EXPERIMENT NO: 01

METHODS OF DIAGNOSIS AND DETECTION OF VARIOUS PLANT DISEASES

This topic is a practical introduction to symptomology with an outlook to prepare for diagnostic work.

Plant disease diagnosis is the identification of nature and cause of diseases based on signs and symptoms. Identification of symptoms and signs and comparative symptomologies of infectious and non-infectious diseases are considered to be most essential for diagnosis of unknown plant diseases. The presence of the pathogens or various structures viz., mycelium, sclerotia, sporophores and spores produced on the surface of the host are called signs whereas symptoms refer to only to the appearance of infected plants or plant tissues.

Diagnosis of a plant disease is one of the most important and useful techniques in plant pathology and familiarity with the basic classification of plant diseases, the characteristics of organisms that cause particular diseases, the symptoms and signs associated with different types of disease is a prerequisite to diagnose a plant disease.

Majority of plant diseases can be diagnosed by a relatively straight-forward procedure involving an evaluation of background information and a macroscopic and often microscopic examination of diseases plant. However, some diseases can be diagnosed correctly through the use of electron microscope and serology. A majority of abiotic and biotic factors may cause similar disease symptoms and the best proof that a particular organism is the cause of disease is fulfilment of Koch’s postulates. Koch's postulates are performed infrequently, except when the disease agent is suspected to be new and previously unreported. Most of the plant disease diagnoses done today involve identification of plant diseases that have been previously described and named. Several techniques may be performed to determine the identity of diseases. Visual studies of symptoms and signs, microscopy, culture media studies and serology techniques are the most frequently used techniques in diagnostic clinics.

1.1 Identification of nature of a disease

In determination of a plant disease the first step is to determine the infectious and non-infectious nature of the disease.
**Infectious diseases**
An infectious disease will spread to other plants in the field by various means and is characterized by the presence of pathogens on the surface of the plants or inside the plant. In diseases caused by pathogens viz., fungi, bacteria, nematodes, viruses, mollicutes, a few or large numbers of these pathogens may be present on the surface of the plants or inside the plants. The presence of such pathogens in an active state on the surface of a plant indicates that they are probably the cause of the diseases. Their detection and identification can be determined with the experienced naked eye or with a magnifying lens and if no such pathogens are present on the surface of a diseased plants then it will be necessary to look for additional symptoms, especially for pathogens inside the diseased plant. Such pathogens are usually at the margins of the affected tissues, in vascular tissues or at the base of the plant or roots. Certain infectious Pathogens like viruses are neither seen nor can be grown on artificial media. They produce symptoms similar to those resulting from nutritional deficiencies.

**Non infectious diseases**
These are the diseases with which no parasite is associated; hence they are called as abiotic diseases. They remain non infectious and cannot be transmitted from diseased plant to healthy plant. If no organism is found in association with the diseased part and if viral symptoms are not present, the diseases may be due to inanimate cause. If symptoms look like those of nutritional deficiencies the identification can be confirmed by spraying a solution of the possible element in its salt form and usually recovery will occur within a week and identification can be confirmed. These non parasitic, non infectious diseases are due to disturbances in the plant body caused by lack of proper environmental conditions of soil and air, low and very high temperatures, unfavourable oxygen relations, unfavourable soil moisture, pH, presence of toxic gases in the atmosphere, mineral excess and deficiencies in the soil etc., are the major causes of non parasitic diseases.

**Examples**
- **Low temperature**: potato tuber injury
- **High temperature**: blossom end rot of citrus fruit
- **Effect of light**: bean scald
- **Excessive moisture**: blossom end rot of tomato
- **Low oxygen**: black heart of potato
- **Air pollution**: ozone on corn
- **Chemical injury**: ammonia on apple
- **Herbicide injury**: 2, 4-D on dicot leaf
- **Nutrient deficiency**: Zn on citrus
1.2 Steps in disease diagnosis

The basic steps involved in plant disease diagnosis are as follows.

1.2.1 Obtain background information on host and disease

- Description of the problem
- Identification of host cultivar
- Planting date
- Source of seed
- Habitat of diseased plant
- Soil type
- Cultural practices
- Disease history
- Environmental conditions
- Pattern of disease

1.2.2 Obtain a good sample of diseased plant or its parts.
Fresh sample in various stages of diseases development

1.2.3 Examine the plant and describe

- Signs/symptoms of disease
- Plant parts affected
- Most common symptom
- Visible signs of the pathogen

By critical examination of signs and symptoms produced in the plant one can identify the broad group to which the causal agent belongs.

1.2.4 Obtain literature description regarding the disease for the particular host

1.2.5 Identify the disease by comparing your description of disease with published description.

1.3 Identification of symptoms and signs: Categories of specimens to provide

- **Necrotic**: Blight, damping off, leaf spot, and root rot.
- **Color change**: mosaic,
- **Wilt**: vascular wilts
- **Hyper-plastic**: club root, scab, root knot nematode
- **Hypo-plastic**: yellows
- **Bacterial**: Wilt (vascular plugging), blight (ooze)
• **Fungal:** Powdery mildew (mycelium), Sclerotia, rusts, smuts, Nematodes (root knot galls).

1.4 Materials for microscopic symptomatology
Microscope, razor blades, lacto phenol-cotton blue, specimens such as vascular wilt, xylem necrosis (*Fusarium* on tomato), root rot (*Rhizoctonia* on beans), bacterial soft rot (*Erwinia*)

Distinguishing the symptomologies of infectious and non infectious diseases

**Materials:** specimens of both infectious and non infectious diseases mixed together including rots, wilts, spots, blights, mineral deficiencies, toxicities, sun burns, etc. and distinguish the differences between infectious and non infectious diseases.

Describe the symptoms of infections and non infectious diseases and made comments on diagnosis with justification.

1.5 Diagnosis of fungal leaf spot disease by visual symptoms, microscopic signs and a moist chamber incubation

Fungal leaf spot identification is accomplished by associating a fungus with the symptoms on the foliage. Fungi are usually identified by spore morphology and spore arrangement on conidiophores or by spore morphology and fruiting bodies observed in the diseased plant tissue. If spores are present on symptomatic tissues, then diagnosis may be completed with light microscopy. If spores are not present, then a moist chamber may be prepared to stimulate spore development and maturation. After 1-7 days, tissues are examined for signs of sporulation. If spores are not produced in a moist chamber, then diagnosis proceeds to fungal isolation in culture. After approximately 3-7 days, culture re-examined microscopically to observe fungal spore development.

**Materials**
Each team of students will require the following material
Plant samples, compound microscope, slides and cover slips, dropper bottle with water, cotton blue stain (0.5%), plastic bags, paper towels.

**Procedure**
1. Select a sample (provided by instructor) for diagnosis.
2. Study and record observations of the symptoms present. Describe leaf spot colour, shape, size, texture etc.
3. Select some leaf spots of varying appearance and observe with the aid of a dissecting microscope. Look for evidence of small black or other coloured bodies scattered over the leaf spots, record observations.

4. If fruiting bodies are observed, cut out tissue sections with bodies and place one or two sections on a microscopic slide, add a drop of water and put cover slip. Observe wet mount for presence of fruiting bodies and spores.

5. If fruiting bodies are not observed, take few leaves with leaf spots and place them onto a plastic bag containing a damp paper towel. Incubate for 7 days at room temperature.

6. Refer to the Illustrated Genera of Imperfect (Barnett and Hunter, 1986 edition) to identify the fungus. Consult host disease indices and compendia of crop diseases to determine whether the fungus has been associated with a previously reported leaf spot disease.

7. Make the diagnosis.

**Observations and record**

Observe the fungus based on spore morphology, fruiting bodies, arrangement of spores on conidiophores.

Determine "whether the symptoms and signs observed have been previously described on the host selected.

Comments should be made on the diagnosis of the problem with justification.
EXPERIMENT NO: 02

METHODS OF PLANT DISEASE MEASUREMENT

2.1 Plant diseases are measured in terms of incidence and severity.

Disease incidence is the number or proportions of plant units that are diseased (i.e. plants, leaves, flowers, fruits etc.) in relation to the total number of the units examined.

It is expressed in terms of percentage as follows: The measurement of disease incidence is relatively quick and easy and is used to measure/assess a disease through a field, region or country.

- Disease severity
  - In a few cases such as cereal smuts, neck blast, brown rot of stone fruits and vascular wilts of annuals where they cause total loss, disease incidence has a distinct relationship with the severity of the disease and yield loss.
  - Disease severity is usually expressed as the proportion of plant area or fruit volume destroyed by the pathogen. It is expressed in the percentage as follows:
  - In many diseases such as most leaf spots, root lesions and rusts in which plants are counted as diseased whether they are exhibiting a single lesion or hundred lesions, disease severity is of greater importance to the growers than the disease incidence.
  - Disease severity is also referred to as disease intensity.
  - For diseases, where the amount of disease varies greatly on different plants in the population, many arbitrary indices and ratings have been in practice.
  - They are usually discouraged and are replaced by percentage scales and standard area diagrams of disease intensity.

Percentage Scales

- In this, usually the number of plants or organs falling into known percentage disease groups is recorded.
- The disease groups are the categories distinguished on the basis of per cent damage seen by human eye.
- A 12 grade scale was suggested by Horsfall and Baratt (1945) who took into the fact that the grades detected by the human eye are approximately equal divisions on a logarithmic scale and generally follow the Weber-Fechner law which states that visual activity depends on the logarithm of the intensity of the stimulus.
- In percentage disease assessment, the eye actually assesses the diseased area upto 50 per cent and the healthy area above 50 per cent.
In Horsfall and Baratt grading system, the categories were as follows: 1= 0%, 2= 0-3%, 3= 3-6%, 4= 6-12%, 5= 12-25%, 6= 25-50%, 7= 50-75%, 8 = 75-87%, 9 = 87-94 %, 10= 94-97%, 11= 97-100 %, 12 =100% disease.

- This is a logarithmic scale and is satisfactory not only for disease measurement, but also for epidemiological studies, because pathogens multiply at logarithmic rate and also for loss appraisal.
- A system using percentage scale was developed by British mycological Society (Anon, 1947) for measuring late blight of potato.

**The percentage scales have many advantages such as:**
- The upper and lower limits of the scale are always well defined.
- The scale is flexible in that it can be divided and subdivided conveniently.
- It is universally known and can be used to record both the number of plants infected (incidence) and area damaged (severity) by a foliage or root pathogen.

**Standard area Diagrams**
- Nathan Cobb developed the first standard area diagram for leaf rust of wheat.
- It divided rust intensity into five grades representing 0, 5, 10, 20 and 50 per cent of leaf area occupied by the visible or sporulating rust pustules.
- The highest grade (50%) represented the maximum possible cover.
- A modified Cobb’s scale was proposed by Melchers and Parleu (1922) for the estimation of stem rust of wheat.
- One of the most practical set of the area diagrams was prepared by James (1971). These diagrams represent the actual area of the leaves, stems, pods and tubers occupied by lesions in terms of per cent area covered.
- A common formula as follows is generally used to calculate the average infection or Infection Index, sometimes also known as Disease Index or Per cent Disease Index, which is calculated as follows:

\[
\text{Percent disease index (PDI)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100
\]

- Severity estimates from fairly small areas can be combined to cover large areas, viz, village, district or state. This overall index can be obtained by using the formula:
Remote sensing

- Remote sensing provides a powerful tool for detection and measurement of diseases.
- It is used for survey of large crop areas by means of aerial photography.
- Diseased plants can be identified as distinct patches in an otherwise uniform picture.
- It is useful for measuring diseases such as late blight of potato where early disease foci can be located and their subsequent spread is followed.
- Remote sensing is also useful when sudden disaster strikes and a rapid appraisal of the situation is required.
- In wheat crop, aerial photography can be used to determine the extent of damage by take-all or eye spot disease. Similarly, the incidence of Heterobasidion can be monitored in pine plantation covering undulating, boggy or rocky land.
EXPERIMENT NO: 03

ASSESSMENT OF CROP YIELD LOSSES

Crop loss can be summarized as the difference between the attainable yield from the healthy crop and that obtained from the diseased crop and is expressed as percentage mostly in terms of money.

- The most important purpose of disease appraisal is the assessment of crop loss.
- Various attempts have been made to utilize disease assessment data for estimation of loss.
- However, such conversion is not easy.
- There is no straight forward way to determine the amount of yield loss.
- While calculating the yield loss from a disease, its nature, extent of damage in terms of yield, quality and loss of market value are to be considered.
- Diseases like smuts, root rots, ergot etc. cause almost 100 per cent damage to the crop and the loss estimates are rather easy to make.
- However, those causing damage to the foliage and other debilitating diseases, thereby affecting the yield partially to different extents pose a great difficulty in assessing losses.
- Sometimes, the crop stage, when the crop is attacked becomes critical in this respect.

3.1 Estimation of yield loss

- For estimating yield loss due to diseases, comparisons between crops grown in different years or localities are not reliable as other factors are not the same.
- For valid comparisons, disease free plots are to be compared with those nearby with varying amount of disease.
- Disease free plots are mostly obtained by use of fungicides with little or no phytotoxicity.
- If the yield loss is to be estimated on a regional basis, data on disease incidence obtained from the disease surveys would be utilized using formulae based on fungicide trials.
- These data are usually employed in the critical point models which are actually the regression equations.
- Multiple point models in which the loss estimation is based on many diseases appear to be more reliable.
- These data are used to produce a multi-dimensional model whose dimensions include the date of disease onset, shape of the disease progress curve, the host cultivar and the yield loss as the dependent variables.
IDENTIFICATION OF DIFFERENT BIOCONTROL AGENTS FOR PLANT DISEASES

4.1 Biological Control- Concept

Biological control of plant pathogens refers to the total or partial destruction of pathogen population by other organisms. It occurs routinely in nature. For example, several diseases in which the pathogen cannot develop in certain areas either because the soil, called suppressive soil, contains microorganisms antagonistic to the pathogen or because the plant that is attacked by a pathogen has also been inoculated naturally with antagonistic microorganisms before or after the pathogen attack. Sometimes, the antagonistic microorganisms may consist of avirulent strains of the same pathogen that destroy or inhibit the development of the pathogen, as happens in hypovirulence and cross protection.

Agriculturalists have increased their efforts to take advantage of such biological antagonisms and to develop strategies by which biological control can be used effectively against several plant diseases.

The most commonly used microorganisms include:

- *Gliocladium virens*, for the control of seedling diseases of ornamental and bedding plants
- *Trichoderma harzianum*, for the control of several plant pathogenic fungi
- *Trichoderma polysporum*, for the control of wood decays
- *Agrobacterium radiobacter* K-84, for the control of crown gall
- *Pseudomonas fluorescens*, against *Rhizoctonia* and *Pythium* causing damping off and other diseases
- *Bacillus subtilis*, used as a seed treatment

4.2 Biological Control of Postharvest Diseases through Fungal and Bacterial Antagonists

- Post harvest rots of several fruits could be reduced by spraying the fruits with spores of antagonistic fungi and saprophytic yeasts at different stages of fruit development, or by dipping the harvested fruit in their inoculum.
- Yeast treatments reduced post harvest rotting of peach and apple.
- *Botrytis* rot of strawberries was reduced by several sprays of *Trichoderma* spores on strawberry blossoms and young fruits. Several antagonistic yeasts protected grapes and tomatoes from *Botrytis*, *Penicillium*, and *Rhizoctonia* rots.
- In bacterial antagonists, *Pseudomonas* protected lemons from *Penicillium* (green mould) and pear from various storage rots.
- Two *Pseudomonas syringae* strains control the post harvest decay in citrus, apple and pear under the trade name Bio-Save.
- Stone fruits such as peaches, nectarines, apricot and plums when treated with suspensions of the antagonistic bacterium *Bacillus subtilis*, they remain free from brown rot, caused by the fungus *Monilinia fructicola* for nine days.
- *Bacillus subtilis* also protected avocado from storage rots.
- *Pseudomonas* protected lemons from *Penicillium* (green mould) and pear from various storage rots.
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• Stone fruits such as peaches, nectarines, apricot and plums when treated with suspensions of the antagonistic bacterium *Bacillus subtilis* remain free from brown rot, caused by the fungus *Monilinia fructicola* up to nine days.

• *Bacillus subtilis* also protects avocado from storage rots.
EXPERIMENT NO: 05

MASS MULTIPLICATION OF *Trichoderma, Pseudomonas*

5.1 Mass multiplication of *Trichoderma viride*

**Preparation of mother culture**

Molasses yeast medium is prepared as detailed below.

- **Molasses**: 30g
- **Yeast**: 5g
- **Distiller water**: 1000 ml

The medium is prepared and dispensed into conical flasks and sterilized at 15 lb pressure for 15 minutes in an autoclave. After the medium is cooled it is inoculated with 10 days old fungal disc of *T. viride* and then incubated for 10 days for fungal growth. This serves as mother culture.

**Mass multiplication:**

Molasses yeast medium is prepared in fermentor and sterilized as described earlier. Then after the medium is cooled, the mother culture is added to the fermentor @ 1.5 lit / 50 lit of the medium and incubated at room temperature for 10 days. Then the incubated broth containing the fungal culture is used for commercial formulation preparation using talc powder.

5.2 Mass production of *Pseudomonas fluorescens*

**Preparation of mother culture**

Mother culture is prepared by using the king’s B medium

- **Peptone**: 20g
- **K$_2$HPO$_4$**: 1.5g
- **MgSO$_4$**: 1.5g
- **Glycerol**: 10ml
- **Distilled water**: 1000 ml

The above broth is dispersed into conical flasks and autoclaved at 15 lb pressure for 15 minutes and cooled and inoculated with a loop of *P. fluorescens* and incubated for 2 days.
Mass multiplication

The kings B medium is prepared and poured into the fermentor and sterilized at 15 lb pressure for 15 minutes. After the broth has cooled below the mother culture of *P. fluorescens* is added to the king’s B medium in the fermentor at the rate of 3 lit for 40 lit of the broth. Then it is incubated in the fermentor for 2 days with frequent mixing of the broth by operating the stirrer. Then the broth containing the bacterial growth is collected in plastic buckets and used for mixing with talc powder for commercial formulation.

5.3 PREPARATION OF TALC BASED PRODUCTS, AIR DRYING OF FORMULATION AND ESTIMATION OF MOISTURE CONTENT

5.3.1 *Tichoderma viride*:

The fungal biomass collected from fermentor is mixed with talc powder at 1:2 ratio. The mixture is air dried in shade and mixed with carboxy methyl cellulose (CMC) @ 5 g / kg the product. It is packed in polythene bags and should be used within 4 months.

**Quality control parameters:**

1. Fresh product should contain hot less than 28 x10⁶ cfu / g
2. After 4 months of storage at room temperature, the population should be 20 x 10⁶cfu/g.
3. Maximum storage period in talc is 4 months.
4. The talc size should be 500 microns
5. The product should be packed in polythene bags
6. Moisture content of the final product should not be more than 20%

5.3.2 *Pseudomones fluorescens*

The broth containing the bacterial growth is collected from fermentor and added @ 400 ml / kg of talc powder. Then CMC is added @ 5 g /kg mixed well air dried to 20% moisture level and packed in polythene bags.

**Quality control parameters:**

1. Fresh produce should contain 2.5 x 10⁸cfu/g
2. After 3 months of storage at room temperature the population should be 8-9 x 10⁷ cfu/g
3. Storage period is 3-4 months
4. Minimum population load should be 1.0 x10⁸cfu /g
5. Moisture content should not exceed 20% in the final product
6. Population per ml of the broth should be 2 x 10⁸cfu /g
Symptoms and signs

Symptoms- External expression or the evidence of the abnormalities in the appearance of the diseased plants brought about by the pathogens after host-pathogen interaction.

Sign- When the pathogen itself becomes visible on the host surface in the form of its organs or structures. eg sclerotia, mycelium etc.

Disease syndrome- A sum total of variety of symptoms produced by the disease.

6.1 Symptoms of Plant Diseases Due to the Character and Appearance of Visible Pathogen, its Structures and Organs

i. Mildews

- Mildews consist of white, grey, brownish or purplish pathogen growth on the host surface.
- Downy mildew is characterized by a tangled cottony or downy growth mostly on the lower surface of the leaves or other plant parts.
- Powdery mildew consists enormous number of spores are formed on superficial growth of the fungus giving a dusty or powdery appearance on the host surface. Black minute fruiting bodies may also develop in the powdery mass.

Fig.6.1. Grapevine downy mildew
ii. **Rust**
- Rust appears as relatively small pustules of the spores, usually breaking through the host epidermis.
- Pustule is a small blister-like elevation of the epidermis, often opening to expose spores. The pustules may be dusty or compact, and red, brown, yellow or black in colour.

iii. **Smut**
- Smut means a sooty or charcoal like powder.
- The affected parts of the plants show black or purplish black dusty areas.
- Symptoms usually appear on floral organs, particularly the ovulary areas.
- The pustules on the leaves and stems are usually larger than those of rusts.

iv. **White Blister**
- **White blister**-like pustules appear on the leaves and other parts of cruciferous plants which break open the epidermis and expose powdery masses of spores.
- Such symptoms are called white rust’, although there is nothing common with them and the rusts.

iv. **Blotch**
- It consists of superficial growth giving the affected plant parts i.e., fruits and leaves smoky (blotched) appearance, e.g. sooty blotch of apple.

v. **Sclerotia**
- A *sclerotium* is a compact, often hard mass of dormant fungus mycelium.
- *Sclerotia* are mostly dark in colour and are found mixed with the healthy grains as in the case of ergot of wheat and rye.

vi. **Exudation**
- Mass of bacterial cells ooze out on the surface of the affected organs where they may be seen as a drop or smear in several bacterial diseases such as bacterial blight of paddy, gummosis of stone fruits and fire blight of apple and pear.
- They form crusts after drying.

vii. **Mycelial growth**
- Appearance of white cottony, mycelial growth of the fungi like *Dematophoranecatrix* on affected roots of apple is an important diagnostic feature of white root rot in the field.
6.2 Symptoms Resulting from Internal Disorders in the Host Plants

i. Colour change
   - **Discolouration** is change of colour from normal. It is one of the most common symptoms of plant diseases. The green pigment of leaves disappears entirely and is replaced by yellow pigments.
   - **Etiolation** is yellowing due to the lack of light.
   - **Chlorosis** is yellowing due to low temperature, lack of iron, excess of the lime or alkali in soil and infection by viruses, fungi and bacteria.
   - **Albilinism** is the phenomenon in which the leaves become devoid of any pigment and look bleached or white.
   - **Chromosis** is change of colour to red, purple or orange.

ii. Overgrowths or hypertrophy
   - **Hypertrophy** is the abnormal increase in the size of the plant organs due to increase in the size of the cells of a particular tissue, whereas
   - **Hyperplasia** is the abnormal increase in the size of the plant organs due to increase in the number of cells of which the tissue or organ is composed, owing to increased cell division.
   - The overgrowths cause galls, curl, pockets or bladders, hairy root, witches’ broom, intumescence etc.

iii. Atrophy or Hypoplasia or Dwarfing
   - **Atrophy** is inhibition of growth and thereby showing stunting and dwarfing effect on the plants.
   - The whole plant may be dwarfed or only certain organs are affected. e.g. rice dwarf, phony peach etc.

6.3 Necrosis
   - Death of the cells, tissues and organs occurs as a result of parasitic activity.
   - The characteristic appearance of the dead areas differs with different hosts, host organs and with different parasites.
   - Necrotic symptoms include spots, streaks or stripes, canker, blight, damping off, burn, scald or scorch and rot.

6.4 Wilt
   - Characterized by drying of the entire plant.
   - Leaves and other green or succulent parts lose their turgidity, become flaccid and droop down.
   - Usually seen first in some of the leaves.
   - Later, the young growing tip or the whole plant may dry up.
   - May be caused by injury to the host system or the conducting vessels.
   - Wilting due to disease is different from the physiological wilting where the plant recovers as soon as the supply of water is retained.

6.5 Die-back or Wither Tip
   - Symptoms are characterized by drying of plant organs, especially stems or branches, from the tip backwards.
   - It is also a form of necrosis caused directly by the pathogen or its toxins.
7.1 Cultural practices
Cultural practices usually influence the development of disease in plants by affecting the environment. Such practices are intended to make the atmospheric, edaphic, or biological surroundings favorable to the crop plant, unfavorable to its parasites. Cultural practices that lead to disease control have little effect on the climate of a region but can exert significant influence on the microclimate of the crop plants in a field. Three stages of parasite’s life cycle namely, Survival between crops, production of inoculum for the primary cycle and inoculation can be control by following preventive measures.

7.2 Survival between Crops
Organisms that survive in the soil can often be controlled by crop rotations with Unsusceptible species. Depending on the system, either of two effects results. Catch crops have been used to control certain nematodes and other soil-borne pathogens. Soil-borne plant pathogens can be controlled by biological methods. Plant parasites may be controlled by antagonistic organisms that can be encouraged to grow luxuriantly by such cultural practices as green manuring and the use of appropriate soil additives. The soil-invading parasite thus becomes an amensal in association with its antagonist. Soil-borne plant parasites may also be killed during their over-seasoning stages by such cultural practices as deep ploughing (as for the pathogen of southern leaf blight of corn), flooding (as for the cottony-rot pathogen and some nematodes), and frequent cultivation and fallow (as for the control of weeds that harbor plant viruses). Plant diseases caused by organisms that survive as parasites within perennial hosts or within the seed of annual plants may be controlled therapeutically. Therapeutic treatments of heat and surgery are applicable here; those involving the use of chemicals will be mentioned later. Removal of cankered limbs (surgery) helps control fire blight of pears, and the hot-water treatment of cabbage seed controls the bacterial disease known as black rot. Heat therapy is also used to rid perennial hosts of plant-parasitic nematodes.

7.3 Production of Inoculum for the Primary Cycle
Environmental factors (particularly temperature, water, and organic and inorganic nutrients) significantly affect Inoculum production. Warm temperature usually breaks dormancy of overseasoning structures; rain may leach growth inhibitors from the soil and permit germination of resting spores; and special nutrients may stimulate the growth of overseasoning structures that produce inoculum.
7.4 Dispersal of inoculum and inoculation
Cultural practices that exemplify avoidance are sometimes used to prevent effective dissemination. A second hierarchy of regulatory disease control is plant quarantine, the legally enforced stoppage of plant pathogens at points of entry into political subdivisions. The Plant Quarantine Act of the United States governs importation of plant materials into the country and requires the state govt. to enforce particular measures. Also, states make regulations concerning the movement of plant materials into them or within them. E.g., Florida imposes quarantine against the citrus-canker bacterium, which was eliminated from the state earlier by means of cooperative efforts led by the Florida Department of Agriculture.

7.5 Sample Inspection
One of the preventive measures to control the diseases is the use of sample inspection method. Laboratory evaluation of the representative sample drawn by the certification agency for the determination of germination, moisture content, weed seed content, admixture, purity, seed borne pathogens.
8.1 Disease predictors

The predictor is a unique outdoor monitoring system designed to take the guess work out of the spray programmes. Just by pushing the button, we can know the current and past environmental conditions, current disease status and history of infection periods or the probability of disease occurrence. It enables us to time the spraying operations for optimum disease control.

**Reuter stokes predictor:** It is an electronic device used to monitor temperature, leaf wetness and relative humidity in apple orchards and was basically programmed to predict apple scab infection periods. A leaf wetness sensor is placed on the tree about 1m above ground and two temperature sensors (linear thermistors) enclosed in a standard weather shelter are connected to the instrument. One thermistor measures dry bulb or ambient temperature, the other, presented in a wet wick measures wet bulb temperature. The other end of the wick is put in the water reservoir through the hole on the top of the sensor shelter. Tipping bucket rain guage, hand held printer, temperature probe and sensor extension cable are optional accessories available with the instrument. Predictor is mounted on 4 cm diameter pipe in the orchard and the reservoir is filled with distilled water. Sensors are connected to the processor. A 6-volt dry battery is used and date and time are keyed in. The instrument measures and compares current environmental conditions with formula developed. It then calculates the onset of specific plant disease for timely application of preventive chemicals. The information is gathered, processed and stored automatically. We can retrieve the information by pushing buttons, which is displayed on Liquid Crystal Display (LCD). It gives current weather data, back data up to 16 days, apple scab history up to last 16 infection periods recorded. Current data on the environment are processed and related to the apple scab status as none, low, medium, and heavy; and fungicide spray options depending upon kick-back action. A degree day accumulator accurately calculates the number of degree days which have transpired for various base temperatures between 0 and 24°C.

![Fig 8.1 Reuter stokes predictor installed in an apple orchard](image)
8.2 Spore traps
Spore traps are used to capture and quantify a broad spectrum of fungal spores (both culturable and non-culturable) present in the air.

i) Burkard’s volumetric spore sampler: A compact unit with built-in vacuum pump, designed to sample airborne particles such as fungus spores and pollens, continuously for periods of up to seven days without attention. Particles are impacted on adhesive coated transparent plastic tape supported on a clock-wise driven drum. Performance of the standard model is similar to the trap described by Hirst in 1952, but interchangeable orifices can be supplied to special order to improve the trapping efficiency for particles in the range 1 to 10 μm diameter.

The pore trap is supplied with one roll of 'Melinex' tape and one roll of double sided tape, together with the laboratory stand and Perspex cutting block.

Fig 8.2. Burkard’s spore sampler

Rotorod sampler: Rotorod sampler is a volumetric rotating arm impaction device capable of obtaining quantitative data on air-borne particulate in the size of 1 to 100 μm at sampling rates up to 120 litres/min. In Plant Pathology, it is used to monitor the amount of inoculum in the form of spores present in the atmosphere in order to assess the disease risk in advance and thus forewarn the managers to get ready for timely spray treatments for effective control of the disease. Its basic components are a constant speed motor and aerodynamically designed collector rods, which are rotated by sampler motor. The type H, V and I rods form the basic collector rod geometry. Sampling for spores in an orchard should be done at a height consistent with level of concern near the fruit, leaves or new woody growth. The collector rods are smeared with silicon grease adhesive before they are put in the place on the fixed compatible sampling heads on the samplers. Mount the coated collector rod on sampler by sliding the centre clip portion of the rod into the slotted hub of the Rotorod motor. Connect the sampler leads to 12 volt battery. The rods will be rotated at the calibrated RPM as long as the DC voltage is in between 9 to 15 volts in a clock-wise direction. The sampler should be operated according to the purpose. For example, in apple scab forecasting, the sampler should be operated immediately after rain for a period of 3-4 hours to trap discharged ascospores in the orchard.
After switching the sampler off, remove the rods and mount them in specially designed grooved stage adapter. The spore deposit is evaluated by counting under light microscope. Nowadays, counting reticules are also available for facilitating the process.

Fig 8.3. Rotorod sampler

**METOS automatic weather station** The heart of METOS system is a central processing computer powered by rechargeable batteries. All full function systems are equipped with a built-in Epson graphics plotter, washable numeric keypad with 9 functional keys and an 80-character LCD screen. This recording unit is housed indoors or in a weatherproof shelter and includes the main power switch and ports for battery charger, optimal external power supply and RS-232 computer interface. The sensor unit is contained in an all-weather, rustproof ventilated housing with standard air temperature, relative humidity and solar radiation sensors and is connected by 15 (5m) cable to a rain gauge with 0.2 mm sensitivity and by 36 (10m) cable to two leaf wetness sensors. The recording and sensor units are joined by a 36 (10m) quick connect cable with optional extensions to 500 (150m). The latest addition to the METOS line is the METOS-DAT, a compact data recorder with multiple channels. This compact version includes many standard and optional sensor inputs, without keyboard, printer or display. Output is channeled to the PC by direct cable or optional cellular or standard phone modem. The METOS system automatically scans all sensors every 12 minutes and stores these data for up to 4 weeks (extendable to 12 months) in internal ROM. In between scans, the METOS remains in standby mode drawing very little power. Each fully functional METOS model contains specific software to convert weather data into easily understandable information on disease conditions, accessed immediately at any time on the LCD by pressing function keys, or automatically at one hour intervals on the graphics plotter. The latest environmental data can be transferred to PC or compatible computer via direct cable or modem.
MET9(R) is a comprehensive software package for IBMTM and compatible personal computers for the calculation of disease management decisions. MET9(R) operated with an easy to use windows TM like format under DOS 3.0 or higher and need the use of the MET8TM database. Temperature, relative humidity rainfall and leaf wetness etc. largely determine the presence of plant diseases. This connection has been established for various harmful organisms. The MET9 TM disease and pest warning programme indicates favourable conditions and gives instant on line warnings for scab of apples, powdery mildew, black rot, downy mildew of grapes, late and early blight of potatoes and Pythium blight, brown patch and dollar spot of turf grasses.

8.3. de Wit leaf wetness recorder:
Leaf wetness recorder is built on hygrothermograph principle with the addition of wetness sensing element and pen arm. de Wit leaf wetness recorder records only leaf wetness. In this, the chart revolves in a clockwise direction. If the weather is dry, the pen makes recording on the margin of the chart. The beginning of a rainy period is indicated by a clear kink in the line. As the moistened sensor shrinks, the pen moves towards the centre of the chart and keeps recording there for the duration of the rain. When the rain stops, the sensor dries again and the pen returns to its former position. Generally, the sensor dries sooner than the leaves and a correction should be made by applying correction of half to one hour. The best spot to mount the instrument is where the leaves remain wet the longest time. The instrument in the orchard is fitted at eye level (approximately 1.5 m high) in a vertical position on a pole with
cross beam with the sensor in a horizontal position. The sensor should not be touched with
the fingers during installation or afterwards. Leaf wetness recorders are efficiently used to
predict the infection periods in case of apple scab.

**Examination and record**
1. Draw and label the parts of these instruments.
2. Understand the principle and working of these instruments.
REFERENCES AND BIBLIOGRAPHY


