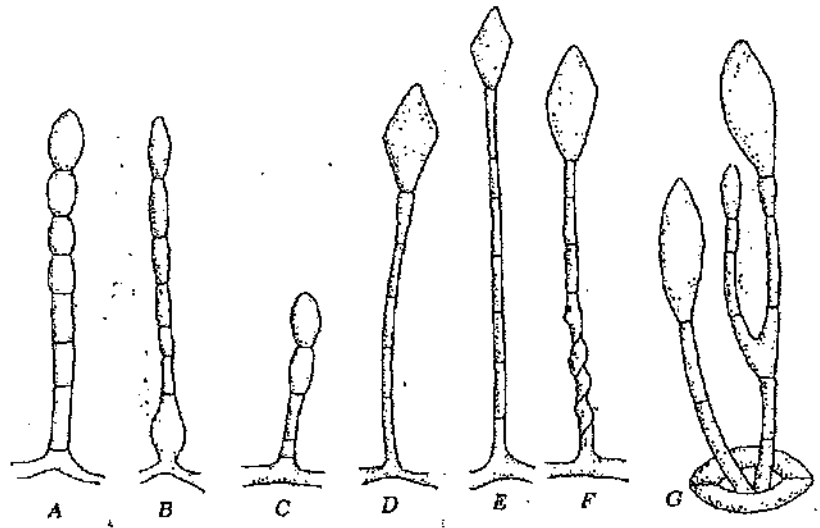


Figure 15-6 Conidiophore types. (A) *Erysiphe cichoracearum*. (B) *Erysiphe graminis*. (C) *Erysiphe polygoni*. (D) *Phyllactinia suffulta*. (E) *Phyllactinia rigida*. (F) *Phyllactinia subspiralis*. (G) *Leveillula taurica*. (Redrawn from Blumer. 1933. By R. W. Scheetz.)

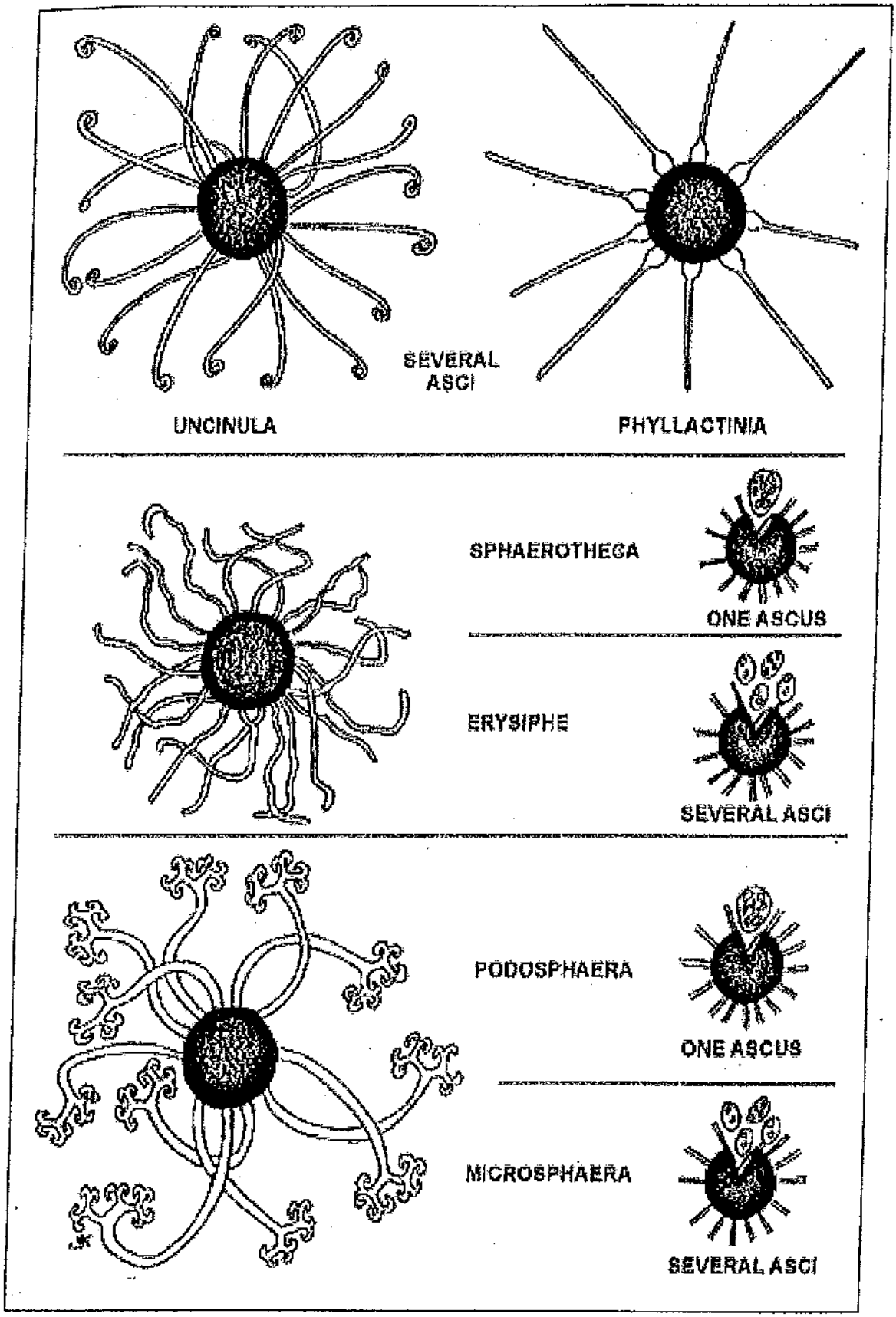
Key to Genera of Powdery Mildew Fungi (based on ascomatal appendages and number of asci)

- Appendages coiled or hooked at tip ----------*Uncinula*
- Appendages simple and straight with bulb-like base----*Phyllactinia*

- Appendages simple or irregularly branched, often enterwined
  - # Cleistothecium contains a single ascus ----------*Sphaerotheca*
  - # Cleistothecium contains several asci ----------*Erysiphe*

  (endophytic mycellum-----*Leveillula*)

- Appendages branching dichotomously at tip, Cleistothecium contains a single ascus ----------*Podosphaera*
  - # Cleistothecium contains several asci ----------*Microsphaera*



Types of Ascomata of powdery mildew fungi

Disease cycle of powdery mildew fungi

1- The fungus can overwinter as dormant mycelium or resting ascospores (in dark cleistothecia) on infected stems or leaves.

2- In spring, dormant mycelia becomes active producing asexual conidia while the cleistothecia produce ascospores.

3- Conidia and ascospores are then carried by wind to susceptible young plant parts.

4- when a conidium germinates on host leaf surface it produces a germ tube which gives an appressorium.

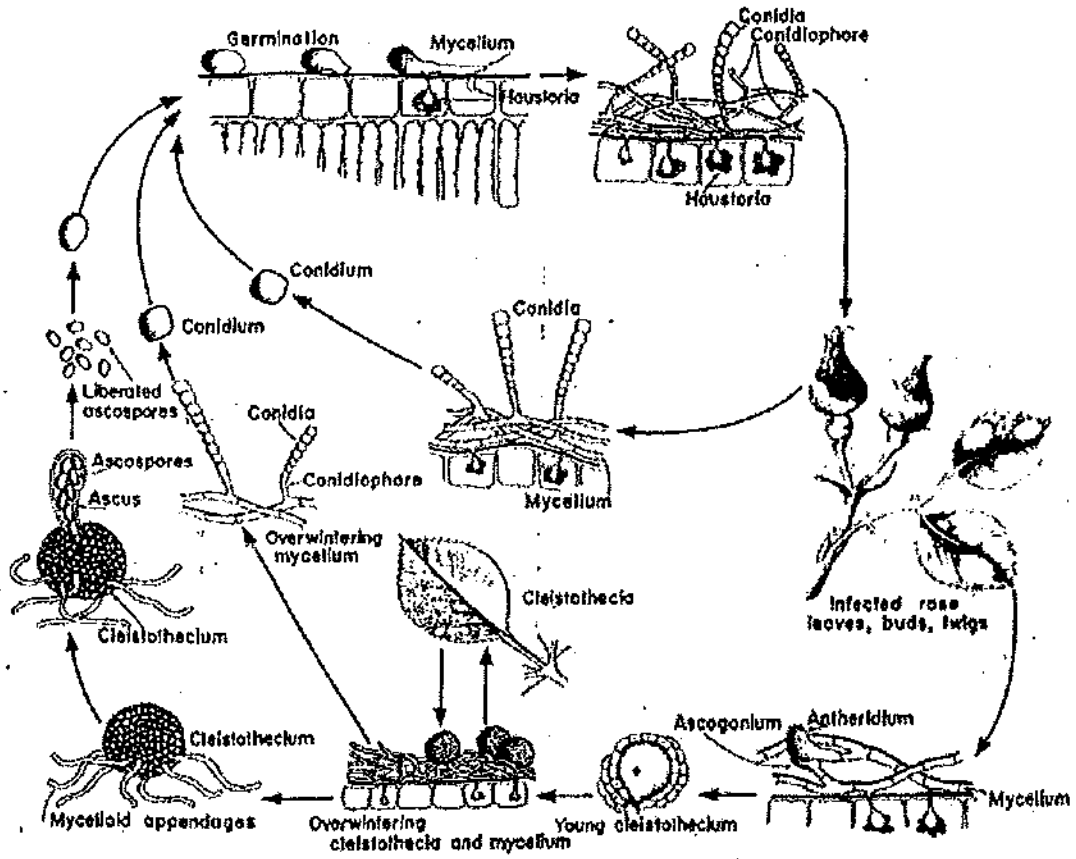
5- From the appressorium a penetration hypha grows through the cuticle and cell wall then swells out in the epidermal cell to form a haustorium (globular or finger-like in shape). The haustorium is a fungus structure that takes the nutrients from the plant

6-Further germ tubes, appressoria and haustoria are produced and the fungus grows out radially from the point of inoculums.

7- About 4 days after inoculation, sporulation starts extending outwards giving conidial chains

8- Dark pin point cleistothecia develop superficially in the same mycelial felt.

9- Cleistothecia overwinter and provide inoculums for infection of next season's crop.



Disease cycle of powdery mildew of rose by *Sphaerotheca pannosa*

Factors influencing disease development

1- Moisture: Powdery mildews are most severe in dry weather. germination of conidia is poor in free water. Spore maturation and release usually occurs during the day when relative humidity is low, at night an increase in relative humidity favors spore germination and penetration of the fungus.

2- Temperature: 11-28 C is favorable for infection. Cool damp nights and warm sunny days favor the development of Powdery Mildew.

3- Light: Higher incidence of powdery mildew on shaded than on exposed leaves. Effects of light include increased conidial germination, negative phototro-pism of germ tubes to white light (+ve to green).

4- Soil fertility: mineral nutrition (K, N, P) affects susceptibility. K- deficiency increases susceptibility.

5- Others: Closely planted gardens with some air movement are ideal conditions for spread of this disease.

Symptoms of powdery mildews:

- Slightly raised blister like areas on the upper leaf surfaces.
- Later, the young expanding leaves become twisted, distorted and covered with a white powdery mass of mycelium and spores.
- Young peduncles, sepals, petals and stems may also show distortion while growing tips and buds may be killed.
- Infected older leaves and stems may remain symptomless

Control of powdery mildew

1- Separation of new plantings from old ones.

2- Application of crop rotation of at least 1 year.

3- Control of weeds especially those related to host plants.

4- Fungicide sprays; e.g. with sulfur, karathane (0.1%), benlate (0.1%), calixin. Seed treatment with bayleton (0.1- 0.2%) or its spray on leaves (200- 500 ug /ml)

5- Breeding of resistant varieties:

3- Apple scab by *Venturia inaequalis*

Ascomycota
Dothideomycetes
Pleosporales
Venturiaceae
Venturia inaequalis

Symptoms:

Dull black or grey-brown lesions on the surface of tree leaves, buds or fruits.
Lesions may also appear less frequently on the woody tissues of the tree.
The disease rarely kills its host, but can significantly reduce fruit yields and fruit quality.
Affected fruits are less marketable due to the presence of the black fungal lesions.

Life cycle

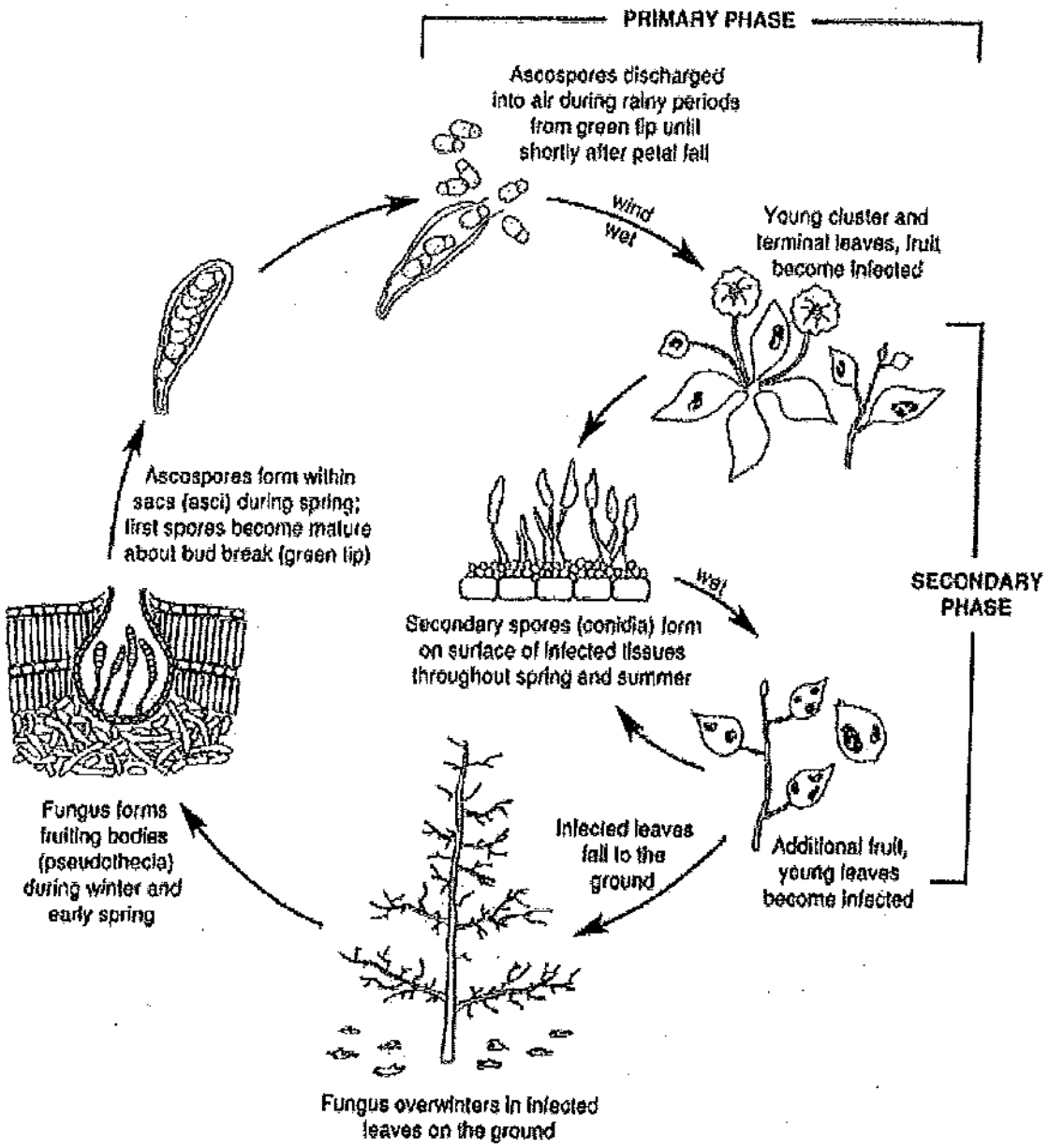
1. The infection cycle begins in the springtime, when suitable temperatures and moisture promote the release of *V. inaequalis* ascospores from leaf litter around the base of previously infected trees.
2. These spores rise into the air and land on the surface of a susceptible tree, where they germinate and form a germ tube that can directly penetrate the plant's waxy cuticle.
3. A fungal mycelium forms between the cuticle and underlying epidermal tissue, starting as a yellow spot that grows and ruptures to reveal a black lesion bearing the asexually as the conidia are released and germinate on fresh areas of the host tree, which in turn produce another generation of conidial spores.
4. This cycle of secondary infections continues throughout the summer, until the leaves and fruit fall from the tree at the onset of winter.
5. Over the winter, *V. inaequalis* undergoes sexual reproduction in the leaf litter around the base of the tree, producing a new generation of ascospores that are released the following spring.
6. Scab lesions located on the woody tissues may also overwinter in place, but will not undergo a sexual reproduction cycle; these lesions can still produce infective conidial spores in the spring.

Control:

a- Resistant cultivars: Breeding programs to develop high quality disease-resistant apple cultivars
b- Sanitation: Prevention of pseudothcial formation in overwintering apple leaves would probably eliminate scab as a serious threat to apple production. Leaf pickup and destruction in late autumn can be employed. Applications of 5% urea to foliage in autumn can hasten leaf decomposition, thus reducing formation of pseudothecia.

c- Chemical treatment: Protectant fungicides prevent the spores from germinating or penetrating leaf tissue. Postinfection fungicides control the scab fungus inside leaves and fruit. These chemicals can penetrate plant tissues to eliminate or inhibit lesion development. Several fungicides are available for controlling apple and pear scab. These include fixed copper, Bordeaux mixtures, copper soaps (copper octanoate), sulfur, mineral or neem oils, and myclobutanil. All these products except myclobutanil are considered organically acceptable

APPLE SCAB DISEASE CYCLE



4- Dutch elm disease (DED) by *Ophiostoma ulmi*

Fungi
Pezizomycotina
Sordariomycetes
Ophiostomatales
Ophiostomataceae
Ophiostoma ulmi

Symptoms:

Dutch elm disease results in the blockage of the water-conducting tissue within the tree.

Initial symptoms include discoloration and wilting of foliage.

This insect (*Scolytus scolytus)* feeds primarily on small branches high in the tree crown.

Foliage on diseased branches turns yellow. .

Wilt symptoms continue to progress on other branches in the tree crown over successive weeks or months.

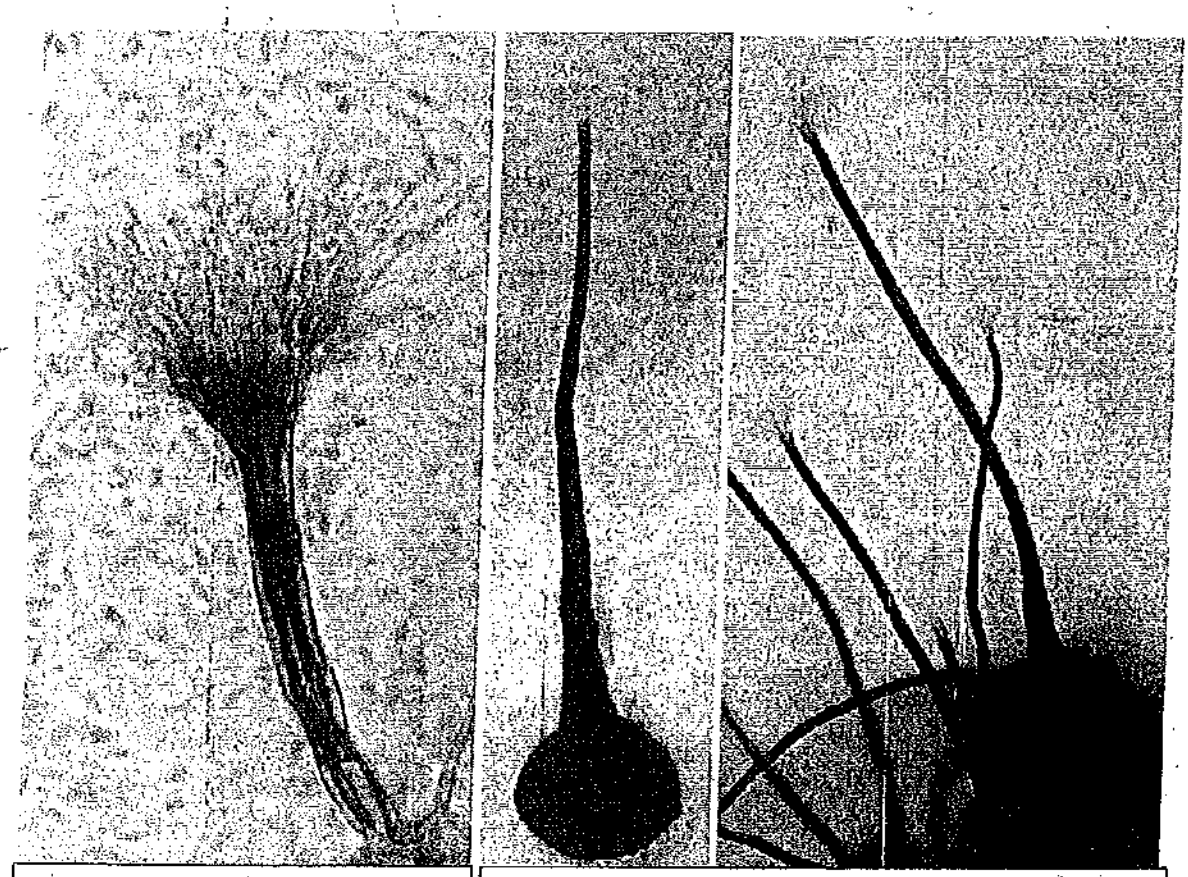
Foliage throughout the crown wilts and the tree dies.

Another diagnostic feature is the formation of brown streaks in infected sapwood.

This is common in trees where infections started by beetle transmission.

Discoloration may occur in the main trunk on trees rapidly killed by root graft infection.

Branches infected with the fungus typically have long brownish red streaks running the length of a branch section.



Graphium state of *O. ulmi*

Ascoma of Ophiostoma ulmi

Control:

Dutch elm disease control involves two different but related programs: (1) community-wide sanitation programs designed to reduce the level of elm bark beetles (principal carriers of the Dutch elm disease fungus); and (2) prevention of the spread of the disease through natural root grafts from infected trees to adjacent healthy trees.

Insecticides: Dursban insecticide spray of tree bases as part of their regular DED control program

Sanitation: destruction of all dead or dying elm wood present in the community.

The only way to prevent transmission through the roots is to create a barrier between diseased and healthy trees by severing or killing those roots between the trees

Chemical Treatment: Systemic fungicides (Arbotect) can be injected into the trunk or root-collar of the affected tree

Therapeutic tree injection is generally only effective where less than 5 percent of the crown of the tree shows symptoms.

Protective Treatment of Healthy Elms: The most effective chemical currently available is Arbotect.

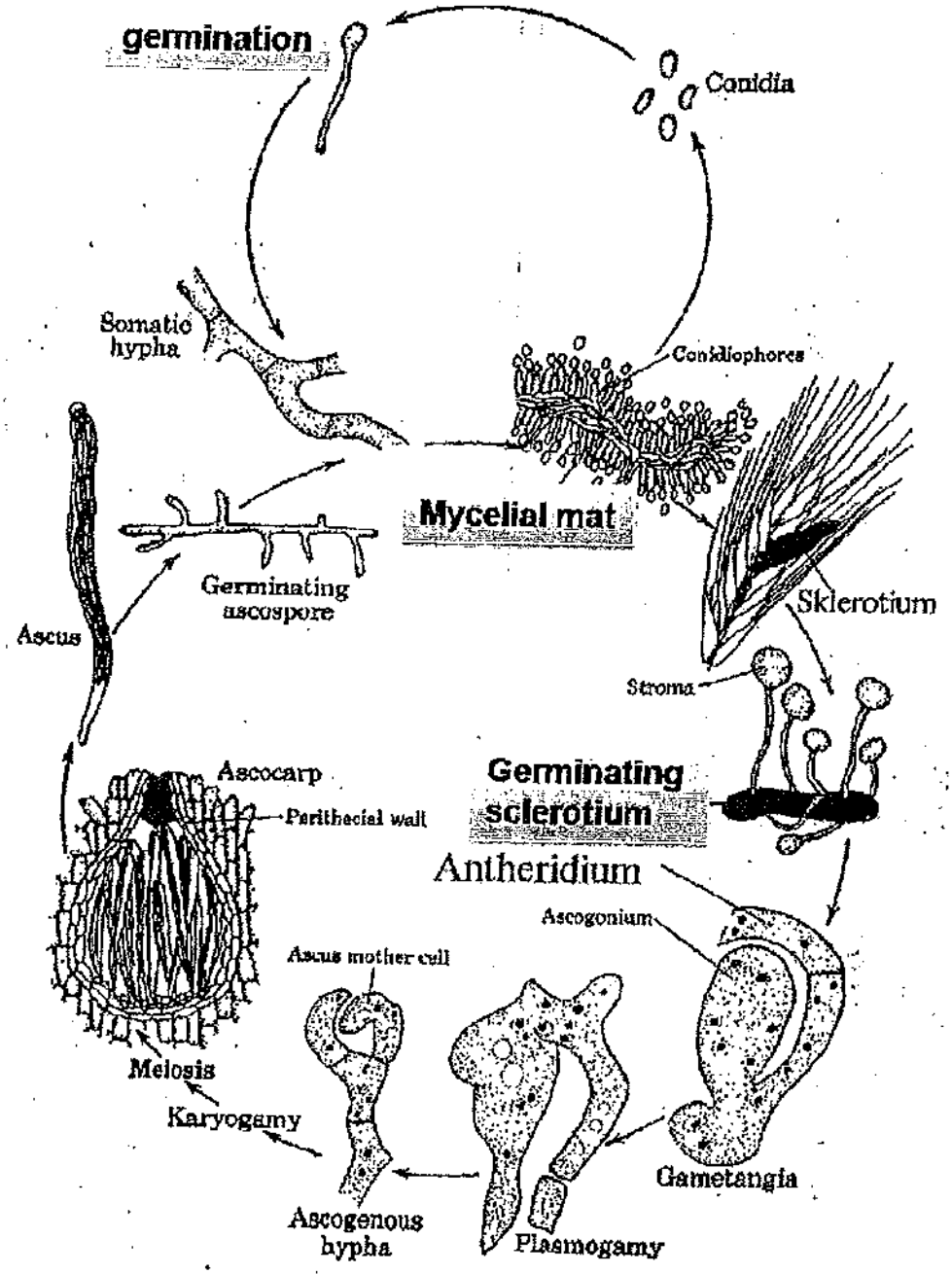
5- Ergot Disease of cereals by *Claviceps purpurea*

Symptoms and Signs

- Dark purple to black sclerotia (ergot bodies) found replacing the grain in the heads of cereals and grasses just prior to harvest.
- The ergot bodies consist of a mass of vegetative strands of the fungus. The interior of the sclerotia is white or tannish-white.
- In some grains, ergot bodies are larger than the normal grain kernels, while in other grains, such as wheat, grain kernels and the ergot bodies may be similar in size .
- Prior to development of the sclerotia bodies, the fungus develops a honey dew stage in the open floret.
- The "honey dew" consists of sticky, yellowish, sugary excretions of the fungus.

- Disease Cycle of *Claviceps purpurea*
- Sclerotia produced in small grain fields or grassy areas fall to the ground and survive on the surface of the soil.
- In the spring and early summer, the sclerotia germinate to produce tiny mushroom-like bodies (stroma) approximately the size of a pin .
- Spores (ascospores) formed by a sexual process in these bodies are shot into the air, and wind currents may carry them to grain heads.
- The first infections are from these wind-borne ascospores which invade the embryo of the developing kernel
- Soon a yellow-white, sweet, sticky fluid ("honey-dew") exudes from the infected flowers. The fluid contains a large number of asexually produced fungus conidia.

- Many species of insects visit the "honey-dew" and become contaminated with the fungus spores.
- These insects visit other grass flowers and spread the fungus.
- Spores may be transferred to other grain heads by rain-splash and direct contact, as well.
- Once the fungus becomes established in the florets, it grows throughout the embryos and replaces them, later producing the dark sclerotia.
- Many sclerotia fall to the ground before harvest and overwinter on the soil surface, serving as potential sources of spores the following year.



Life cycle of *Claviceps purpurea*

6- Nectria canker of hardwoods

Causal agent: *Nectria galligena* Bres.

Host: beech, white and yellow birch, red and sugar maple, poplar, and willow.

Symptoms:

A depressed or flattened area of bark near small wounds or at the base of dead twigs or branches is the first indication of the disease.

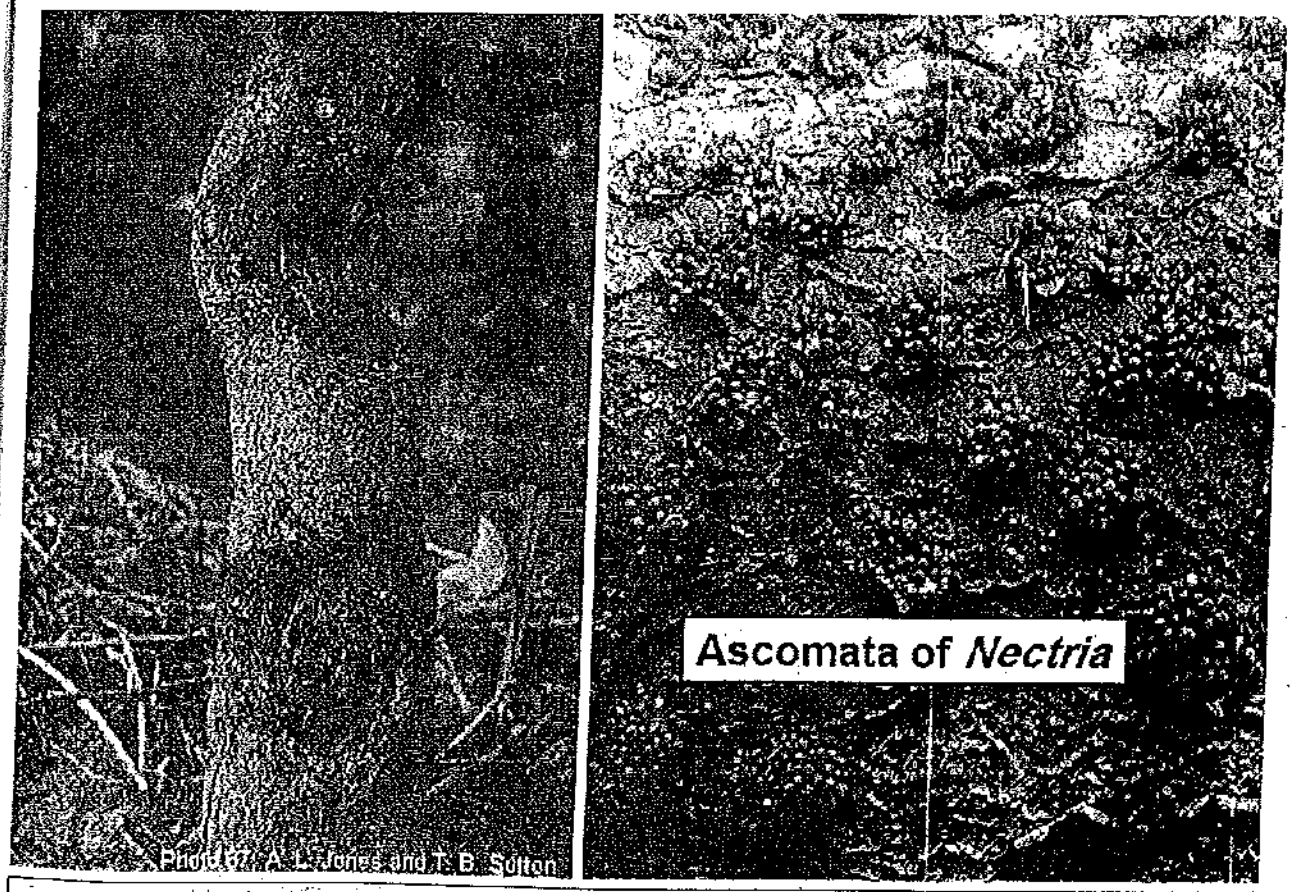
These areas may have a darker color and a water-soaked appearance.

The older and larger cankers may be concentric or target-shaped with callous ridges evident and the bark completely sloughed off or irregular in shape and lacking evidence of callous tissue.

The tiny, red, balloon-like fruiting bodies may be evident on the canker margin.

Cankered area is partially or completely covered by a roll of callus, (the tree is overcoming the infection.

The resulting deformation reduces the value of the tree



Nectria canker on apple tree caused by *Nectria galligena*

7- Soft rot Diseases by *Sclerotinia sclerotiorum*

Hosts: Cabbage, bean, citrus, celery, coriander, melon, squash, soybean, tomato, lettuce, carrots,, onions, peas, pumpkins and cucumber.

Symtoms:

Water-soaked spots on fruits, stems, leaves, or petioles which usually have an irregular shape.
These spots enlarge and a cottony mycelium covers the affected area.
The fungus spreads and the plant becomes a soft, slimy, water-soaked mass.
The cottony mycelium usually produces numerous sclerotia, black seed-like reproductive structures, a reliable diagnostic sign of *Sclerotinia* (these usually do not form until after host death).
In contrast to the water-soaked symptoms, the host may exhibit "dry" lesions on the stalk, stems, or branches, with an obvious definition between healthy and diseased tissues.
The lesions enlarge and girdle the plant part.

Distal portions of the plant become yellow, then brown, then die.

The girdled portion is often the base of the plant which causes the plant to die.

Sclerotia form within the stem pith cavities, fruit cavities, or between tissues (i.e., bark and xylem).

Apothecia of *Sclertiorum*

VI- Diseases caused by Basidiomycota

- These are the most structurally complex fungi, and include what we commonly call mushrooms, toadstools and bracket fungi. Rust and smut fungi are plant parasitic basidiomycetes.
- Basidiomycetes are characterized by a septate mycelium.
- The septa are highly complex and are pierced by a particular kind of pore termed a dolipore.
- The dolipore does not allow nuclei to pass through the septum.
- Consequently, hooked outgrowths called clamp connections are formed to ensure the proper distribution of nuclei as the hyphae grow.

The Basidiomycota have three classes:

a) Hymenomycetes

- Mushrooms and toadstools, composed of highly complex fruiting bodies (basidioma) and networks of dikaryotic mycelia.
- Basidioma have pores or gills, which are lined with basidia.
- Mushrooms as *Armillaria mellea* attack roots and trunks of many trees.
- Bracket fungi as *Ganoderma* grow on solid substrates such as tree trunks.

b) Uredinomycetes

- These are highly specialized plant pathogens which can only grow and reproduce on their host species or closely related species.
- Over 6000 members of the Uredinomycetes (commonly known as rusts) are important members of these sub-phyla.
- Wheat and bean rusts are economically important diseases.

Rust Diseases caused by Order: Uredinales

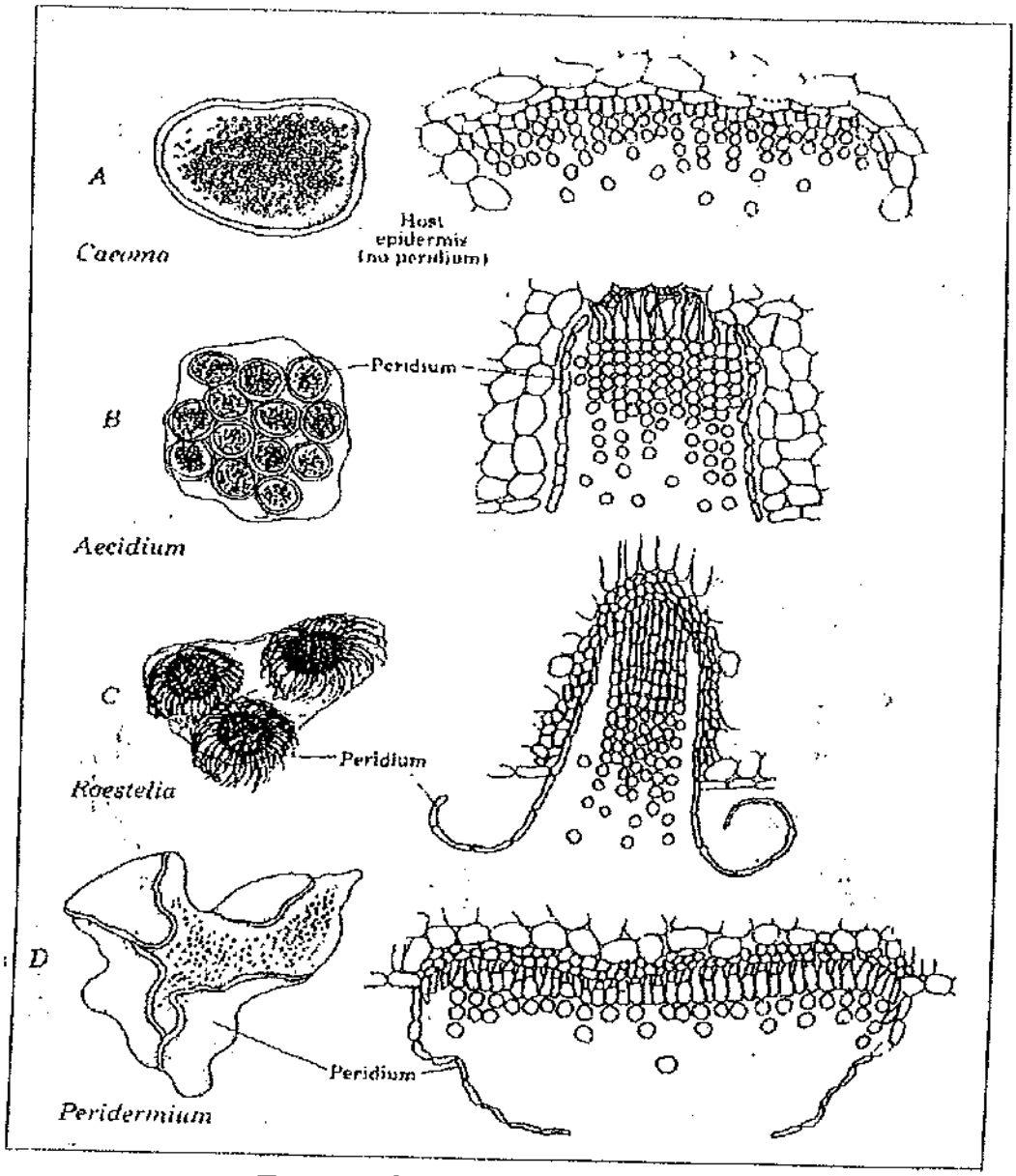
1- *Family Pucciniaceae*: (teliospores stalked)
   a. *Uromyces fabae, U. appendiculatus*
   b. *Puccinia graminis tritici*
   c. *Hemilea vastatrix*
   d. *Gymnosporangium junperi- virginianae*
   e. *Phragmidium mucronatum*

2- *Family Melampsoraceae*: (teliospores sessile)
   a. *Melampsora lini*
   b. *Cronartium ribicola*

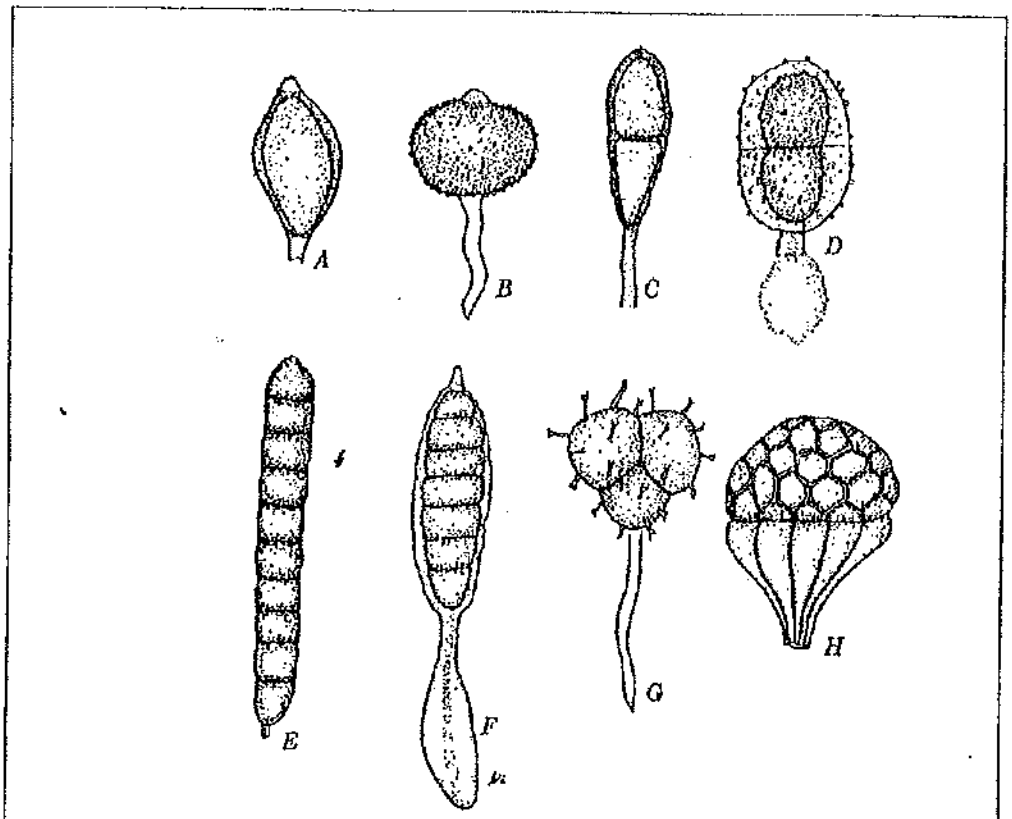
The life cycle of a typical rust species is among the most complex found anywhere in nature, consisting of five different spore stages (macrocyclic) on two plant hosts which are taxonomically entirely unrelated to each other.

<u>The macrocyclic life cycle is consisting of:</u>

A- Spermogonium or pycnial stage
B- Aecial stage
C- Uredial stage
D- Telial stage
E- Basidial stage (in soil)

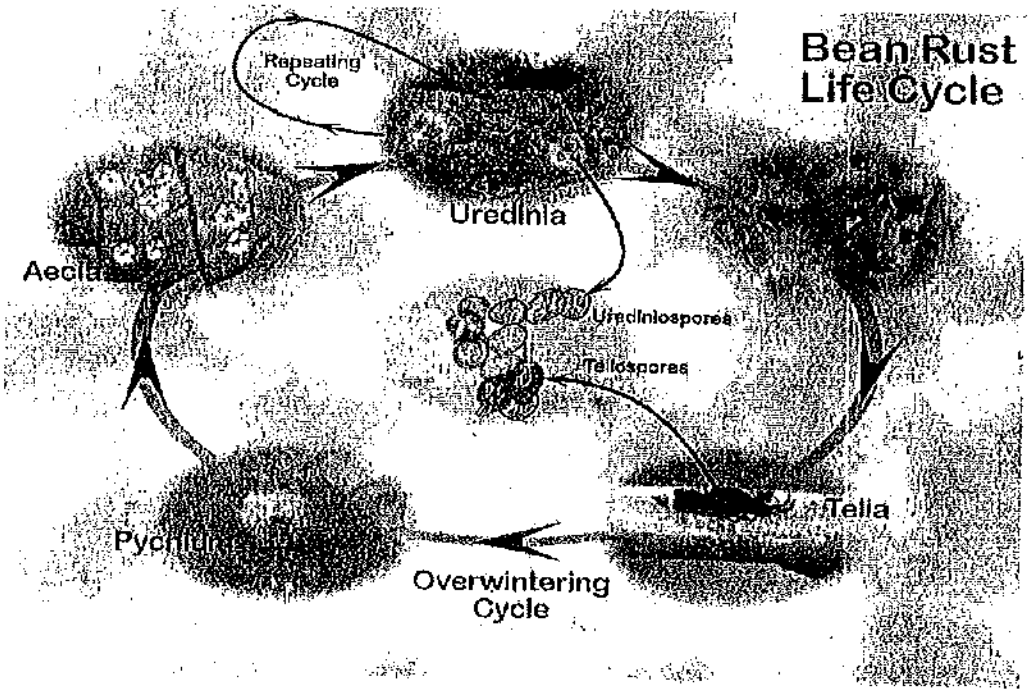


Types of aecia of rust fungi



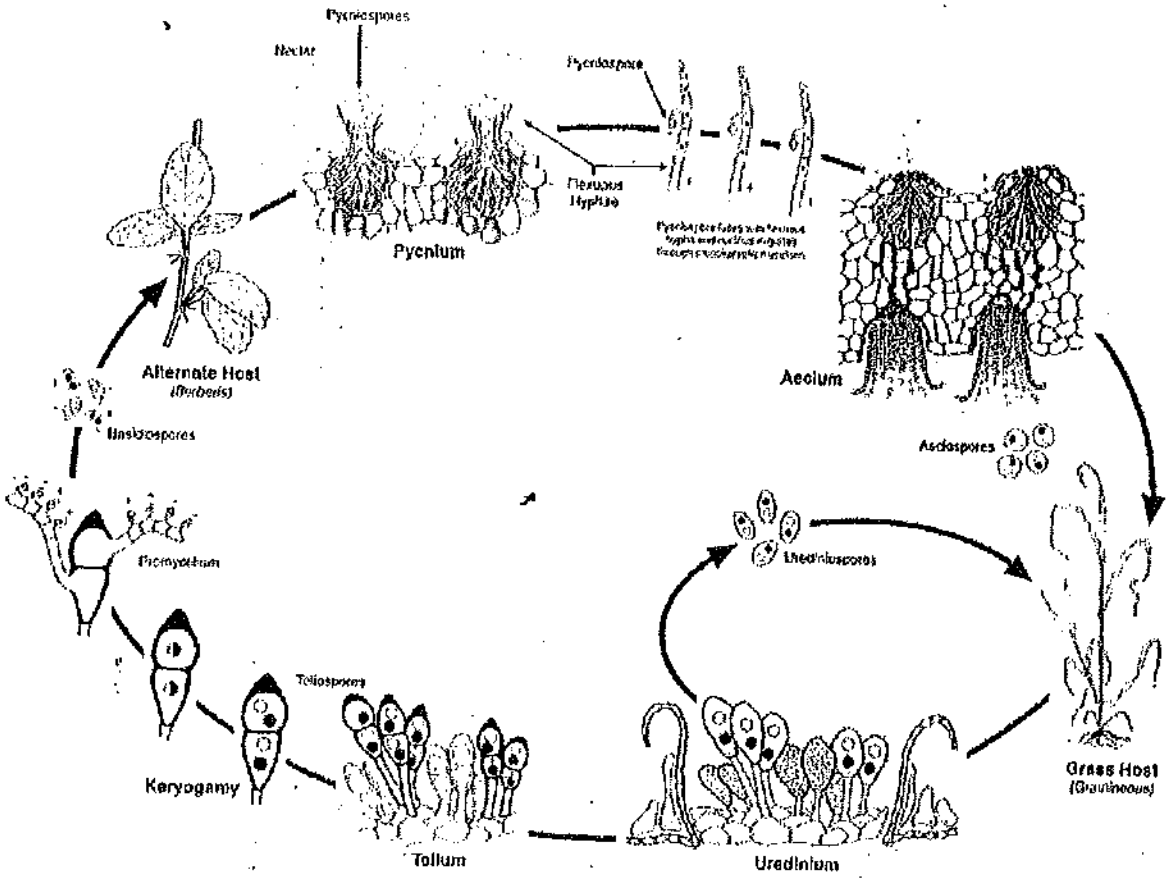
Various types of teliospores representing eight genera of rusts: (A) *Uromyces*, (B) *Pileolaria*, (C) *Puccinia*, (D) *Uropyxis*, (E) *Xenodochus*, (F) *Phragmidium*, (G) *Nyssospora*, (H) *Ravenelia*. [(B, D, E, H) Redrawn from Cummins by permission from *Manual of the Rusts of the United States and Canada*, by J. C. Arthur (1934). Purdue Research Foundation, Lafayette, IN.]

A- Rust of broad bean by *Uromyces fabae*



B- Black stem rust of wheat caused by *Puccinia graminis*

- Life cycle: (macrocyclic, 5 stages)
  - A- Spermogonium or pycnial stage: on upper srface of *Berberis vulgaris* leaves
  - B- Aecial stage :(on lower surface of *Berberis* leaves)
  - C- Uredial stage (early on wheat stem)
  - D- Telial stage (late on wheat stem)
  - E- Basidial stage (In soil)



Disease cycle of black stem rust of wheat caused by *Puccinia graminis tritici*

C- Yellow (stripe) rust of wheat by *Puccinia striiformis*
D- Orange rust on wheat leaves by *Puccinia recondite*
E- Rust of garlic by *Puccinia allii*
F- Peanut rust by *Puccinia arachidis* on the underside of leaves
G- Aple rust by *Gymnosporangium clavariaeforme*
H- Rust of rose by *Phragmidium mucronatum*
I- White pine rust by *Cronartium ribicola*
J- Rust of flax by *Melampsora lini*

c) Ustilagomycetes

They are commonly known as smuts, and over 1000 members of this class live in a similar manner to the rusts, as obligate biotrophic fungi – they can only grow on living plants.

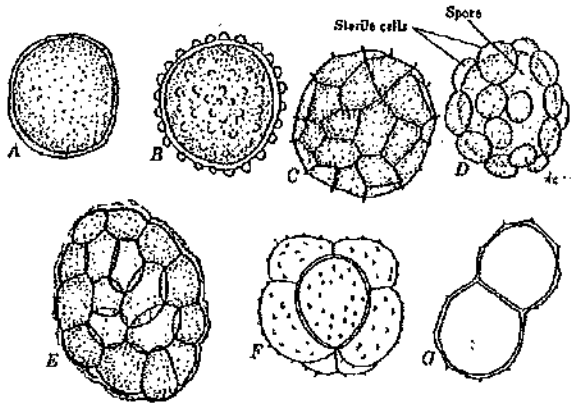
Maize Smut, caused by *Ustilago maydis* is an economically important disease

Smut Diseases caused by Order: Ustilaginales

1- *Family Ustilaginaceae*
Ustilago maydis, Ustilago tritici,
Ustilago hordei, Ustilago avenae,
Sphacelotheca sorghi, S. reliana
Tolyposporium ehrenbergii

2- *Family tilletiaceae*
Tilletia caris
Urocystis cepulae

3- *Family: Graphiolaceae*
Graphiola phoenicis



Modes of infection by smut fungi

1- Embryo infection:
- Loose smut of wheat by *U. nuda*

2- Seedling infection:
- Loose smut of oats by *U. avenae*
- Covered smut of barley by *U.hordei*
- Stripe smut of grasses by *U. striiformis*
- Dwarf bunt of wheat by *Tilletia contraversa*
- Onion smut by *Urocystis cepulae*

3- Shoot or local infection:
- Smut of anthers of *Melandrium album* by *U. violaceae.*
- Sugarcane smut by *U. scitaminae*
- Long smut of sorghum by *Tolyposporium ehrenbergii*
- Common smut of corn by *U. maydis*
- Rice bunt by *Tilletia barclayana*

Examples of smut diseases

1- Loose smut of wheat and barley by *U. nuda*

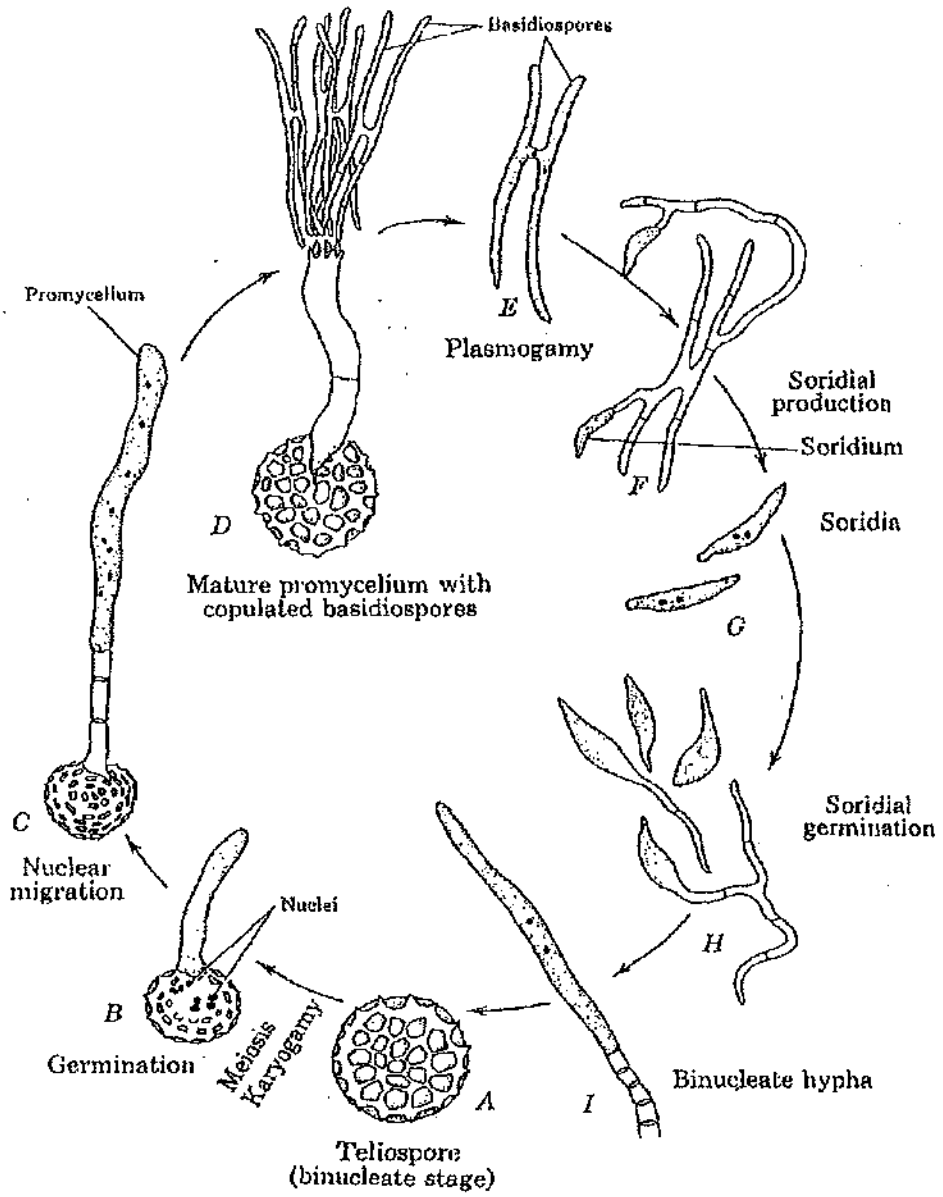
- Embryonic infection.
- It is not possible to determine whether a plant is diseased or not until the ears emerge when in infected plants the inflorescence is replaced by a mass of black, smut spores.
- Flag leaf may be infected in highly susceptible plants.
- Only glumes are affected in resistant plants
- Once spores are exposed they are blown by wind to flowers of healthy plants.
- Infection by *U. nuda* occurs through ovary walls, hyphae cross pericarp, enter the testa (intracellular), move towards the embryo (intercellular).
- Infected grains appear as healthy.
- When grains germinate, fungal mycelium becomes active, passes into the crown node of the seedling and is carried up during growth to the inflorescence primordea.
- Spore formation begins some weeks before the ears emerge and is complete at emergence.

2- Loose smut of oat:

- caused by *Ustilago avenae*
- Seedling infection.
- Spores are dispersed at flowering.
- On germination, the mycelium becomes established in glumes and pericarp.
- Embryo not invaded.
- When seeds are planted the dormant mycelium becomes active and invades the young seedlings.
- Subsequent development of the fungus is similar to that of *U. nuda*.

3- Bunt of wheat (stinking smut)

- caused by *Tilletia caries*. Seedling infection.
- Spores have an odor of bad fish (trimethylamine).
- All parts of the grain except the coat are replaced by smut spores.
- Infected grains (Bunt balls) are shorter and plumper than healthy.
- Broken bunt balls release millions of spores which contaminate healthy grains
- When contaminated grains are sown, spores on the grain coat germinate and the binucleate hyphae formed by fusion of sporedial cells infect the young coleoptile.
- Subsequent events are similar to those in U. nuda

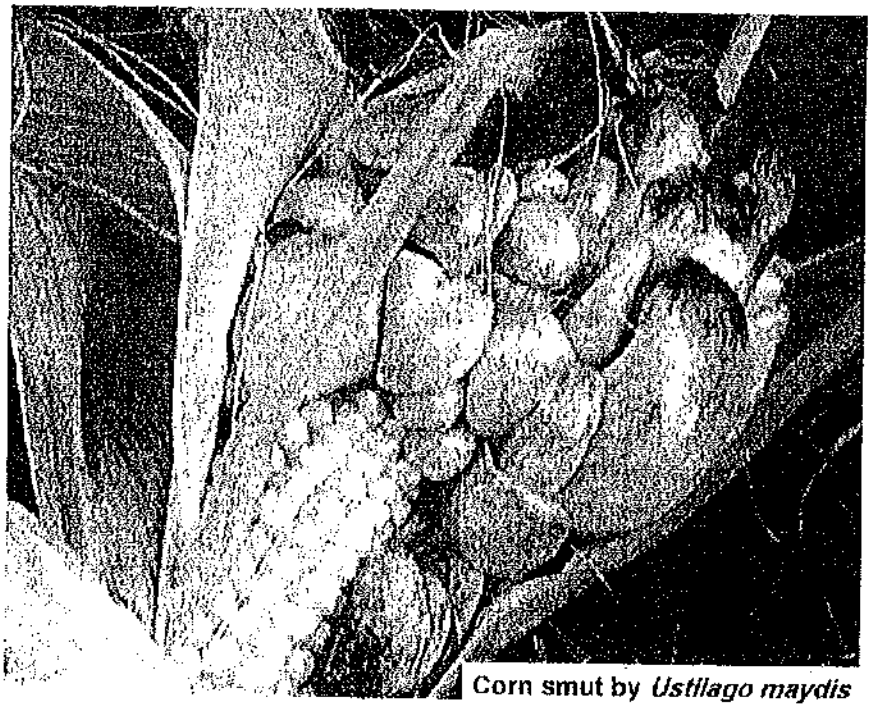


Life cycle of *Tilletia carie* cause of bunt of wheat

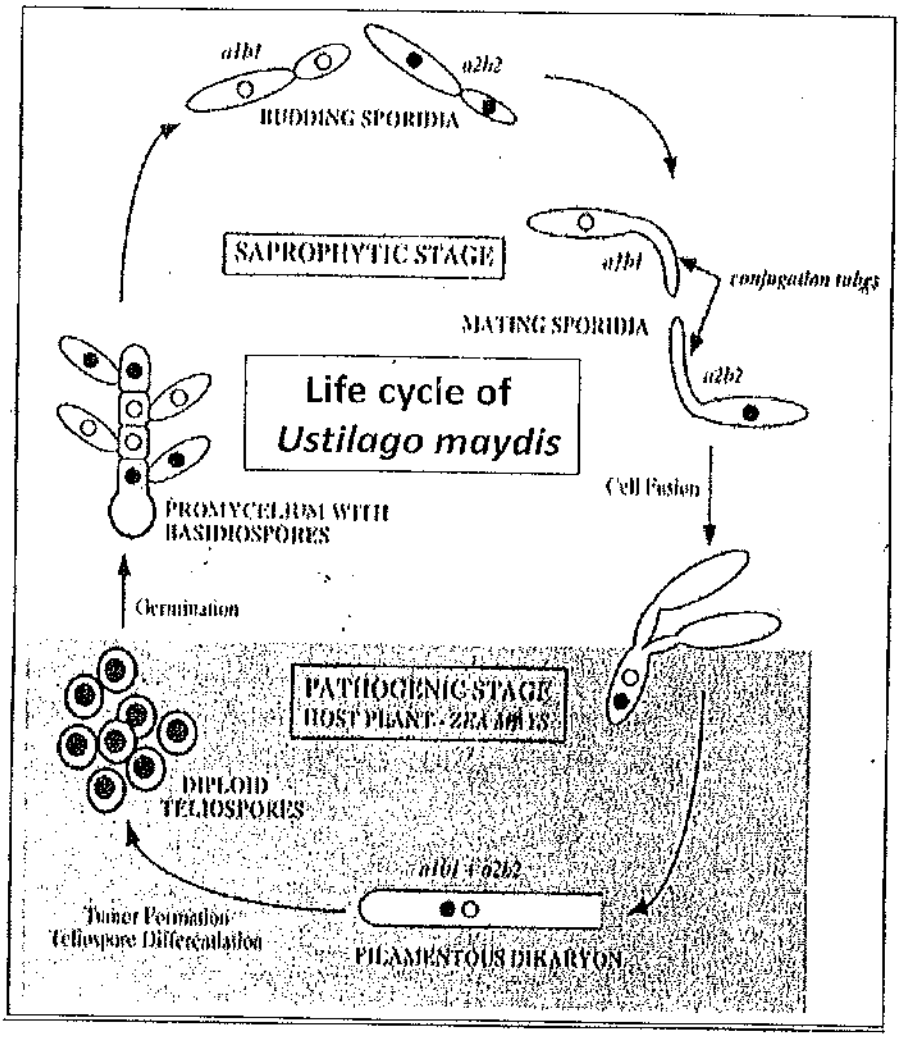
4- Sugarcane smut: Black, whiplike sorus arising from the terminal meristem of a stalk infected by *Ustilago scitaminea.*

5- - Onion smut by *Urocystis cepulae*

6- Common smut of corn by *Ustilago maydis*



Corn smut by *Ustilago maydis*



VII- Diseases caused by Deuteromycetes

1- Fusarium and Verticillium wilts

Fungus	Host
F. oxysporum f.sp. lycopersici	Tomato, potato, eggplant
F. oxysporum f.sp. vasinfectum	Cotton
F. oxysporum f.sp. conglutinans	Cabbage
F. oxysporum f.sp. Cubense	banana
F. oxysporum f.sp. betae	sugarbeet
Verticillium alboatrum (dark resting mycelium)	Tomato, alfalfa
Verticillium dahliae (microsclerotia)	*Potato, tomato,*
Verticillium nigrescens (chlamydospores)	Tomato

Mode of infection and survival:

- Wilt inducing forms of *Fusarium* and *Verticillium* enter their hosts through uninjured young roots or injured old roots.
- Plants infested by nematodes are severely attacked by wilt fungi.
- Fungi invade root cortex but do not damage it to a great extent. They become established in xylem vessels.
- Fungal enzymes disintegrate walls of xylem vessels.
- Fungal toxins are considered as a cause of wilt.
- Survival: by resting mycelium, chlamydospores or microsclerotia.

Disease symptoms:

- Lower leaf-petioles bend downwards (epinasty)
- Slight vein clearing and yellowing of the lower leaves.
- Chlorosis and death of leaves.
- Similar symptoms develop on younger leaves.
- During hot days, leaves wilt ,then recover at night.
- Wilt becomes permanent and plants die.
- Browning of vascular system.
- Water supply to leaves is plugged with fungal mycelium, conidia, tyloses and gums
- Some collapse of the vessels and disintegration of adjoining parenchyma.

4- Bean Anthracnose by *Colletotrichum lindemuthianum*

- Symptoms and Signs
- Seedlings grown from infected seeds often have dark brown to black sunken lesions on the cotyledons and stems.
- Severely infected cotyledons senesce prematurely, and growth of the plants is stunted. Diseased areas may girdle the stem and kill the seedling.
- Under moist conditions, small, pink masses of spores are produced in the lesions. Spores produced on cotyledon and stem lesions may spread to the leaves.
- Symptoms generally occur on the underside of the leaves as linear, dark brick-red to black lesions on the leaf veins. As the disease progresses, the discoloration appears on the upper leaf surface.
- On pods. Small, reddish brown to black lesions.
- Mature lesions are surrounded by a circular, reddish brown to black border.
- During moist periods, the interior of the lesion may exude pink masses of spores.
- Severely infected pods may shrivel, and the seeds they carry are usually infected.
- Infected seeds have brown to black sunken lesions.

5- Post harvest diseases

Disease	Causal agent
Spoilage of corn grains	*Aspergillus flavus,*
Rot on peanut kernels	*Aspergillus flavus*
Blue rot of apple fruits	*Penicillium expansum*
Blue rot of Citrus fruits	*Penicillium italicum*
Green rot of Citrus fruits	*Penicillium digitatum*
Cladosporium rot of corn	*Cladosporium cladosporioides*
Penicillium rot of corn	*Penicillium oxalicum*
Fusarium rot of corn	*Fusarium graminearum*