AGC 3342: Crop Protection - Plant Pathology Component

UNIVERSITY OF ZAMBIA

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FUNGI, BACTERIA, VIRUSES, NEMATODES

PLEASE NOTE THAT THE NOTES IN THIS HANDOUT ARE NOT EXHAUSTIVE AND MUST BE STUDIED IN COMBINATION WITH MATERIALS GIVEN IN THE MOST RECENT POWERPOINT SLIDES.

General Introduction on Pathogens

Do you remember that time you were not well and went to the hospital? What did the doctor say was the cause of your ailment? Bacteria, virus, fungi? You probably did not realise that plant diseases are also caused by microbes coming from these very groups of living things. Disease is a condition involving abnormal changes in the form, physiology, integrity or behaviour of a plant. Such changes may result in partial impairment or death of the plant or its parts. The subject of Plant Pathology is entirely dedicated to this area. Plant pathology is the living entities and the environmental conditions that cause disease in plants, the mechanisms by which these factors produce disease in plants, the interactions between the disease-causing agents and the diseased plant, and the methods of preventing or controlling disease and alleviating the damage it causes. Why would you say is the reason for studying and wanting to control diseases? Well, all of our food, much of our clothing, building materials, and much of the beauty in nature is provided by plants. Hence Plant diseases are a constant threat to the world's food and fibre crops, forests, and landscape plants. They affect the plant's performance in particular yield, causes famines (history has records to that effect), loss of farmer's income affecting the social welfare of the farmers household e.g. no money for school fees.

How Do You Tell That A Plant Has Disease?

Just like you and I, a diseased plant will show symptoms. One way in which you will know that the plant is diseased is by understanding the differences between a healthy plant, and one which has symptoms. Disease symptoms refers to the damage on plant parts as a result of the activities of pathogens. Figure 7 below illustrates the difference between a healthy plant and one showing symptoms of different diseases.

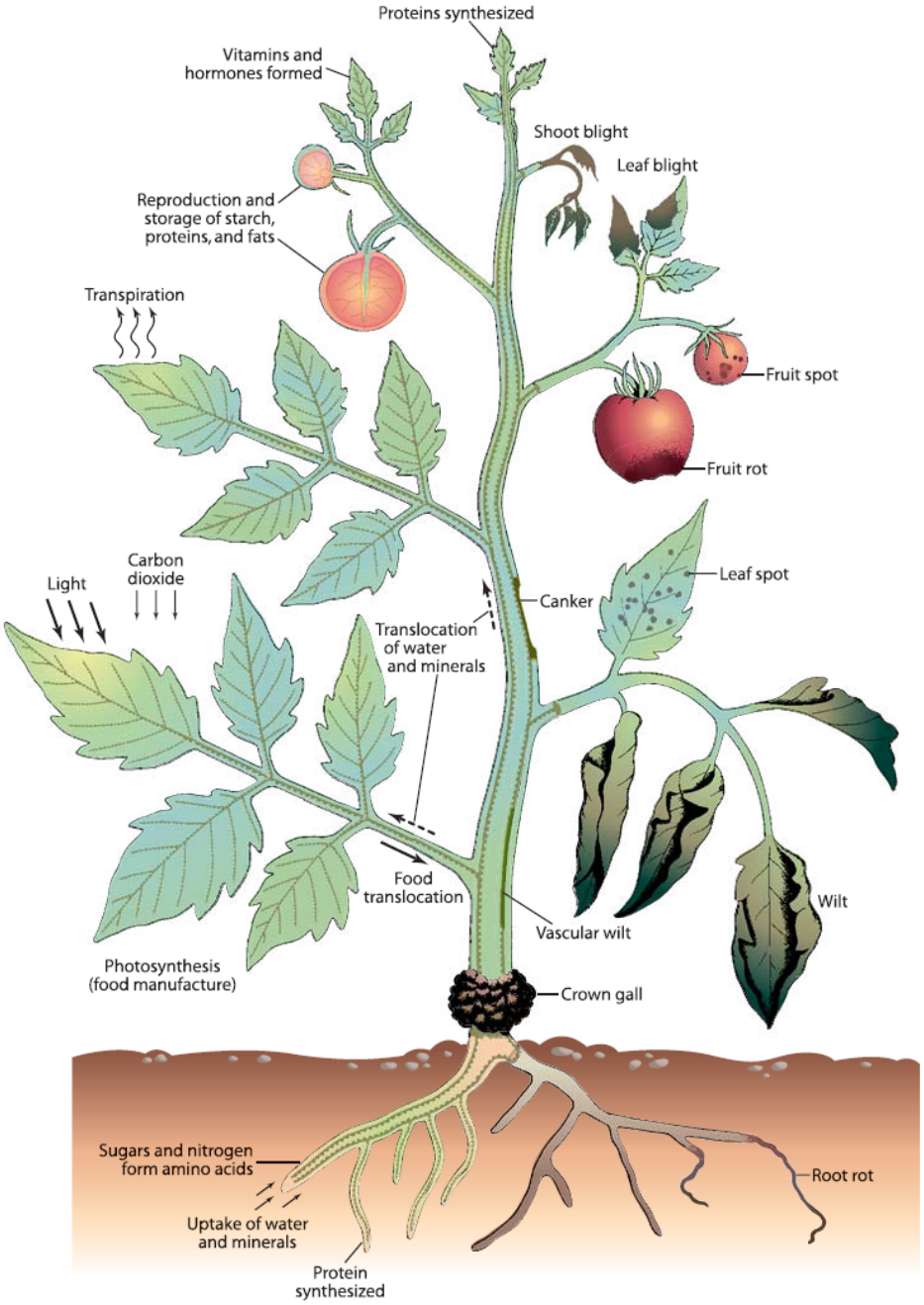

Figure 7. Schematic representation of a normal plant with normal functions (left) and diseased plant (right). Source: Agrios 2005 (Plant pathology. Elsevier)

The visible presence of the pathogen or products of the pathogen on the host plant are referred to "Signs of the pathogen". These are physical manifestation of the causal agent maybe in the form of spores (microscopic seed-like structures), mycelia (threadlike/cotton-wool-like), bacterial ooze (when the bacterium is present). In contrast, symptoms are the external and internal reactions or alterations of a plant as a

result of disease. They are physical manifestations of the host's response to pathogen. These are profound physiological and morphological changes that are visible in a diseased plant. The symptoms also change as the disease progresses. Symptoms may be localized (confined to a small area of the plant) or systemic (spread throughout the plant). Primary symptom: symptom proximal to the infection site produced as a direct response to the causal agent. A common primary symptom is a lesion; a well-defined localized diseased area. Secondary symptom: symptoms which result from indirect or "chain of events" interactions and occur some distance from the infection point and in many cases distal to the causal agent. **Symptoms** of disease include death and destruction of host tissue, wilting, abnormal growth and differentiation and discolouration of host tissue. Some parasites, called necrotrophs, secrete enzymes that kill host tissue, extract nutrients from the cells and then live in the dead tissue. The necrotic lesions caused by pathogens can be localised or extensive. Local necrotic **lesions** appear as discrete necrotic areas, while extensive, or spreading lesions spread until the whole organ or plant is killed. **Wilting** occurs when water loss is greater than water intake. It results from either: interference with water and nutrient absorption at the roots, interference with water conduction within the plant (i.e. infection of the vascular tissue), or loss of control of transpiration. **Abnormal growth and differentiation** results from deviation from the complex balance of interrelated reactions that take place in plants. Parasites can alter the hormonal balance in plants causing an abnormal increase in the size or number of cells, resulting in abnormal growth and differentiation, for example, and formation of galls. **Discolouration** of tissue is most commonly by chlorosis or mosaics of leaves, both of which can have a number of causes. Anything that interferes with the production of chlorophyll causes leaves to turn yellow, or chlorotic. Mosaicism is a symptom typical of many virus infections and is characterised by alternating light and dark green areas on the leaves. Normal tissue colours may be any of this: green, dark green, light green or multiple hues of red. While any deviation from this may be symptoms of the disease. Symptoms aid in the correct diagnosis of a plant disease

Soft versus Dry Rots: Soft rots are usually soft, usually watery and often odoriferous or produce a bad smell. Dry rot, or un-watery rot, are largely firm dry decaying caused by fungal infection, e.g. Fusarium dry rot on potato tuber or Fusarium dry root

rot of beans. The table below gives additional symptoms that you may find in your garden or crop field.

Table 2. Some symptoms of plant diseases and their descriptions.

Blight	A disease characterised by widespread death of plant tissue.
Local necrosis	The necrosis sites are limited and only involves a part or a few plant cells/tissues.
Canker	A sunken necrotic lesion often of a main stem, branch or root.
Damping-off	Collapse and rot of seedlings near soil level before emergence or soon after emergence caused by *Pythium* spp., *Phytophthora* spp., *Fusarium* spp., and *Rhizoctonia* spp.
Dieback	Partial defoliation, twig and branch death and even complete death of plants.
Downy mildew	White or grey 'bloom' on leaves and stems caused by production of sporangiophores and sporangia by members of the Peronosporales (downy mildew fungi).
Gall	An abnormal growth or swelling produced as a result of pathogenic invasion.
Mosaic	Patchy variation of normal green colour in leaves, usually light and dark green mosaic, symptomatic of many viral diseases.
Powdery mildew	White powdery 'bloom' on the plant surface caused by the production of fungal mycelium, conidiophores and conidia by members of the Erysiphales (powdery mildew fungi).
Pustule	A blister-like spore mass breaking through a plant epidermis.
Rot	Disintegration of tissue, often caused by enzymes or toxins produced by pathogens.
Rust	Rust-coloured pustules formed by members of the Uredinales (rust fungi).
Scab	A discrete, superficial roughened lesion. E.g: Citrus scab – *Elsinoe fawcettii,* Powdery scab of potatoes – *Spongospora subterranea*
Smut	A disease characterised by black spore masses on leaves, stems or inflorescences, caused by members of the Ustilaginales (smut fungi).
Vascular wilt	A disease in which the pathogen is confined to the vascular system of the host and in which wilting is a characteristic symptom; plants lose their turgidity and become flaccid, leaves collapse.

Abiotic vs biotic causes of diseases

Have you noticed that when you forget to water your plants on a hot day it wilts? What you may not know is that root damage due to a microbe that has degraded plant roots in the soil may also manifest as wilting above ground. Unfortunately, if the wilting has been caused by root damage to due microbes underground, irrigating the plant will not take away the wilting symptoms. Which means, you need to be able to know the real cause of the wilting, for you to be able to apply a correct intervention. So, how do you tell the difference between abiotic and biotic diseases? Symptoms caused by biotic agents usually are random in the field. Such symptoms also do not spread rapidly, that is, where one or a few plants are infected, it is unlikely that you will find the entire field covered with symptoms two or three days later. Random distribution of symptoms on injured plants is usually caused by a biotic factor, such as infectious disease pathogens or an insect/animal. Uniform patterns are generally associated with abiotic or non-infectious agents like pesticides, fertilizers, environmental or site stress and mechanical damage. Nonliving or abiotic agents can indirectly result in plant problems. Additionally, several factors in the plant's environment can produce disease-like symptoms: weather extremes, high winds, high or low temperatures, nutrient deficiencies, physical damage and poor cultural conditions. Frost often damages buds and leaves in early spring. Hail can cause leaf spotting or holes. Drought and high winds result in wilting and in extreme cases, browning and curling. Air, water and soil pollution affect plant health and can produce disease-like symptoms. Soil imbalance, resulting from construction or other dumping, or misapplied garden chemicals can cause damage and disease-like symptoms. Dumping of household, automotive and industrial chemicals can also produce plant damage. Plant disease can result from a combination of abiotic agents and biotic agents. Plants may be initially placed under stress by nonliving agents. This creates susceptibility in plants for attack by living agents. Drought may damage roots which then are more likely to be infected by fungal diseases. Let us now look at each of the groups of disease-causing organisms closely in the sections below.

Fungi

Fungi are eukaryotic, spore bearing, achlorophyllous organisms that generally reproduce sexually and asexually, and whose usually filamentous, branched somatic structures are typically surrounded by cell walls containing chitin or cellulose, together with many other complex organic molecules. They include microorganisms such as yeasts, molds and mushrooms. There are four major classes of fungi:

1. Oomycetes-aseptate fungus-like organisms characterised by production of sexual spores called oospores. An example is *Phytopthora infestans* causing late blight of tomato and Irish potatoes. (Another example is *Pythium spp*, causing several root rots)
2. Ascomycetes-sexually and asexually reproducing fungi with varying morphological features. Sexually reproducing ones characterised by production of ascospores. An example is *Colletotrichum lindemuthianum* causing bean anthracnose disease
3. Basidiomycetes-mostly smuts and rusts, characterised by production of sexual spores called basidiospores (typically 4) on a basidium. An example is *Ustilago maydis* causing maize smut disease and *Uromyces appendiculatus* causing bean rust
4. Zygomycetes (old name)-sexually and asexually reproducing fungi with varying morphological features and coenocytic hyphae. Sexually reproducing ones characterised by production of zygospores. An example is *Rhizopus stolonifer* causing soft rot of sweet potato during storage, packaging and sale.

Symptoms of fungal diseases

You need to be able to properly identify a symptom of a fungal disease so that you pick the correct control measure for that fungal disease. Otherwise, you will be treating something using a wrong remedy. A preliminary diagnosis of a fungus disease is sometimes possible from symptoms. Though non related pathogens may produce similar types of damage. Infection of the root and collar by soil-inhabiting fungi may lead to damping-off of seedling or wilt or sudden death of mature plants. Stem necrosis may take the form of die-back, or limited anthracnose lesions or cankers. On leaves a shot-hole effect is produced when lesions become necrotic and dead parts fall away. Blight commonly occurs when leaf damage is sudden and serious. Mildew is

used when the pathogen produces a visible growth over host surface. Hyperplasia i.e. over development (galls, witches broom, scab lesions) , or hypoplasia (dwarfing or rossetting). Transformation of host tissue are characteristic of smut diseases and ergot. Sometimes symptoms may appear on parts of a plant remote from that actually attacked, as in diseases of certain fruit trees and certain wilts, where toxic substances are secreted by the pathogen and transported in the host. Considerable variability in type or severity of symptoms or in parts attacked is commonly observed, for instance in *Phytophthora* diseases. It is important to decide whether the symptom variability is due to local environment, host variety or different strains of the pathogen. Reproduction of the original symptoms upon inoculation of a healthy plant is one of the requirements for proving the pathogenicity of any fungus suspected of causing a disease.

Figure 8 below illustrates the various methods through which fungi (and bacteria, viruses, nematodes) may spread. If you understand how they spread, then you can better understand why some of the control measures mentioned above have been chosen.

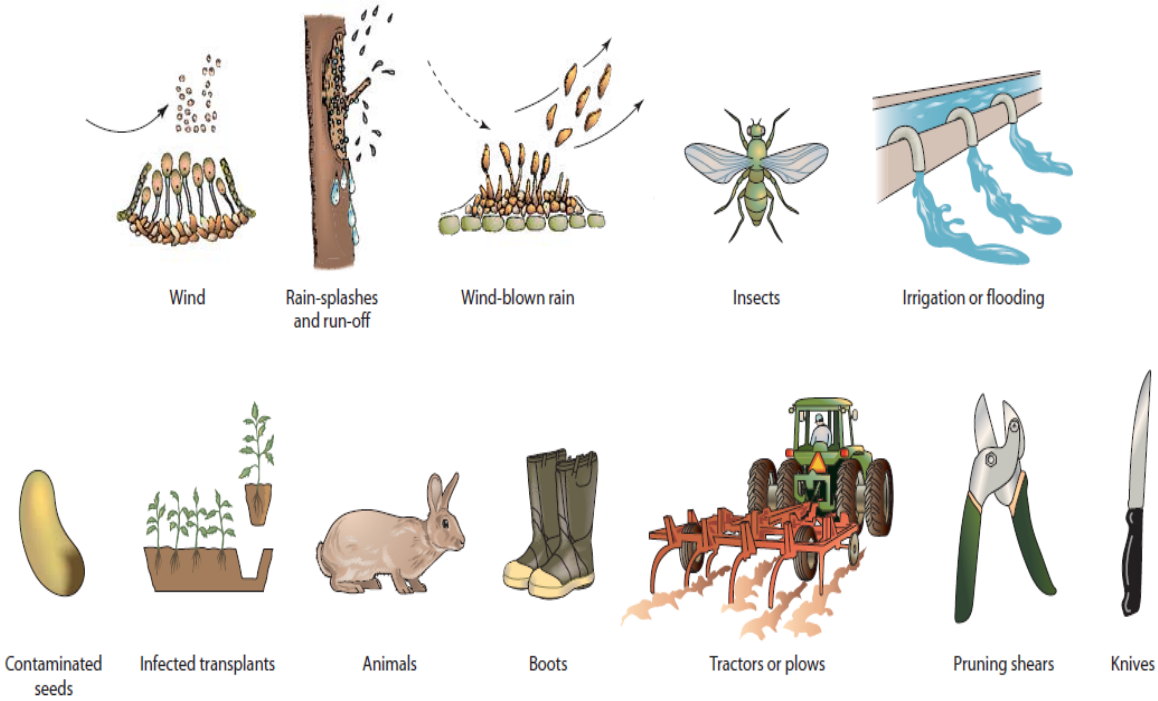

Figure 8. Methods by which fungi spread (please note that some of these also apply to the spread of viruses, bacteria and nematodes. We cove these in the sections below). Source: Agrios 2005 (Plant pathology. Elsevier)

Bacteria

When you and I have a bacterial infection, the doctor prescribes an antibiotic to deal with the disease. Plants also do get bacterial infections and diseases. Bacteria are microscopic prokaryotes with a cell membrane, rigid cell wall and one or more flagella. Figure 9 below illustrates the main structures found in a bacterium. Bacteria are everywhere, a single teaspoon of healthy topsoil from your garden contains about a billion bacterial cells. Of the known species, of almost 200 are plant pathogenic bacteria, many of them consisting of numerous pathovars, that is strains differing only in the plant species they infect. Some bacteria have thick, rigid cell walls which will retain dye from a cell staining method developed by Christian Gram, while other bacteria will not accept this stain. This method of staining results in the bacteria being classed as Gram-positive or Gram-negative and is an important factor in identification and classification. Gram-positive bacteria appear purple and Gram-negative bacteria appear pink under magnification.

Almost all plant-pathogenic bacteria develop mostly in the host plant as parasites and partly in plant debris or in soil as saprophytes. However, there are great differences among species in the degree of their development in one or other environment.

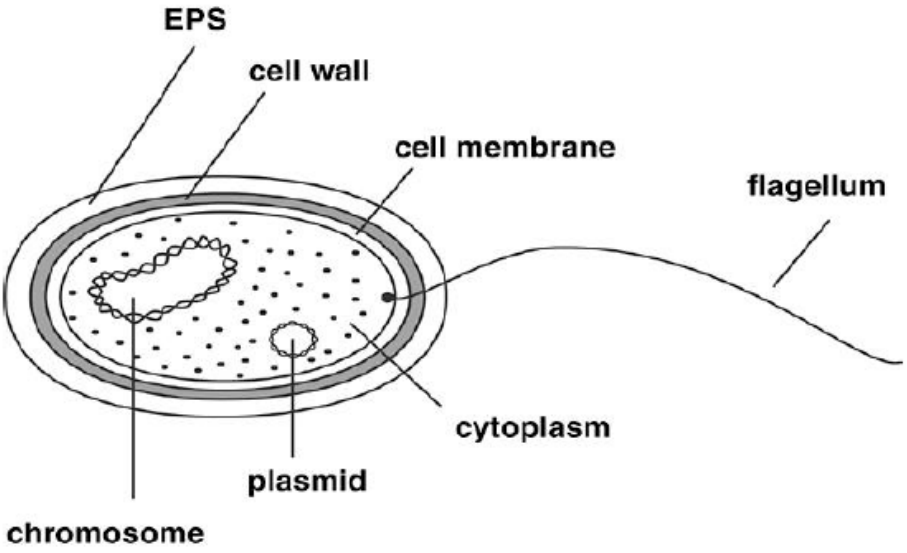

Figure 9. General features of bacteria
Source: Agrios 2005 (Plant pathology. Elsevier)

Some bacterial pathogens, such as *Erwinia amylovora* (cause fireblight) produce their populations in the host plant but in the soil, their number decline rapidly and don't contribute to the propagation of the diseases from season to season. They rely on their association with vegetative propagating organs of the host or seed, existence of insect vectors and the perennial nature of the host for the plant to plant infection cycle to occur; as a result, they have lost the requirements for survival in the soil. Others are Soil inhabitants such as *Agrobacterium tumefaciens* (crown gall), *Pseudononas solanacearum* (Bacterial wilt in solanaceous crops), *Steptomyces scabies* (common scab of potato). They build their population with the host but their population only gradually declines when released into the soil. In the presence of the susceptible host over a period, sufficient high numbers of bacteria could be released to cause a net increase of bacterial populations in the soil from season to season. Most pathogenic bacteria are soil invaders that enter the soil in host tissue or as free cells, because they are poor competitors as saprophytes, only persists as long as the host tissue resist decomposition. In the soil, bacteria live mostly on plant material and less often freely or saprophytically, or in their natural bacterial ooze, which protects them from various adverse factors. Bacteria may also survive in or on seeds, other plant parts or soil insects. On plants, bacteria survive epiphytically, in buds, on wounds, in their exudates, or inside the various tissues or organs that they infect. Even species with flagella can move only very short distances in water on their own power. Most plant pathogenic bacteria, however, are disseminated by water (include rain), soil water movements and insects, and to a lesser degree by humans (handling, cultural practices and transportation and introduction of infected plant materials in new areas) and other animals (birds, rabbits etc.). It is important to know the survival characteristics of bacteria for effective management strategy and intervention in the dissemination of plant pathogenic bacteria.

Similar to what you studied under fungi, bacteria do cause symptoms on plants and you can only deal with the diseases correctly, if you can identify the cause of the symptoms correctly. We will now look at the symptoms associated with bacterial disease below. Symptoms of bacterial infection in plants are much like the symptoms in fungal plant disease. They include leaf spots, blights, wilts, scabs, cankers and soft rots of roots, storage organs and fruit, and overgrowth. Most common symptoms of

bacterial diseases are leaf spots and blight, soft rots of fruit, root and storage organs, wilts, galls, scabs, cankers.

- **Leaf spots**

Spots appear on leaves, blossoms, fruits and stems. If the spots appear and advance rapidly the disease is considered blight. Spots on leaves of dicotyledonous plants often have a rotten or fishy odour, are water soaked and are initially confined between the leaf veins and will appear angular. In some cases, bacterial ooze will be present; this is diagnostic for bacterial infections. Sometimes a chlorotic halo will surround the bacterial lesion of an infected leaf (unlike with fungi). Spots may coalesce causing large areas of necrotic tissue. Bacterial spots will appear as streaks or stripes on monocotyledonous plants. Almost all bacterial leaf spots and blights are caused by the genera *Pseudomonas* and *Xanthomonas*.

- **Cankers**

Primarily *Pseudomonas and Xanthomonas* spp. cause canker disease of stone fruit and pome fruit trees, and canker disease of citrus respectively. Canker symptoms can appear on trunks, stems, twigs and branches. The most conspicuous symptom of a bacterial canker disease in stone and pome fruit trees is the development of cankers and gum exudation (gummosis). Cankers can be slightly sunken, dark brown and much longer than broad. The cortical tissue of the canker can be orange brown to dark brown. Gum is produced in most cankers and some branches and twigs. Cankers that do not produce gum may have a sour odour and be soft, sunken and moist. Cankers that girdle trunks and branches can result in leaf stress and eventual dieback of the portion of the tree distal to the canker.

- **Bacterial Galls**

Bacterial galls can be produced by the genus *Agrobacterium and certain species of Arthrobacter, Pseudomonas, Rhizobacter and Rhodococcus. Agrobacterium tumefaciens, A. rubi and A. vitis alone are* responsible for galls in over 390 plant genera worldwide. Galls of these genera have been referred to as crown gall, crown knot, root knot and root gall. Species of these bacteria are thought to be present in most agriculture soil. A wound in the host is required for the pathogen to gain entry into the host tissue. Gall tissue is composed of disorganized, randomly proliferating

cells that multiply in the intercellular (between the cells) spaces in the vicinity of the wound. In the presence of the pathogen rapid and continuous cell division (hyperplasia and hypertrophy) of the plant tissue persists. Gall damage can be deadly. Crown gall first appears as small, whitish, soft round overgrowths typically on the plants crown or at the main root. The colour of galls (tumors) caused by *A. tumefaciens* can be orange-brown and as it enlarges the surface can become convoluted and dark brown. This is most often found in high value nurseries.

- **Bacterial Vascular Wilts:**

Vascular wilts caused by bacteria primarily affect herbaceous plants such as vegetables, field crops, ornamentals and some tropical plants. The causal pathogen enters, multiplies in, and moves through the xylem vessels of the host plant and interferes with the translocation of nutrients and water by producing gum. The pathogen will often destroy parts of the cell wall of the xylem vessels resulting in pockets of bacteria, gums and cellular debris. The symptoms of bacterial wilt disease include wilting and death of the aboveground parts of the plant. In some cases, bacterial ooze seeps out through stomata or cracks onto the surface of infected leaves. Usually this ooze does not occur until the infected plant tissue is dead.

- **Bacterial Soft Rots**

Primarily the bacteria that cause soft rots in living plant tissue include *Erwinia spp., Pseudomonas spp., Bacillus spp.* and *Clostridium spp.* Many soft rots are caused by non-phytopathogenic bacteria which are saprophytes that grow in tissue that has been killed by pathogenic or environmental causes. Soft rots attack a large number of hosts and are best known for causing disease in fleshy plant structures both above and below ground. These bacteria are almost always present where susceptible plants under stress are in the field or in storage. Soft rot pathogens enter the host through wounds. After entering the host tissue these bacteria produce enzymes that break down the middle lamella causing separation of the cells at the site of the infection. The cells die and disintegrate. Rotting tissue becomes watery and soft and bacteria will form slimy foul smelling ooze that will ooze out of infected tissue. Bacterial ooze is diagnostic of soft rot diseases.

- **Bacterial scabs**

Bacterial scabs primarily infect belowground parts of plants such as potatoes. Common scab of potato is caused by *Streptomyces scabies* which cause localized scabby lesions on the outer surface of the tuber. Typically, corky tissue will form below and around the lesion. Rot pathogens can gain entrance into the host tissue through these lesions and further degrade the host.

VIRUSES

Viruses are very small (submicroscopic) infectious particles (virions) composed of a protein coat and a nucleic acid core. They do not consist of cells, but of particles made up of a nucleic acid core of either single-stranded or double-stranded RNA or DNA, surrounded by a protein coat.

Symptoms of viral infection

If you were to look at virus-infected plants, you would notice a wide range of symptoms depending on the disease but often there is leaf yellowing (either of the whole leaf or in a pattern of stripes or blotches), leaf distortion (e.g. curling) and/or other growth distortions (e.g. stunting of the whole plant, abnormalities in flower or fruit formation). An abnormal appearance in plant is usually the first indication that a plant is virus-infected. If you looked at some virus symptoms, you might confuse them for genetic abnormalities of a plant, and show great diversity and severity. They include mosaic, flecking or spotting on foliage, Some viruses are host-specific, but others have a broad host range e.g. tobacco mosaic virus (TMV) may infect Tomato, Tobacco, Eggplant etc. Sometimes the virus is restricted to certain parts of the plant (e.g. the vascular system; discrete spots on the leaf) but in others it spreads throughout the plant causing a systemic infection. Infection does not always result in visible symptoms, and yet they might, at the same time, be spreading from one area of your farm to another.

Spread of viruses

Viruses in plants spread through some of the methods you will notice with viral diseases in your livestock, for example. It is important for you to know how they spread, so that you may effectively control them in your crop. They methods of spread include (i) mechanical transmission through sap by plants touching one

another, through root grafts, and manhandling, (ii) vegetative propagation and grafting, (iii) seed, (iv) pollen and (v) Insects. The major insect vectors include:

- **Aphids**: transmit viruses from many different genera, including *Potyvirus, Cucumovirus* and *Luteovirus*.The green peach aphid Myzus persicae, the vector of many plant viruses, including Potato virus Y.

- **Whiteflies**: transmit viruses from several genera but particularly those in the genus Begomovirus. *Bemisia tabaci*, the vector of many viruses including Tomato yellow leaf curl virus and Lettuce infectious yellows virus.

- **Hoppers**: transmit viruses from several genera, including those in the families Rhabdoviridae and Reoviridae. The picture shows Micrutalis malleifera, the treehopper vector of Tomato pseudo-curly top virus.

- **Thrips**: transmit viruses in the genus Tospovirus. The picture shows Frankinella occidentalis, the western flower thrips that is a major vector of Tomato spotted wilt virus

- Other organisms that transmit viruses that you need to be aware of are mites, nematodes, dodder, fungi (carried by zoospores and mycelium) and insects. Nematodes are root-feeding parasites, some of which transmit viruses in the genera *Nepovirus* and *Tobravirus*. E.g. *Paratrichodorus pachydermus*, the vector of Tobacco rattle virus.

NEMATODES

As you may know by now, the soil is a complex world where many living things are found, including some we cannot see with our naked eye (without a microscope), such as nematodes. Nematodes are a group of disease-causing agents that you will probably find more complicated than all others. Generally, nematodes are microscopic, worm-like animals that live saprophytically in water or soil, or as parasites of plants or animals. The most important nematodes in the tropics and subtropics such as Zambia are root knot nematodes (genus: *Meloidogyne*) and cyst

nematodes (genera: *Heterodera* and *Globodera*). Figure 10 below gives a generalized drawing of a nematode.

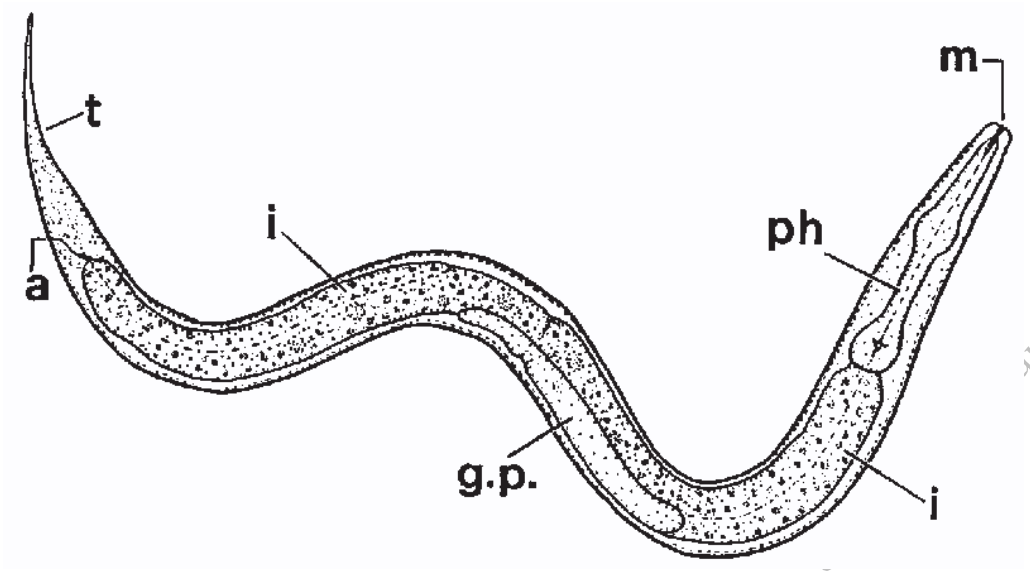

Figure 10. Schematic representation of a nematode. m = mouth, ph = pharynx, gp = genital primordium, i = intestine, t = tail, a = anus.
Source: Poletti et al 2004 (DOI: 10.1140/epjd/e2004-00084-4)

When you look at the nematode under a microscope (especially a microscope with an 'internal' measuring rule) or using hand lenses, you will observe that these wormlike creatures are 300 to 1000 µm long by 15 to 35 um wide, with a transparent cuticle. The external colourless cuticle is marked by surface striations. The cuticle is produced by the hypodermis (a group of cells the extends into the body cavity as four chords separating four bands of longitudinal muscles. The longitudinal muscles enable movement. Additional muscles are located at the mouth, digestive tract, and reproductive structures. The body cavity contains fluid through which circulation and respiration occurs. The digestive system is a hollow tube extending from mouth through esophagus, intestine, rectum and the anus. Lips, usually six, surround mouth - may be useful for identification. All plant parasitic nematodes have a hollow stylet or spear that is used to puncture cells an allow nutrients to be obtained. The reproductive systems are well developed. The female nematodes have one or two ovaries, followed by oviduct and uterus terminating in a vulva. The male has testis, seminal vesicle and a terminus in a common opening with the intestine. Males have copulatory spicules to aid in mating process. Reproduction may occur either sexually by sperm and egg, hermaphroditic or parthenogenically. Some species have no males. The life histories

of plant parasitic nematodes in general are quite similar. An egg hatch to produce a single juvenile. The first juvenile stage may occur in the egg. Each juvenile stage ends with a moult. Differentiation into female and male occurs after the first moult. Differentiated males and females are fertile and may reproduce. A life cycle from egg to egg may be completed within 2 to 4 weeks under optimum environment al conditions and may be modified by cool temperatures. The infective stages must feed on susceptible host(s) or starve to death absence of susceptible hosts may result in elimination of a nematode population.

Spread of Nematodes

Do you remember the methods we discussed above as being important to the spread of bacteria, fungi and viruses? Some of them apply to nematodes as well. It is possible for you to understand how nematodes are spread so that you can understand why some of the control measures are targeted at these methods of spread. Spread of nematodes is facilitated by water, wind, cultivation equipment, soil dispersal, mankind. A few nematodes that attack aboveground plant parts are not only spread through the soil by may be splashed onto the plants by falling rain or overhead watering or ascend wet plant stem or leaf surfaces by their own power.

Symptoms of nematode diseases

The symptoms of nematode attack are often non-specific, such as poor growth, stunting or wilting, and a close examination may be necessary to determine the cause of the trouble. Symptoms may be deformed roots or galls, this so if when the nematode is a root-feeder, and the above -ground symptoms are due to root nematode. Various nematodes induce gall formation, especially species of *Meloidogyne* e.g. *M. Incognita* on tomato presumably due to the production or induction of plant hormones. Only small part of the damage is due to mechanical processes. In summary, the root symptoms may appear as root knots, galls, root lesions, excessive root branching, injured root tips and root rots. While, the above ground symptoms include stunted growth, yellowing, wilting, reduced yields and poor quality of products.