- By Prof. James Muthomi

Contents

1. Abiotic Disorders In Seed Production
2. Diagnosis Of Plant Diseases
3. Pest Risk Analysis

SEED ENTERPRISE MANAGEMENT INSTITUTE (SEMIs)

Seed Production Field Diagnostics

Short Course

22nd – 27th June 2015

Abiotic Disorders In Seed Production



Prof. James W. Muthomi

Department of Plant Science and Crop Protection

University of Nairobi

- Abiotic plant problems are sometimes termed “physiological disorders
- Abiotic disorders” refers to a wide array of plant problem
- “Abiotic” to indicate that the symptom is not caused by a biological agent such as an insect, mite or pathogen.
- Abiotic disorders are associated with non-living causal factors such as weather, soils, chemicals, mechanical injuries, prolonged drought, cultural practices and, in some cases, a genetic predisposition
- Abiotic stressors can also predispose plants to pathogens

Abiotic Disorders In Seed Production

- Genetic mutations and reversions
- Chimeras - Leaf variegation
- Low-temperature injury
- Sunscald and frost cracking
- Frost injury
- Drought and heat
- Flooding

- Lightning and hail
- Nutrient deficiencies and excesses
- Salt injury
- Herbicides
- Pesticides
- Air pollution

- Plants suffering from nutrient or physiological disorders, the plant exhibits disease-like symptoms
- Nutrient disorders are sometimes mistaken for a disease
- Nutrient deficiencies lack visible signs, they are often mistaken for virus diseases
- Nutrient disorders may result in a reduction in yield

Soil nutrients

Macro-nutrients
Constitute main elements required by plant for basic functioning

- Phosphorous (P),
- Potassium (K),
- Nitrogen (N),
- Calcium (Ca),
- Magnesium (Mg)
- Sulfur (S).

Micro-nutrients (trace elements)
Required in very small amounts but are essential for normal growth

- Iron (Fe),
- Zinc (Zn),
- Manganese (Mn),
- Boron (B),
- Molybdenum (Mo)
- Copper (Cu)

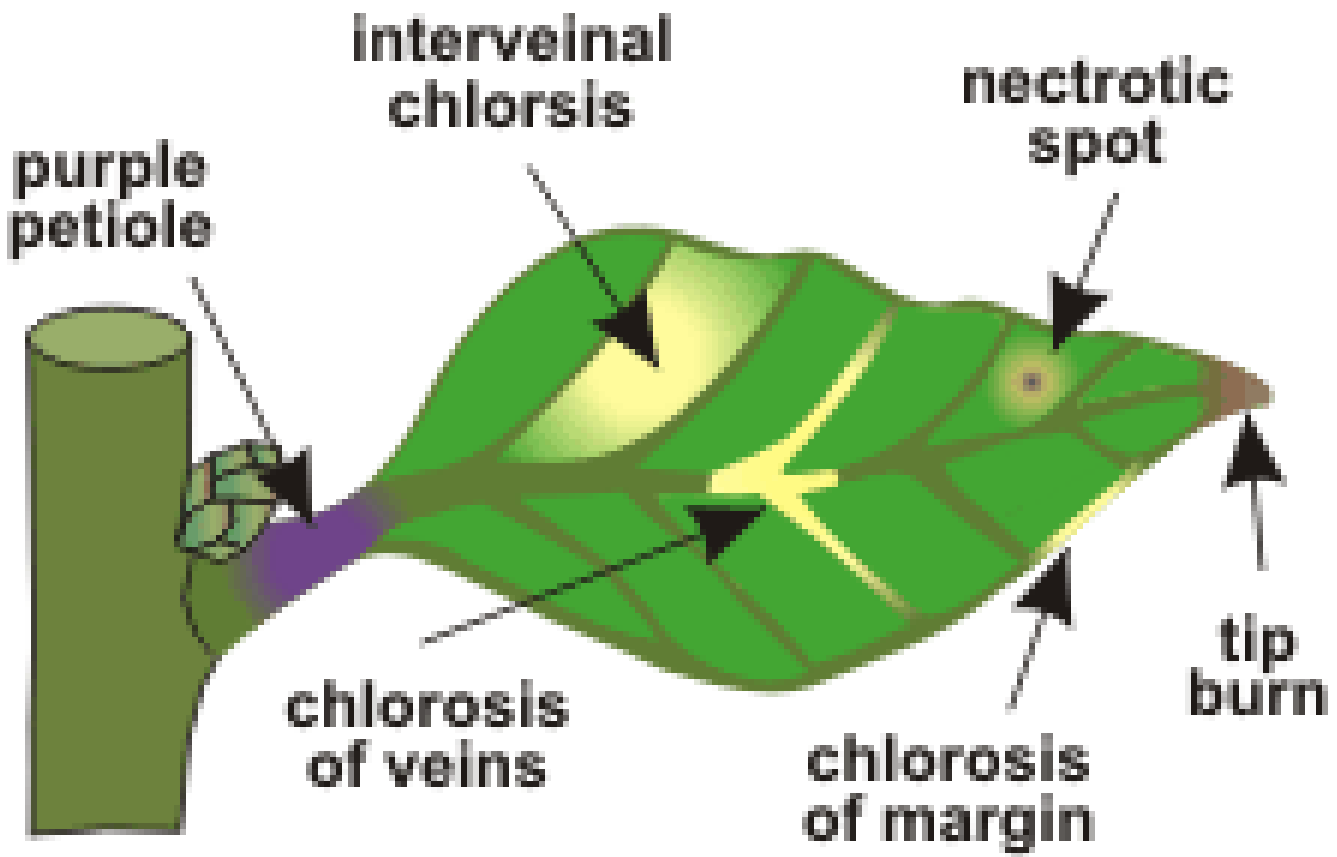


Fig 15.1 Some common leaf abnormalities resulting from nutrient deficiencies.

Nutrient deficiencies

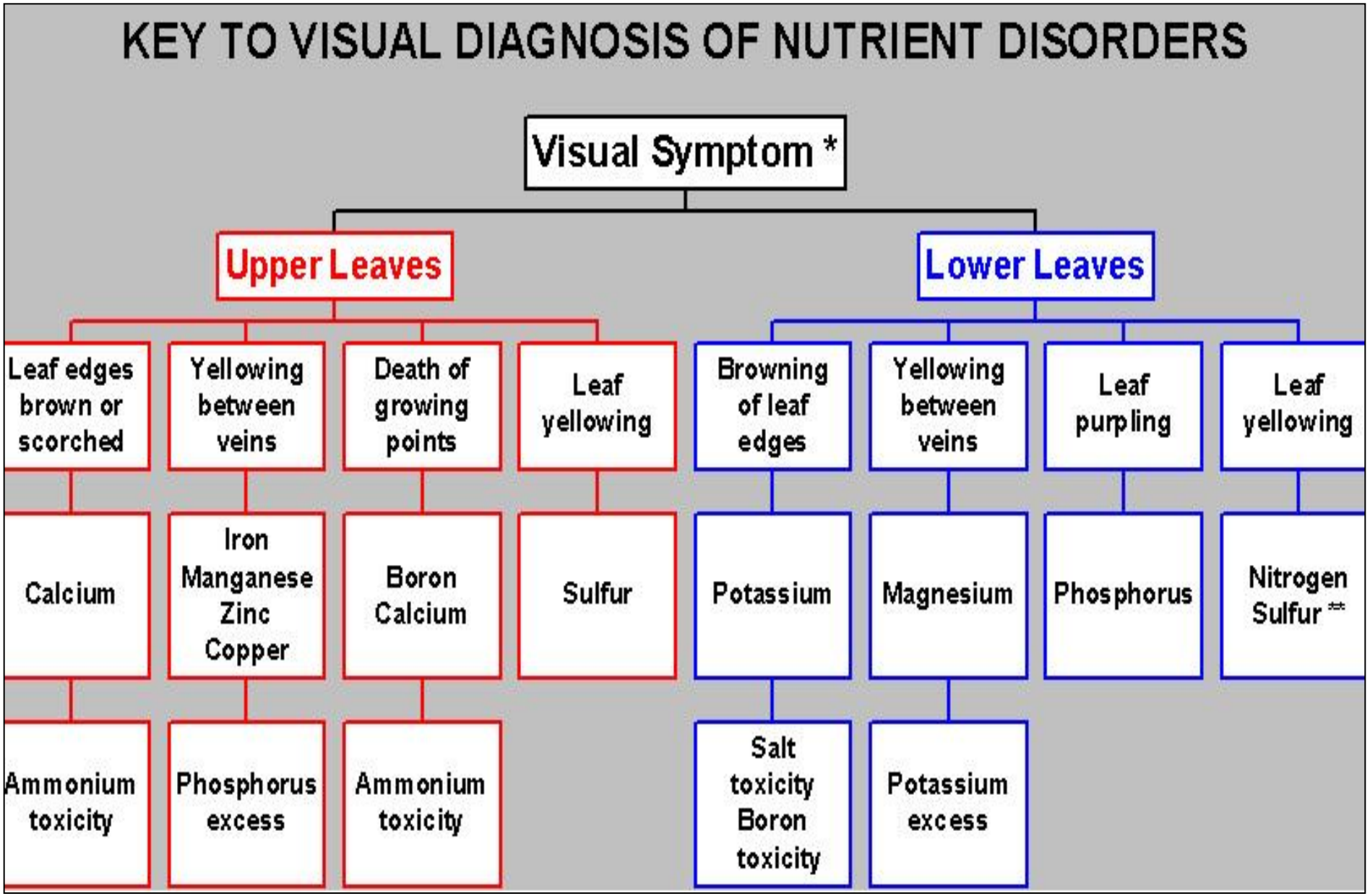
- Symptoms of nutritional disorders occur in defined patterns and are specific for each nutrient
- Symptoms are first seen in older leaves for some deficiencies, and in young leaves and/or tissues for others
- Mobile nutrients (n, p, k and mg) deficiencies are first seen in older leaves;
- Immobile nutrients (ca, b, cu, zn and fe) deficiencies are first seen in youngest leaves and/or growing tissue

Pesticide toxicity or disease symptoms may resemble nutrient deficiencies or toxicities

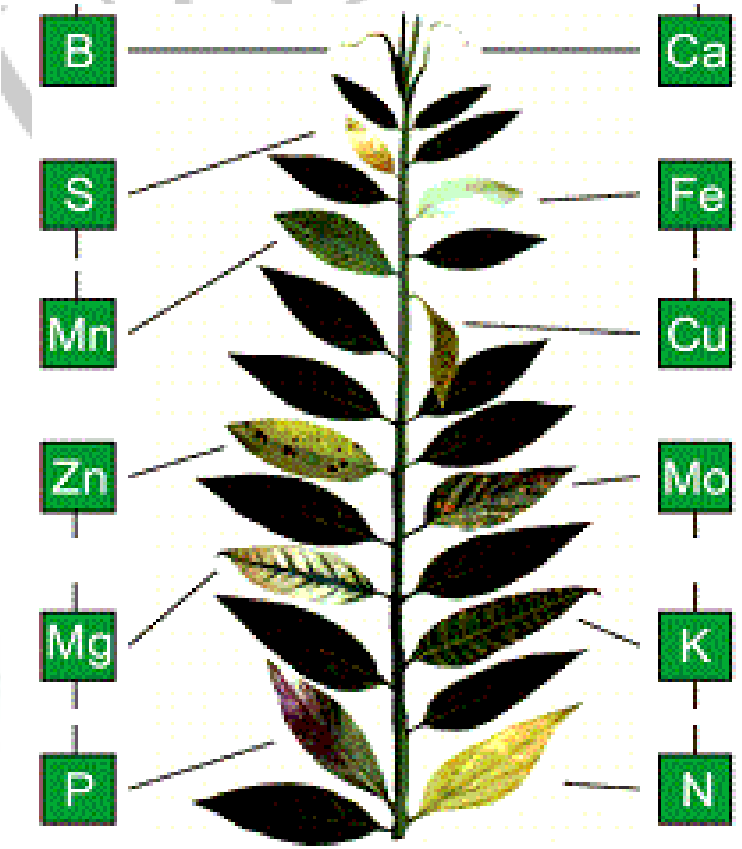
Symptoms of nutritional disorders are often species or variety dependent

Soil and plant tissue analysis should be used to help confirm whether the symptoms truly are nutritional

Magnesium deficiencies are often confused with viruses and other nutrient problems. However, symptoms of viruses are typically manifested in the young, growing part of the plant.


KEY TO VISUAL DIAGNOSIS OF NUTRIENT DISORDERS
Visual Symptom *
Upper Leaves
Lower Leaves
Leaf edges brown or scorched
Yellowing between veins
Death of growing points
Leaf yellowing
Browning of leaf edges
Yellowing between veins
Leaf purpling
Leaf yellowing
Calcium
Iron Manganese Zinc Copper
Boron Calcium
Sulfur
Potassium
Magnesium
Phosphorus
Nitrogen Sulfur **
Ammonium toxicity
Phosphorus excess
Ammonium toxicity
Salt toxicity Boron toxicity
Potassium excess


Calcium
Iron
Potassium
Nitrogen
Carbondioxide
Manganes
Phosphate
Magnesium


B
Ca
S
Fe
Mn
Cu
Zn
Mo
Mg
K
P
N

SYMPTOMS OF ABIOTIC DISORDERS

LEGUMES

Iron Deficiency of Peanut

Iron deficiency in cowpea

Iron

Iron deficiency

Iron deficiency in soybean, upper leaves

Manganese Deficient Soybean

Manganese Deficiency of Peanut

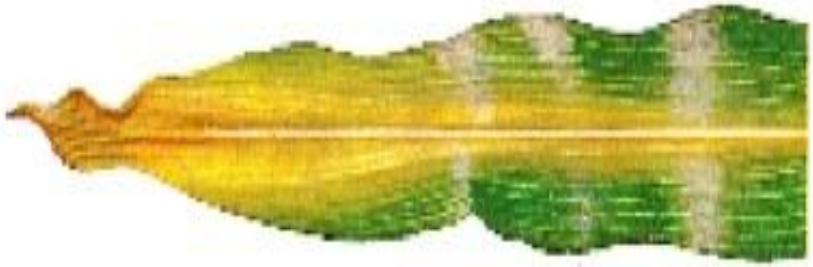
Molybdenum

Molybdenum Deficiency of Peanut (Right) Grown in Strongly Acid Soil (PH

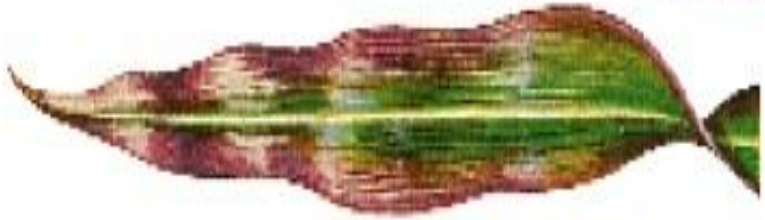
Molybdenum Deficiency of Peanut (Right) Grown in Strongly Acid Soil (PH 4.5)

SYMPTOMS ON CEREALS

a healthy corn plant leaf is deep green and glossy



a leaf from a plant with nitrogen deficiency yellows down the midvein starting at the tip and moving back towards the stem



a leaf displaying phosphorus deficiency turns red-purple along the leaf margins

a leaf from a potassium-deprived plant features firing and yellowing along the leaf margins

Phosphorus

Potassium

Potassium

Potassium deficiency in corn, lower leave

Potassium deficiency
Not chiseled (left), chiseled (right)

Potassium

Nitrogen

Nitrogen

Wheat

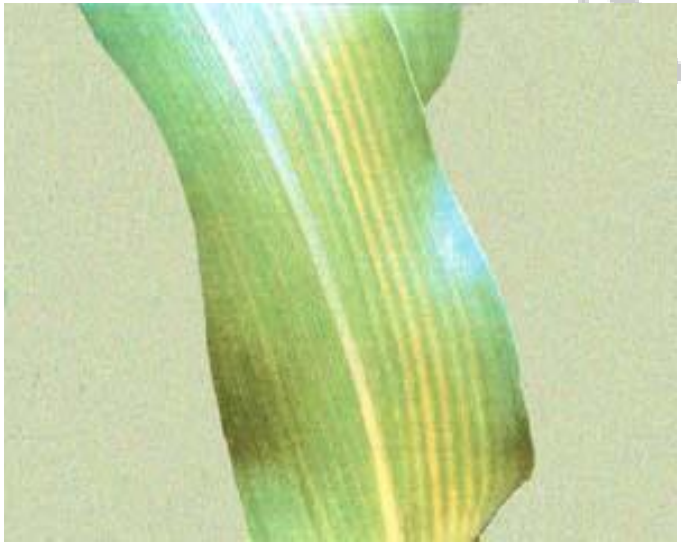
Nitrogen deficiency

Potassium deficiency

Phosphorus deficiency

Magnesium

Sulphur



Boron

Manganese

Manganese deficiency

Zinc

Zinc Deficiency of Rice

Zinc Deficiency of maize

Zinc

Iron

Copper

Calcium

Boron Deficiency in Papaya

MANAGEMENT OF NUTRIENT DEFICIENCIES

Conditions leading to nutrient deficiency

Nitrogen –

- Infection by root pathogens such as root-knot nematodes
- Nitrogen deficiencies can cause increased susceptibility to certain leaf pathogens such as *alternaria solani,*
- Excessive plant N levels may result in increased susceptibility to other pathogens

Phosphorus –

- Acid and clay soils are particularly prone to P deficiency.
- Cool conditions or poor oxygen availability to the roots can lead to p deficiency

Iron (Fe) –

- Most soils have adequate supplies of fe;
- Availability decreases as soil ph increase

Potassium (K) –

- Availability reduced by presence of competing cations such as ca)$^{2+}$ and $NH_4^{+;}$
- Potassium can also be readily leached from sandy soils.
- Plant uptake of k may be reduced by certain environmental conditions including temperature, soil moisture, and oxygen availability.

Deficiency	Symptoms	Remedies
Phosphorous (P)	Poor germination, seedling establishment & plant growth; leaves may be dull bluish/greyish-green or have red pigment in leaf bases and dying leaves; oldest leaves may turn yellow & drop.	Apply phosphorus fertilisers & manure
Potassium (K)	Yellowing on older leaves; scorching of edges and/or interveinal region	Apply K fertilizer rate
Nitrogen (N)	Poor plant growth; older leaves are pale green to yellow and they eventually dry and drop; fruit and tubers are small.	Add N fertilizer improve irrigation management.

Calcium (Ca)	**Retarded growth; yellowing & distortion of young leaves; blossom end rot in cucurbits and tomatoes**	**Side dress with a Ca fertilizer**
Magnesium (Mg)	Growth retarded; chlorotic patches between the veins of older leaves; a triangle of green remains at base of leaf; leaf margins may burn.	Application of fertilizer or weekly foliar sprays
Sulfur (S)	Yellowing of young leaves while older leaves remain dark green; growth stunted.	Application of sulfate compounds.
Boron (B)	Bushy stunted growth & dying growing tips; internal brown rot; brittle plant tissue & split easily; hollow areas in stems.	Application of boron-fertilizers

Iron (Fe)	**Leaves turn yellow/bleached between vein margins; stunting & abnormal growth; fruit may not mature.**	**Spray iron sulphate; reduce soil pH below 7.5**
Manganese (Mn)	Yellow patches between veins; reduced flower formation.	Foliar sprays with manganese sulphate
Molybdenum (Mo)	stunted, pale green or yellow stunting & pale green or yellowish green colour between the veins & along edges of leaves; leaf tissue of margins dies;	Liming to increase soil pH to 6.5; foliar applications of sodium or ammonium molybdate.
Zinc (Zn)	Stunted & pale with creamy yellow interveinal area; distorted young leaves.	Application of Zn foliar spray
Copper (Cu)	Chlorosis in young leaves; tips of leaves distorted; stunted growth.	Apply a copper fertiliser

NUTRIENT TOXICITIES AND CHEMICAL INJURY

Nutrient toxicities

- Chloride toxicity – Caused by saline water and soil conditions; plants wilt when soil moisture seems adequate; test and monitor irrigation water quality; plants vary in their tolerance to salinity.

- Manganese toxicity – Yellowing of margins of older leaves; poor root development; favoured by acidic, waterlogged soil; lime soil to correct pH.

- Ammonium toxicity “jelly butt” – Poor emergence followed by wilting and death of seedlings; browning of the central root tissue; favoured by excess ammonium from fertiliser or poultry manure in cold wet soil.

Nutrient toxicity

Broadcast nitrogen solution

Broadcast solution nitrogen

Nutrient toxicity

Seed-placed urea stand loss and biuret damage

Granular urea

Nutrient toxicity

Two examples of improper use of non-selective herbicide.

Salt injury on taxus.

Leaf cupping/ curling due to a growth regulator herbicide.

Physiological disorders

- Tipburn (physiological/nutritional) – a result of a calcium transport problem within the plant.
- Blossom end rot (physiological/nutritional) – caused by a deficiency of calcium or insufficient calcium uptake and translocation to growing points.
- Riciness of cauliflower.
- Gomasho (grey speck) of cabbage and Chinese cabbage.
- Measles on smooth skinned melons and cucumbers.

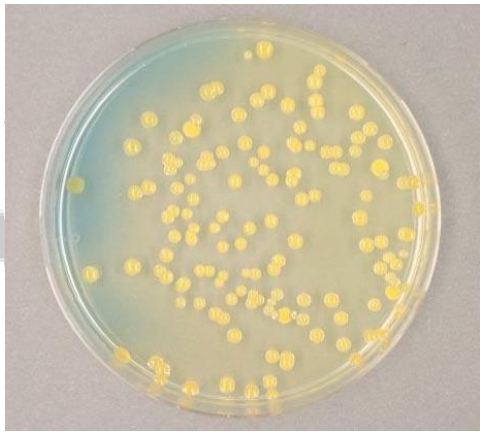
Management of abiotic disorders

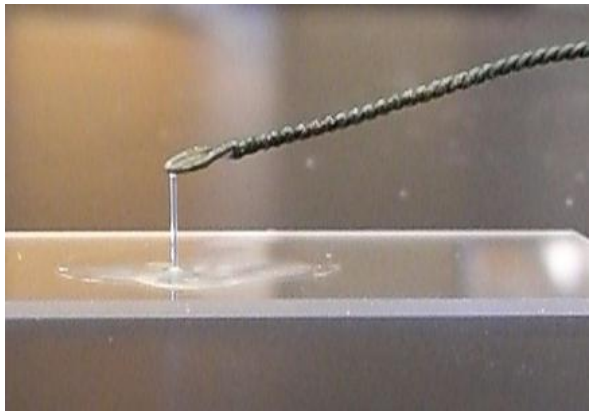
- Investigate weather patterns
- Analyze plant nutrient status
- Look for drainage and compaction
- Check for irrigation problems

- Get a chemical use history
- Plant nutrient deficiencies are best diagnosed using plant tissue analysis.
- As opposed to soil nutrient analysis, plant tissue analysis allows one to determine plant nutrient uptake rather than plant nutrient availability


THANK YOU FOR THE AUDIENCE

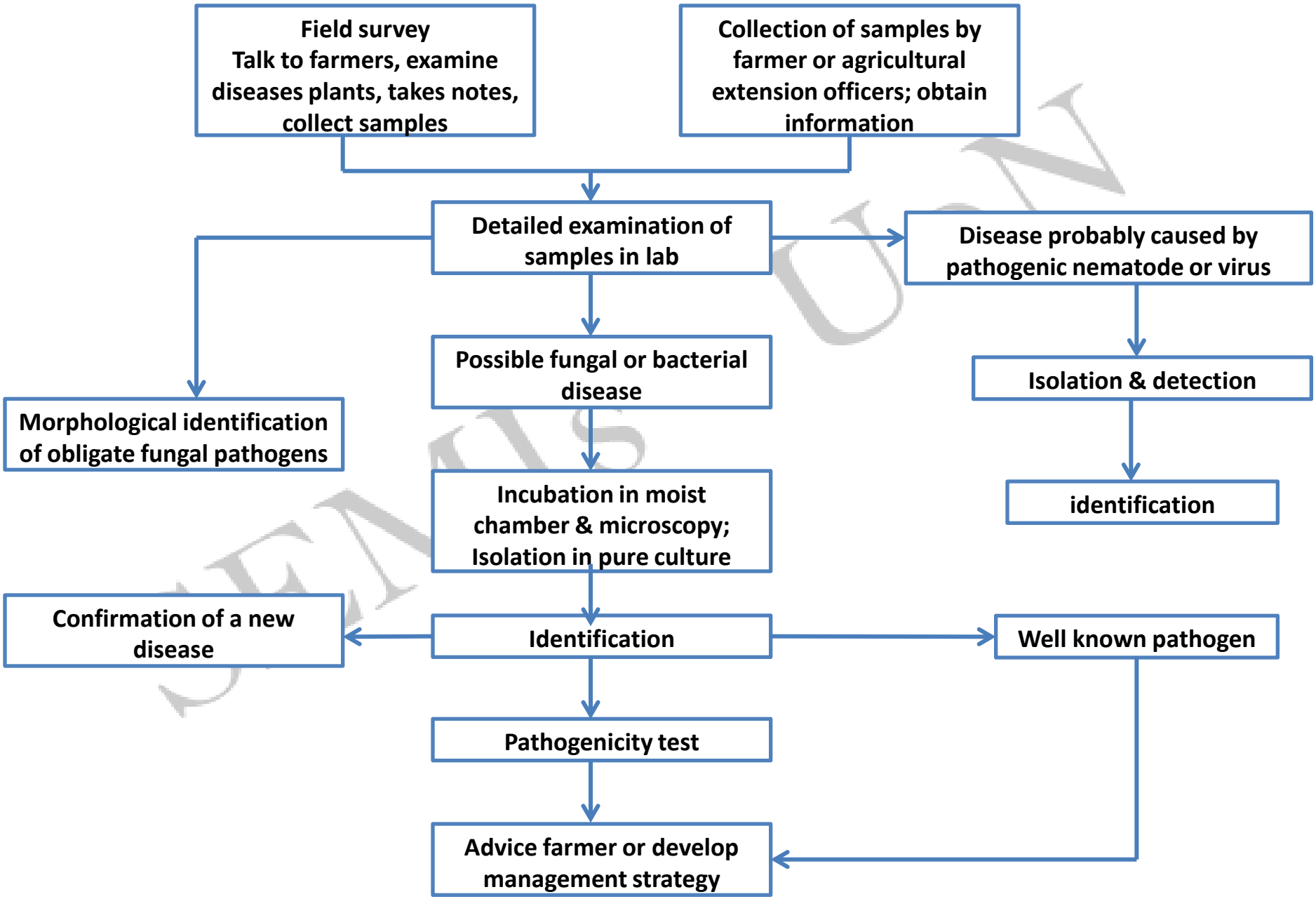
DIAGNOSIS OF PLANT DISEASES





James W. Muthomi
Department of Plant Science and Crop Protection
University of Nairobi

Process in Diagnosis of plant diseases



Diagnosis of diseases of plants

1. Check for symptoms and signs of the disease (Preliminary diagnosis)

signs - physical evidence of the pathogen e.g. fungal fruiting bodies (spores, sclerotia, perithecia, cleistothecia, mycelia); bacterial exudates.. Examples of plant diseases showing signs – leaf rust, stem rust, powdery mildew; bacterial wilt

symptoms - visible effect of disease on the plant e.g. detectable change in color, shape or function of the plant (wilting yellowing, scab, stunting, mosaic, malformations). Examples of plant diseases showing symptoms – damping off, leaf spot, chlorosis

i. **Look for signs of biotic causal agents**

ii. **Identify Plant Part Affected - Are symptoms associated with specific plant parts?**

iii. **Observe Patterns - Check distribution of symptoms; Check for host specificity; Review the cultural practices and growing environment**

2. Laboratory Tests

Sometimes neither symptoms nor signs provide enough specific or characteristic information to decide the cause of an infectious plant disease. In such cases, it may be necessary to bring a sample back to the laboratory for further tests to isolate and identify the causal agent.

i. **Incubation of plant material**

ii. **Isolation and identification of biotic plant disease causal agents – Koch's postulates**

iii. **Diagnostic tests for identification of biotic causal agents – selective media, serological & biochemical tests, PCR**

iv. **Diagnostic tests for identification of abiotic plant disease causal agents – soil & water tests (pH, nutrient composition, salinity, pesticide residues)**

USE OF SERIAL DILUTION IN ISOLATION AND ENUMERATION OF BACTERIA IN SOIL SAMPLES

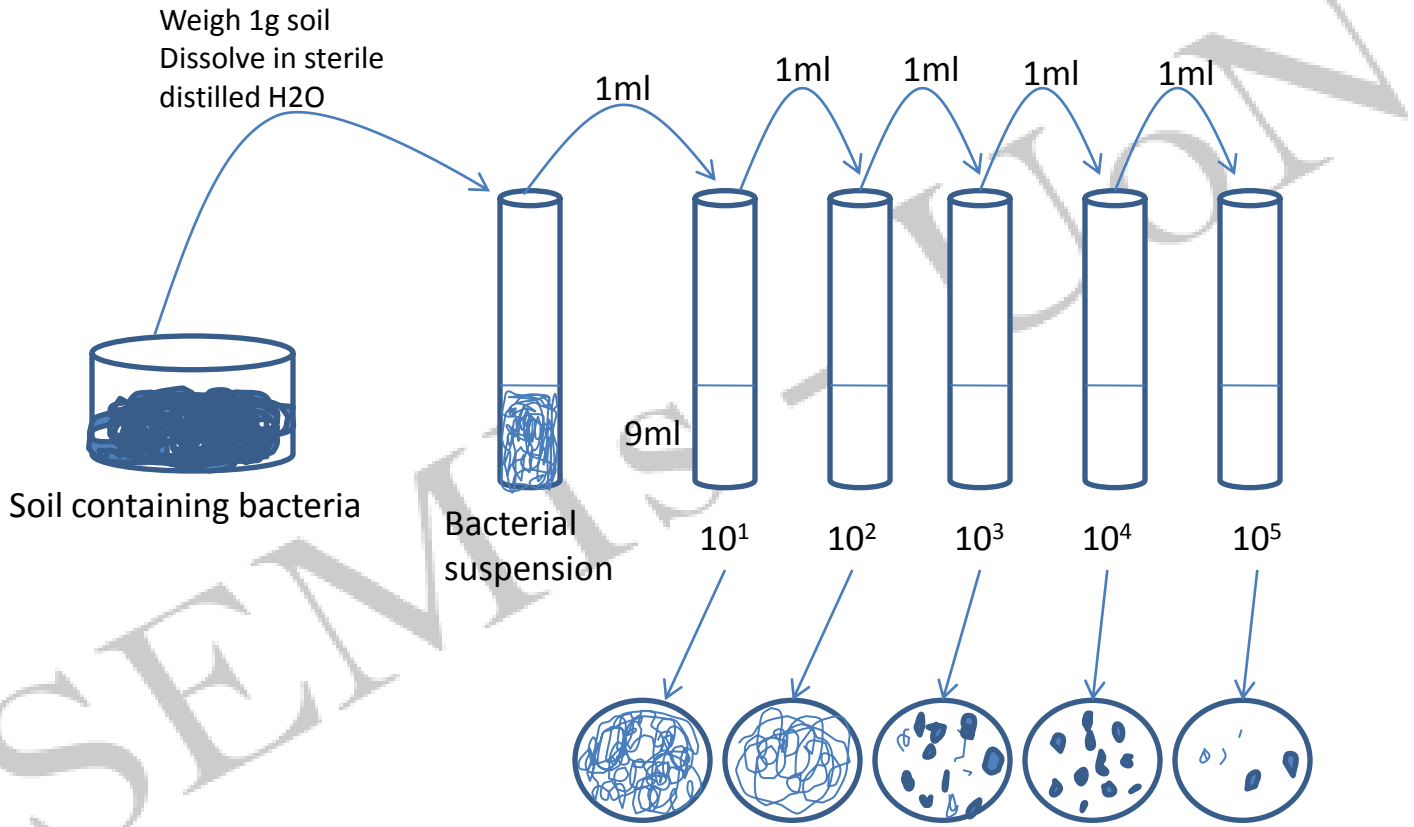
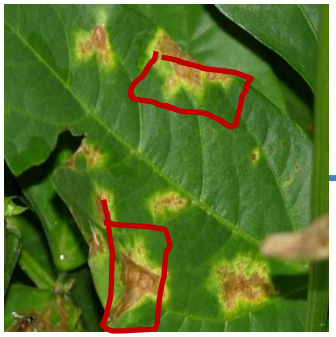


Plate 1ml of each dilution in molten agar medium. Incubate and count the number of colonies for each dilution. Determine bacterial population by multiplying the number of colonies by the dilution factor

Diagnosis of diseases of plants – look for characteristic symptoms

Diagnosis of diseases of plants – isolation & purification of fungi

ISOLATION OF PLANT PATHOGENIC FUNGI



Diseased plant

Cut 5mm^2 tissue pieces at edge of lesion to include diseased and healthy tissues

Surface sterilize in 3% NaoCl for 3min

Rinse in 3 changes of sterile distilled water

Plate the surface sterilized plant tissues on appropriate agar medium

Incubate for 5 to 14 days. Observe growth of fungal colonies around the plated plant tissues

Subculture by teasing out mycelial fragments from advancing edges of the colonies on fresh agar media to obtain

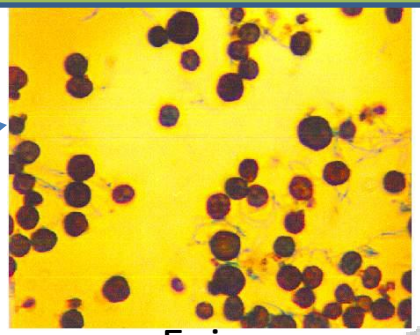
FRUITING STRUCTURES

Induction of perithecia in culture

Perithecia on carnation leaves on agar

Diagnosis of diseases of plants – microscopic examination of fungi

ISOLATION AND MICROSCOPY



Epicoccum

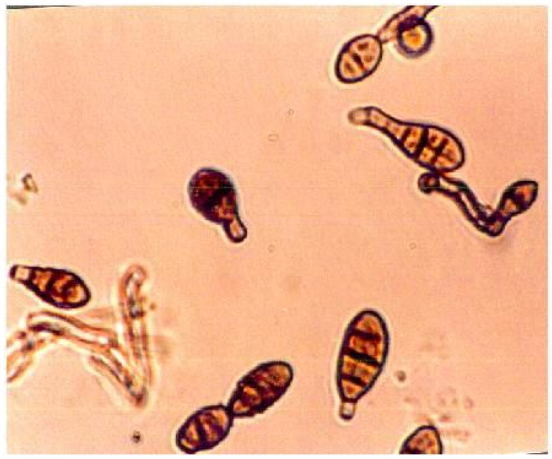
Penicillium

Fusarium

Spores

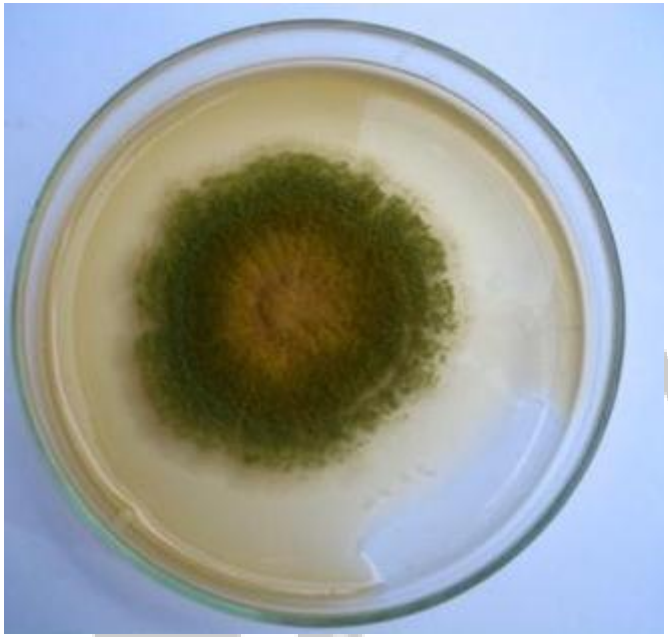
Chlamydospores

Alternaria

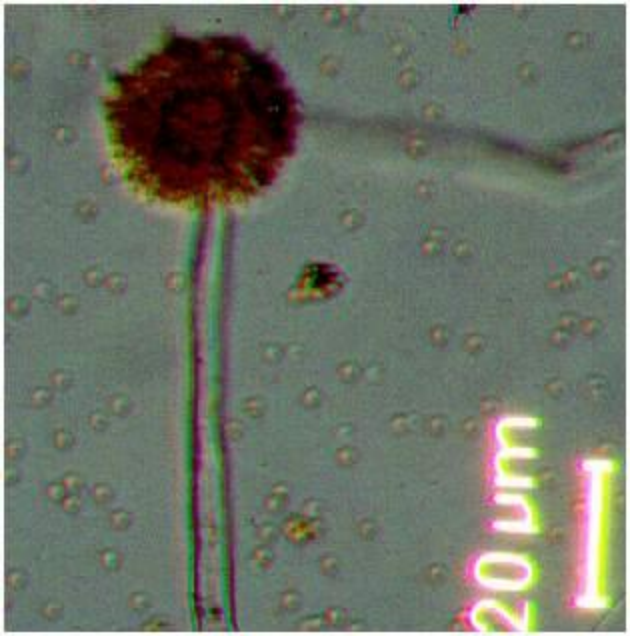


Aspergillus flavus

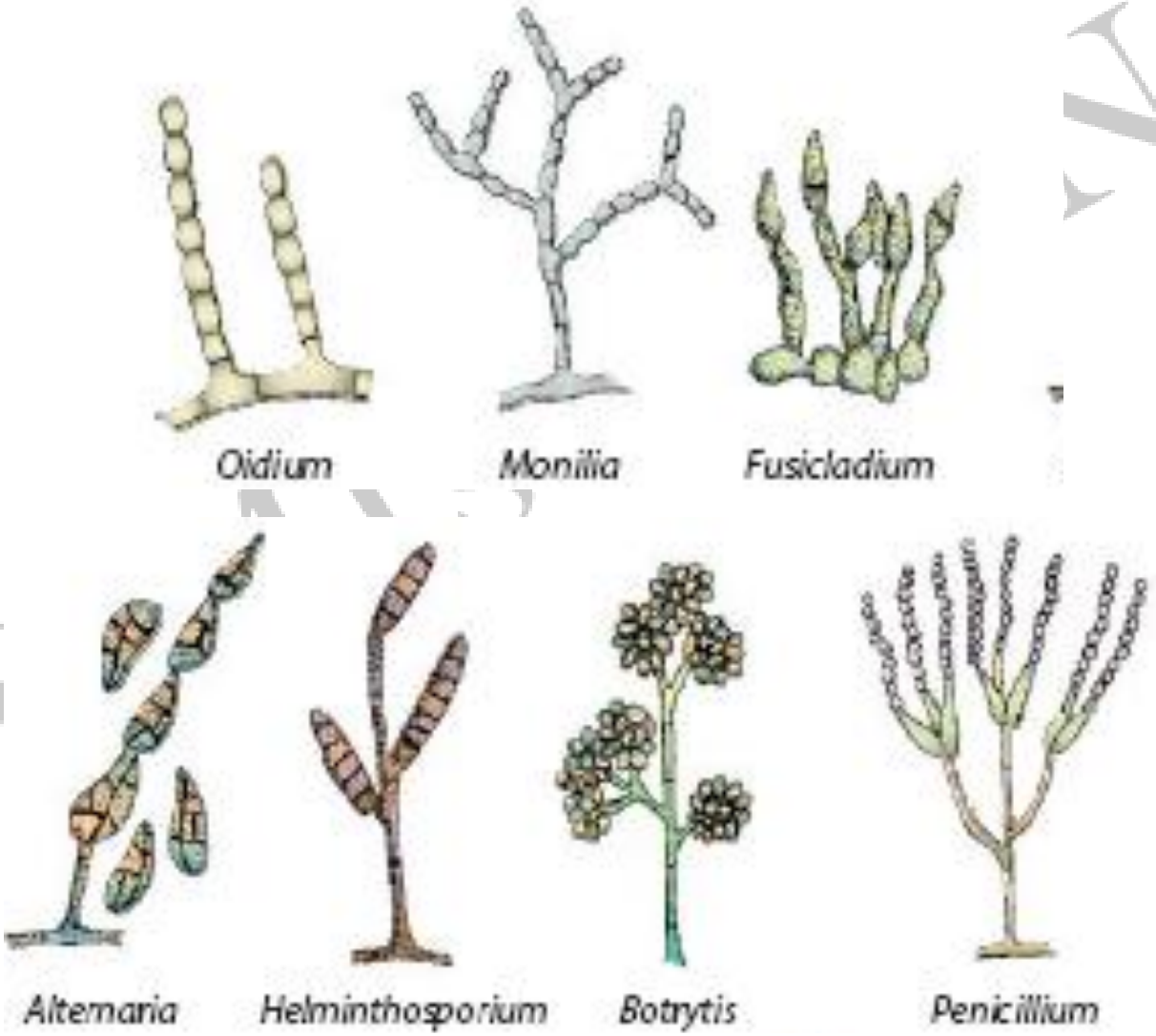
Culture



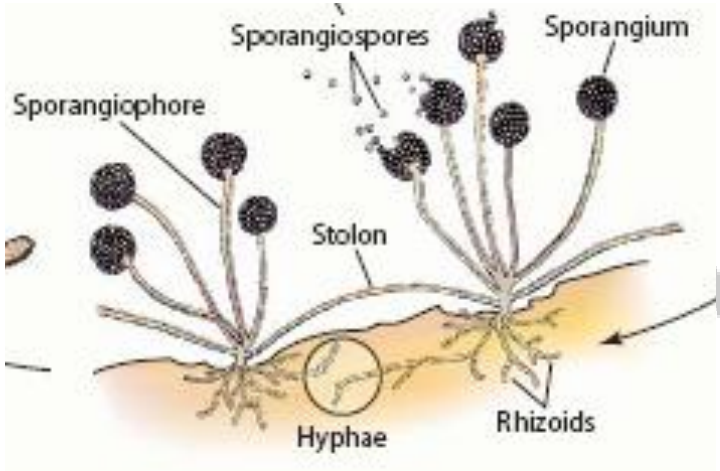
Conidial head



Micro-morphological characteristics of fungi



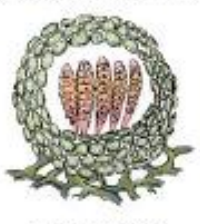
Micro-morphological characteristics of fungi



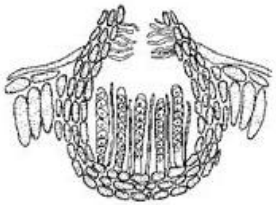
Structure of fungi

Ascus containing ascospores

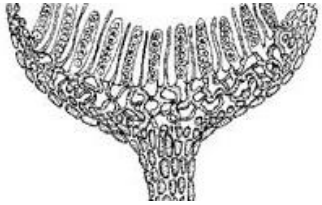
Naked asci



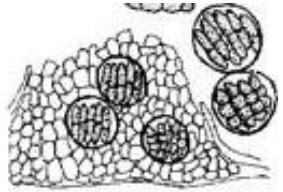
Cleistothecium



Perithecium



Apothecium



Ascostroma

Fruiting structures

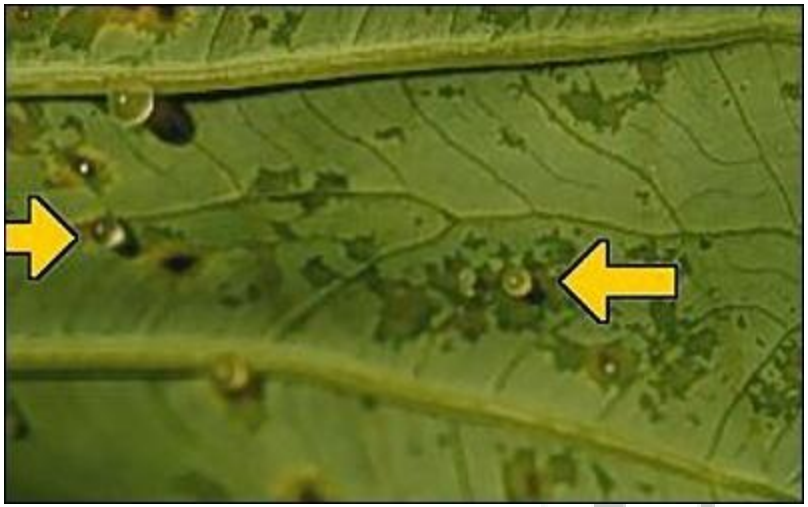
DIAGNOSIS OF BACTERIAL DISEASES

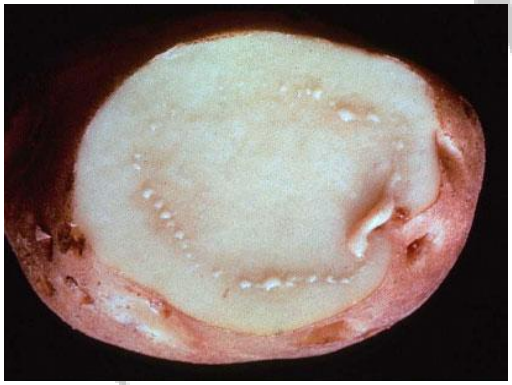
Diagnosis of diseases of plants – characteristic symptoms for bacteria

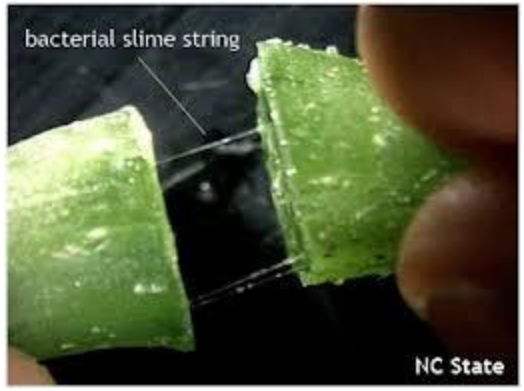
Water soaking

Diagnosis of diseases of plants – examination of signs (bacterial ooze)

Bacterial ooze





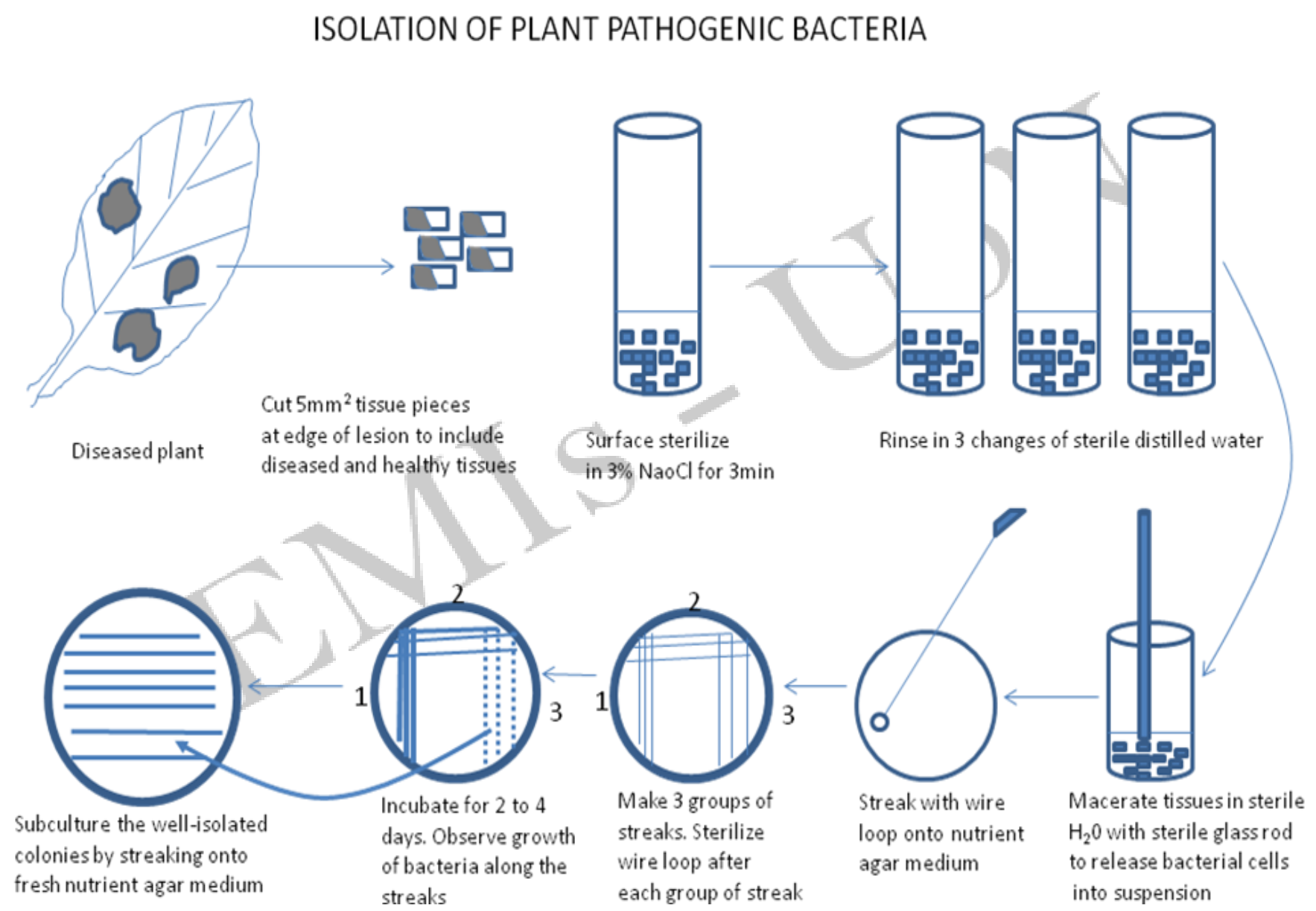


bacterial "ooze" or exudate seen coming out of water soaked lesions (see arrows). The "ooze" forms in the readily seen droplets. These droplets are a sign of the pathogen, being composed mostly of bacterial cells

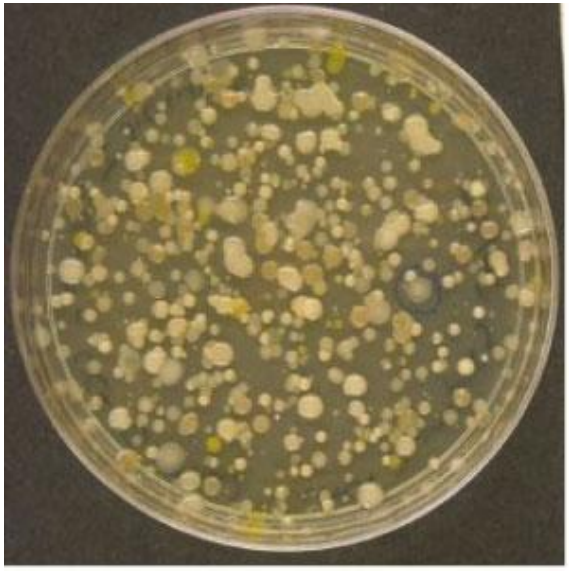
Bacterial streaming test – diagnosis of bacterial wilt

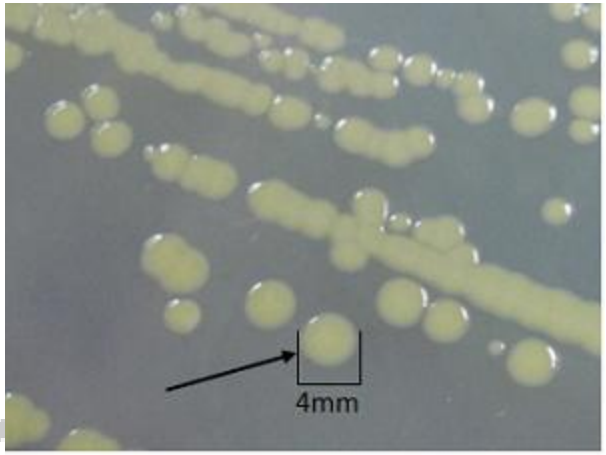
To properly diagnose bacterial wilt, a horizontal cut was made in the lower stem and the cut stem immersed partway in water. Within a few minutes, copious amounts of bacterial exudate emerged from the cut end, forming the white streamers you see in the water. This only occurs with bacterial wilt and not with any other type of pathogen or abiotic cause

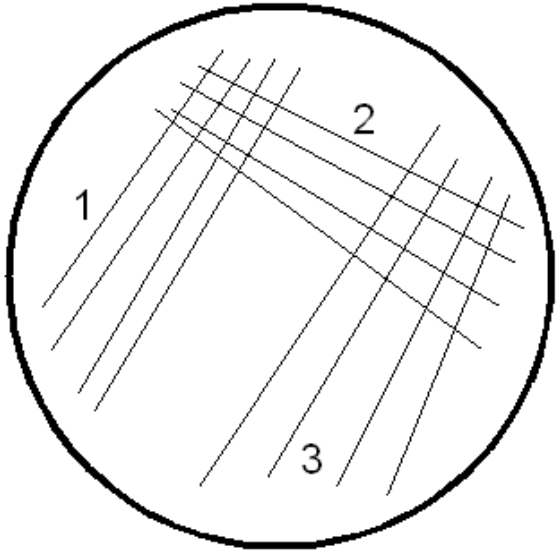
Diagnosis of diseases of plants – isolation of bacteria

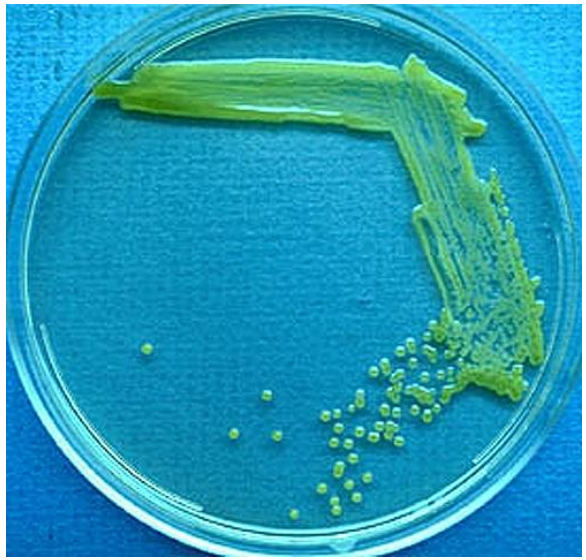


Isolation of bacteria

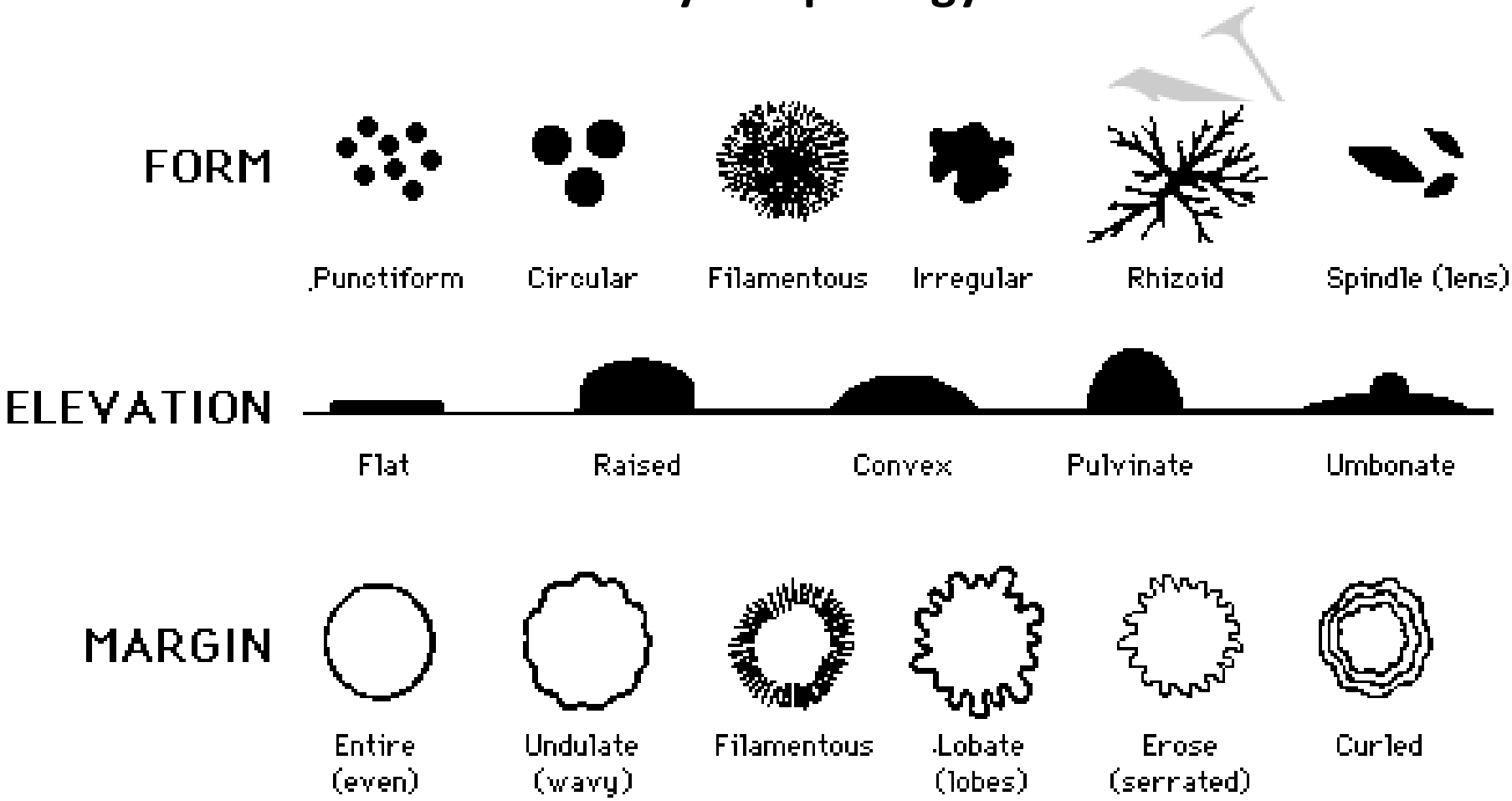




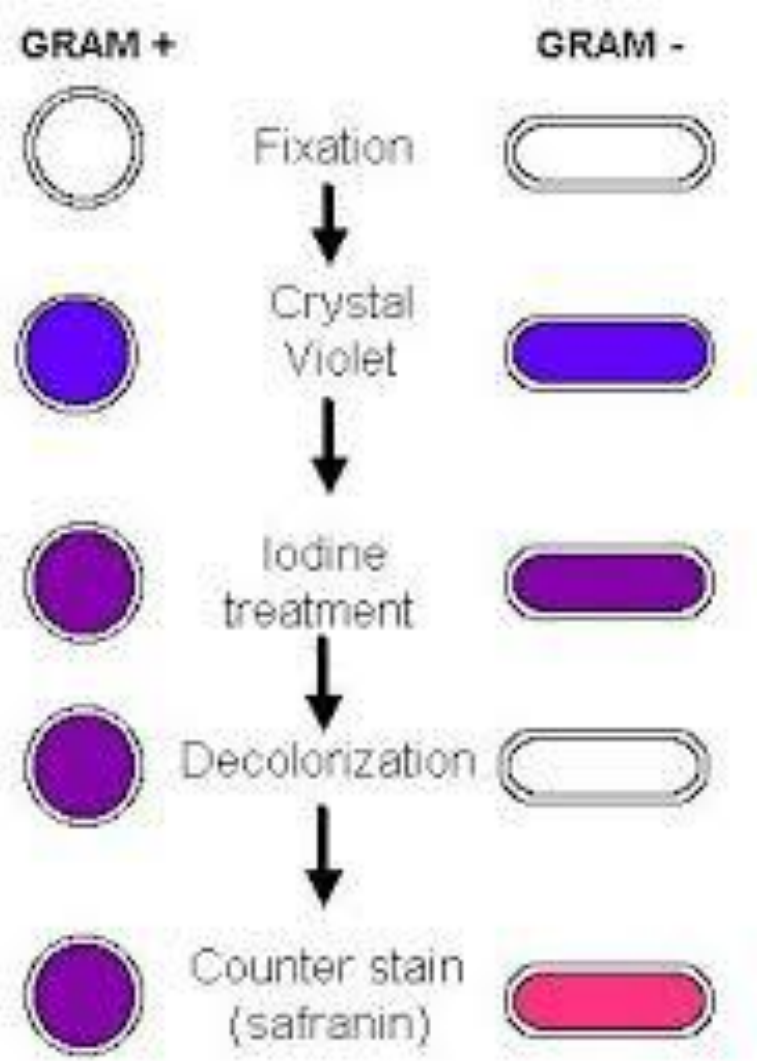


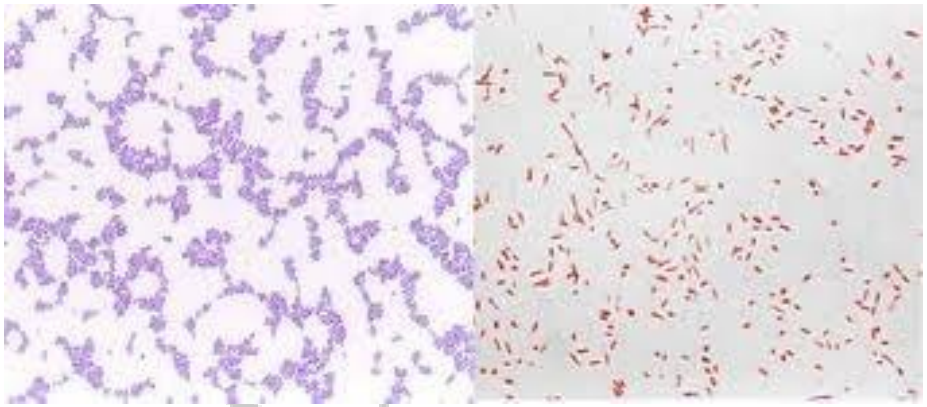


Colony morphology

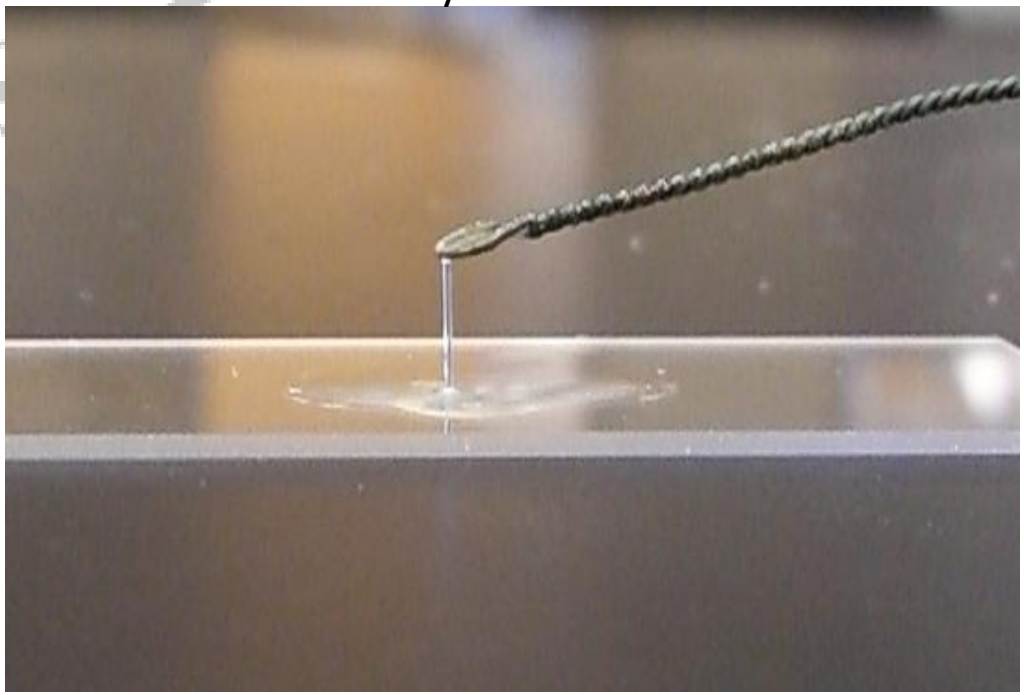


Gram stain test





KOH solubility test for Gram stain



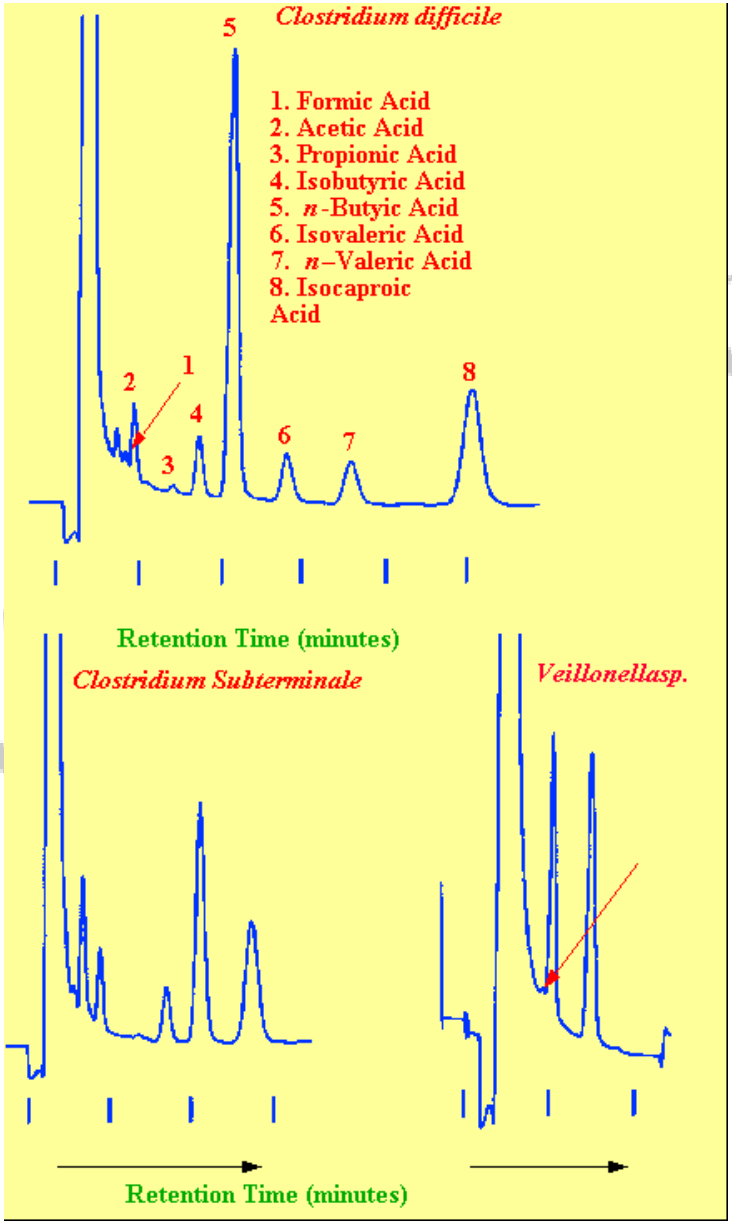
Diagnosis of plant diseases – fatty acid analysis

- Serology – ELISA
- Fatty acid analysis
- PCR-based analysis (molecular markers)
- Determination of pathogenicity
  - Inoculation of detached fruits
  - Stem inoculation for canker and gall inducing pathogens
  - Leaf inoculation for leaf spot pathogens
  - Cotyledon inoculation
  - Root inoculation
  - Inoculation of storage organs for soft rot bacteria

Fatty acid analysis

- Bacterial cell membranes contain many different fatty acids
- Types and relative proportions of these are unique to a particular species.
- Fatty acids are extracted & analyzed by gas chromatography and the fatty acid profiles are used to identify the bacteria genus, species, and, in some cases, to strain level

Gas Chromatogram fatty acid profiles



DNA – based analysis

- Methods are based on gene sequences in the DNA of the bacterial chromosome.
- Gene sequence is unique to a particular species or strain.
- The steps in DNA analysis include:

i. Bacterial DNA is first extracted from the cell

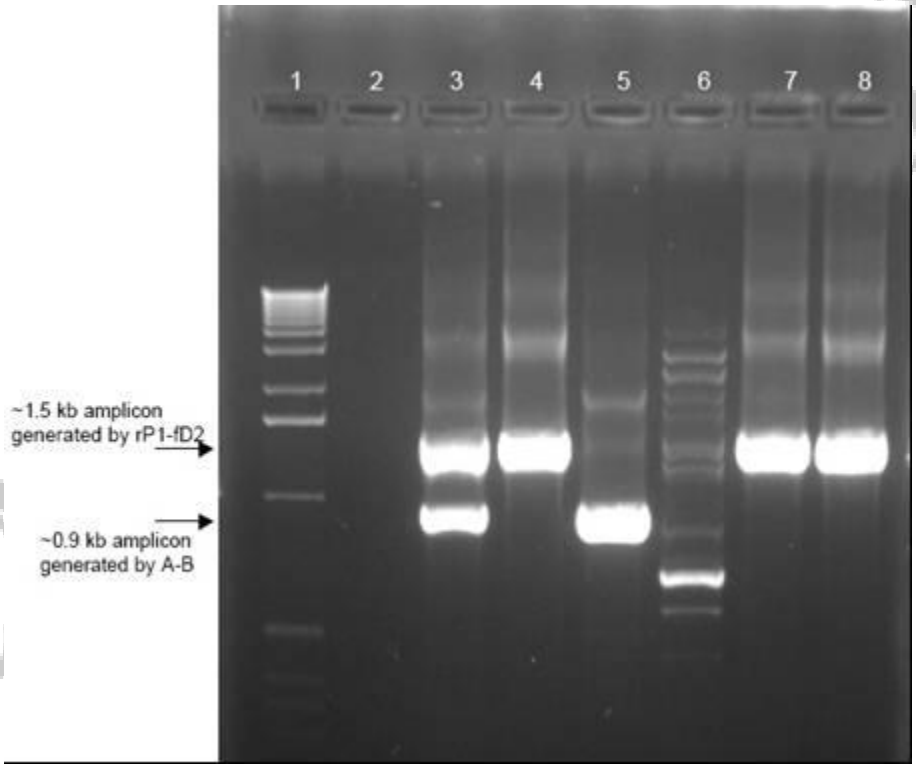
ii. The extracted DNA is amplified by polymerase chain reaction (PCR),

iii. The amplified DNA fragments are separated by electrophoresis on agarose gel to produce finger prints (DNA profiles).

iv. Based on the banding pattern produced on the DNA profiles, the bacteria are identified by comparison to profiles of known DNA

Nucleic acid-based analysis

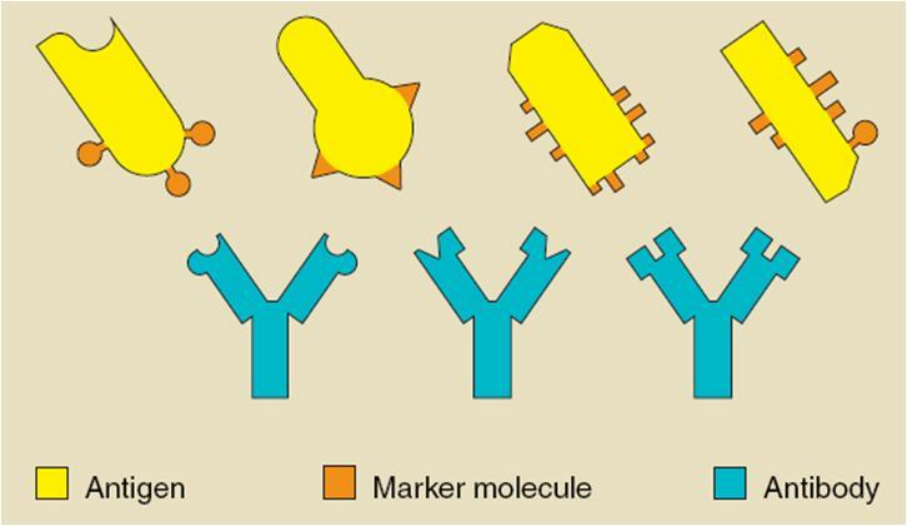
PCR analysis of bacteria



Diagnosis of diseases of plants – Serology (ELISA)

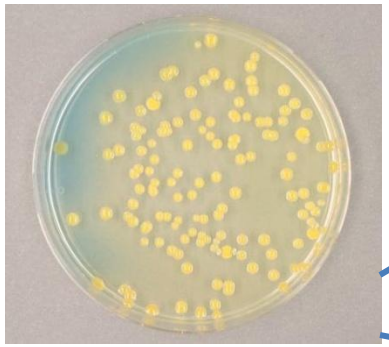
- A specific antibody is conjugated to polystyrene wells and a suspension of the test bacterium is placed in the well and allowed to react with the antibody.
- A positive reaction is indicated by colour change, which can be detected by eye or measured by spectrophotometer (ELISA reader).

Antigen-antibody reaction



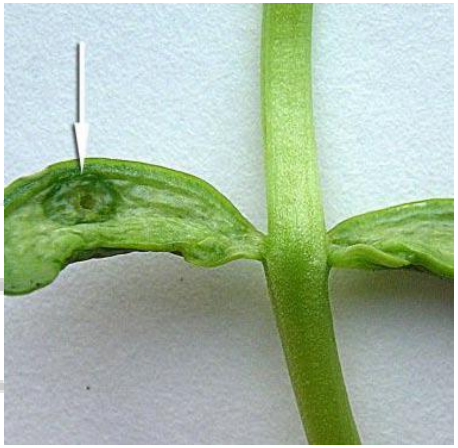
ELISA plate

Pathogenicity test

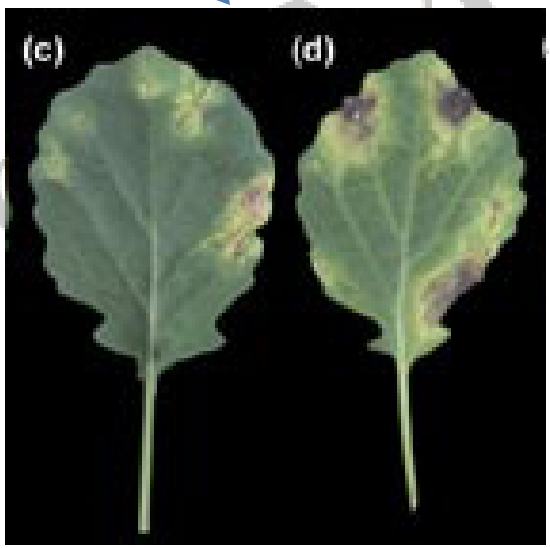


Isolated bacteria

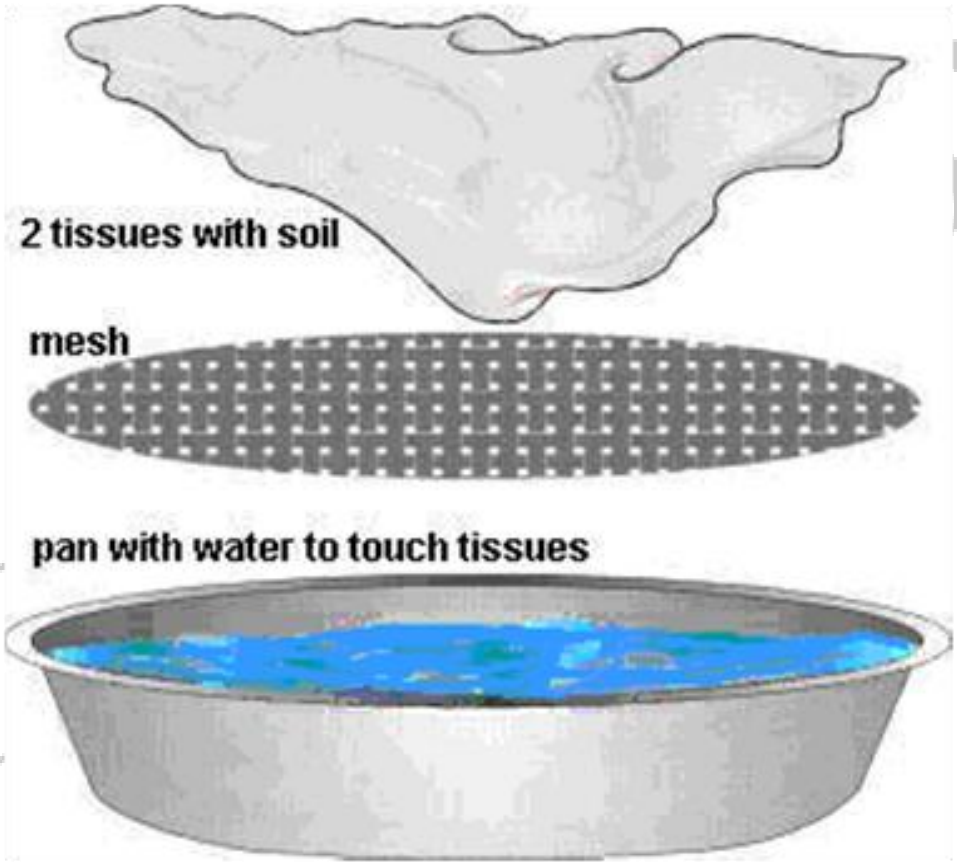
Inoculate on germinated bean cotyledons



Water soaking sympom



Diagnosis of diseases of plants – isolation of nematodes



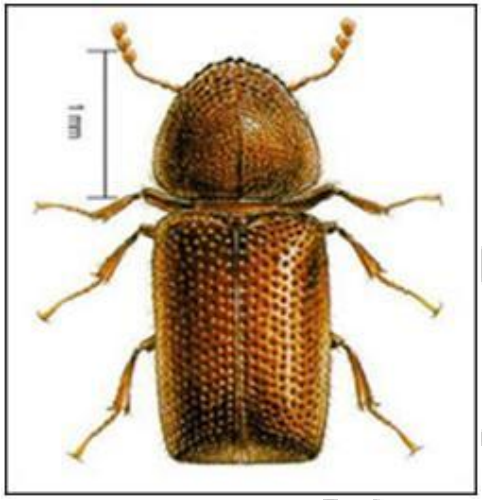
SEED ENTERPRISE MANAGEMENT INSTITUTE (SEMIs)

Seed Production Field Diagnostics

Short Course

22nd – 27th June 2015

Pest Risk Analysis



Prof. James W. Muthomi

Department of Plant Science and Crop Protection

University of Nairobi

Definition:

Pest Risk Analysis (PRA) is a process of:

- investigation,
- evaluation of information and
- decision making

with respect to a certain pest to avoid or reduce the probability

of entrance or establishment of the pest into the country

Why and when is a PRA done?

- Pest Risk Analysis (PRA) is done to protect the country's agriculture from damages that can be caused by harmful (quarantine) pests which can be brought in along with imported commodities

- PRA evaluates the likelihood of the entry, establishment, or spread of a pest and the associated potential biological and economic consequences

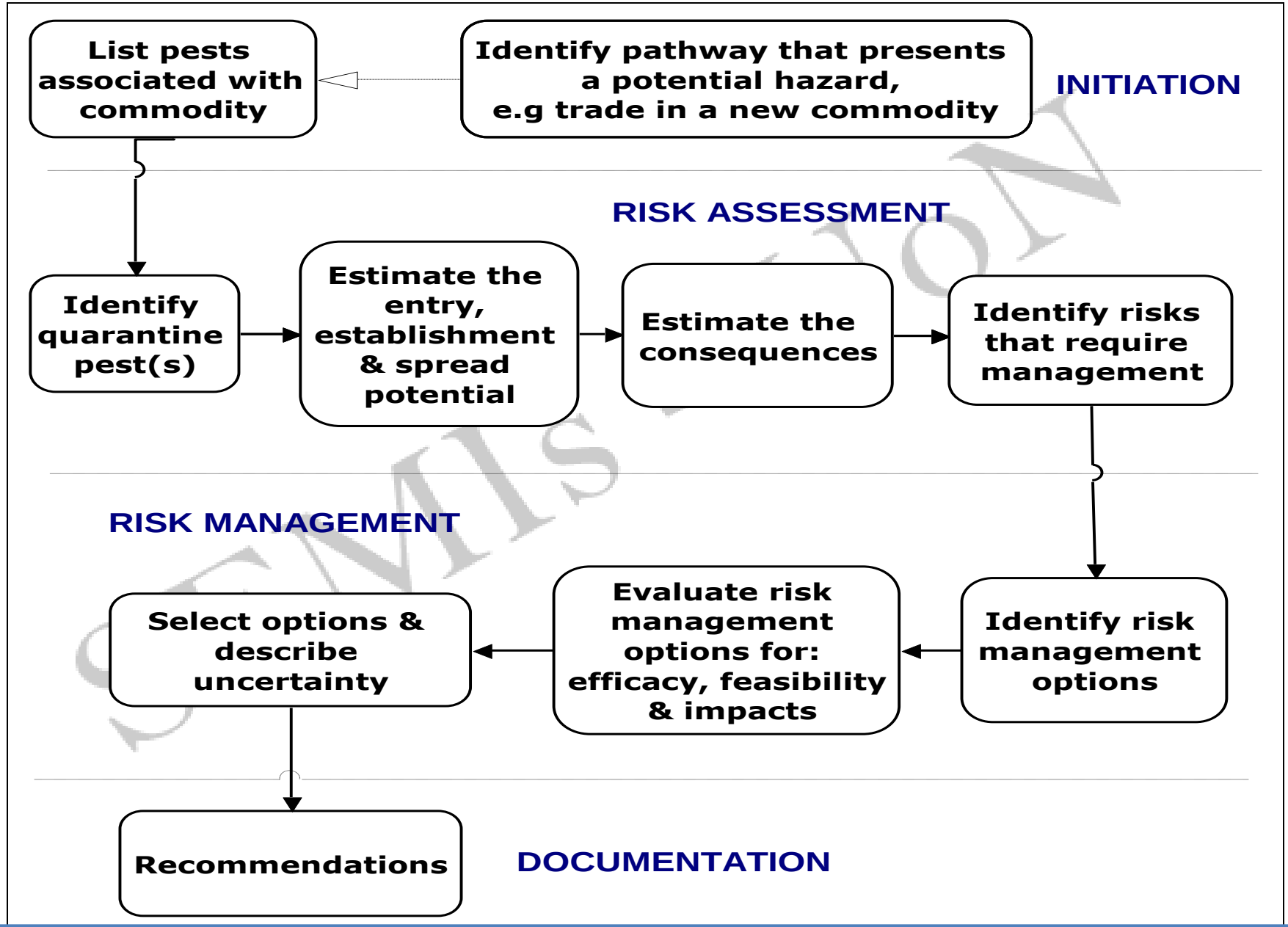
Stages in Pest Risk Analysis (PRA):

- Stage 1 – PRA initiation => identifying the pest(s) & pathways of quarantine concern.

- Stage 2 - Risk assessment => begins with categorization of individual pests to determine whether the criteria for a quarantine pest are satisfied; followed by evaluation of probability of pest entry, establishment, and spread, and potential economic consequences.

- Stage 3 - Risk management => identifying management options for reducing the risks identified at stage 2. Management options are evaluated for efficacy, feasibility and impact in order to select those that are appropriate.

Pest Risk Analysis



Stage 1: PRA Initiation

Aim of the initiation stage is to identify the pest(s) and pathways which are of quarantine concern

The PRA process may be initiated as a result of:

1. The identification of a pathway that presents a potential pest hazard
2. The identification of a pest that may require phytosanitary measures
3. The review or revision of phytosanitary policies and priorities.

1. PRA initiated by the identification of a pathway

- Trade is initiated in a commodity not previously imported into the country
- New plant species are imported for selection
- A pathway is identified e.g. Natural spread, packing material etc

2. PRA initiated by the identification of a pest

A requirement for a new or revised PRA on a specific pest may arise in the following situations:

- Discovery of an established infestation or outbreak of a new pest
- Interception of a new pest on an imported commodity
- A new pest risk is identified by scientific research
- A pest is introduced into an area

Pest Risk Analysis

- A pest is reported to be more damaging in an area other than in its area of origin
- A pest is repeatedly intercepted
- A request is made to import an organism
- An organism is identified as a vector for other pests
- An organism is genetically altered in a way which clearly identifies its potential as a plant pest.

3. PRA initiated by the review or revision of a policy

A requirement for a new or revised PRA originating from policy concerns will most frequently arise in the following situations:

- Review phytosanitary regulations, requirements or operations
- A proposal made by another country or by an international organization (RPPO, FAO) is reviewed
- A new treatment or loss of a treatment system, a new process, or new information impacts on an earlier decision
- A dispute arises on phytosanitary measures
- The phytosanitary situation in a country changes, a new country is created, or political boundaries have changed.

Stage 2: Pest Risk Assessment

Pest Risk Analysis

The process for pest risk assessment can be broadly divided into three interrelated steps:

1. Pest categorization
2. Assessment of the probability of introduction and spread
3. Assessment of potential economic consequences (including environmental impacts).

1. Pest Categorization

- Quarantine pest
- Regulated non-quarantine pest

Conclusion of pest categorization

- Pest has the potential to be a quarantine pest, the PRA process should continue.
- Pest does not fulfill all of the criteria for a quarantine pest, the PRA process for that pest may stop.
- Insufficient information, the uncertainties should be identified and the PRA process should continue

2. Assessment of the probability of introduction and spread

This involves assessment of the following:

i. Probability of entry of a pest

ii. Probability of establishment

iii. Probability of spread after establishment

i) Probability of entry of a pest

- Identification of pathways for a PRA initiated by a pest
- Probability of the pest being associated with the pathway at origin
- Probability of survival during transport or storage
- Probability of pest surviving existing pest management procedures
- Probability of transfer to a suitable host

ii) Probability of establishment

- Availability, quantity and distribution of hosts in the PRA area
- Environmental suitability in the PRA area
- Potential for adaptation of the pest
- Reproductive strategy of the pest
- Method of pest survival
- Cultural practices and control measures

iii) Probability of spread after establishment

- Suitability of the natural and/or managed environment for natural spread of the pest
- Presence of natural barriers
- The potential for movement with commodities or conveyances
- Intended use of the commodity
- Potential vectors of the pest in the PRA area
- Potential natural enemies of the pest in the PRA area

3. Assessment of potential economic consequences

Direct pest effects - types, amount & frequency of damage, crop losses (yield and quality), rate of spread, rate of reproduction, control measures (including their efficacy and cost), and effect on existing production practices

Indirect pest effects - effects on domestic and export markets, changes to producer costs or input demands, changes to domestic or foreign consumer demand feasibility and cost of eradication or containment, capacity to act as a vector for other pests, resources needed for additional research and advice, social and other effects.

Conclusions from Pest Risk Assessment

- Used to decide whether risk management is required and the strength of measures to be used.
- Since zero-risk is not a reasonable option, the guiding principle for risk management should be to manage risk to achieve the required degree of safety that can be justified and is feasible within the limits of available options and resources.

Stage 3: Pest Risk Management

Pest Risk Analysis

- **Pest Risk Management** - process of identifying ways to react to a perceived risk, evaluating the efficacy of these actions, and identifying the most appropriate options.

- Overall risk is determined by the examination of the outputs of the assessments of the probability of introduction and the economic impact.

- If the risk is found to be unacceptable, then the first step in risk management is to identify possible phytosanitary measures that will reduce the risk to, or below an acceptable level.

- Measures are not justified if the risk is already acceptable or must be accepted because it is not manageable (as may be the case with natural spread).

Identification and selection of appropriate risk management options

Appropriate measures should be chosen based on their effectiveness in reducing the probability of introduction of the pest.

The choice should be based on the following considerations:

- Phytosanitary measures shown to be cost-effective and feasible
- Principle of "minimal impact" – not restrict trade
- Reassessment of previous requirements
- Principle of "equivalence" - different phytosanitary measures with the same effect
- Principle of "non-discrimination" - phytosanitary measures should not be more stringent than those applied within the PRA area

Qualitative risk analysis matrix

Likelihood	Economic/environmental/social consequences				
	Negligible	Low	Medium	High	Extreme
Extremely high	H	H	E	E	E
High	M	H	H	E	E
Medium	L	M	H	E	E
Low	L	L	M	H	E
Negligible	L	L	M	H	H

E – Extreme risk; H – High risk; M – Moderate risk; L – Low risk; N – Negligible risk

Pest Risk Analysis

E = Extreme risk - specific action is immediately required to reduce risk

H = High risk - specific action is required, generic risk treatment plans should be adopted as soon as possible in the interim.

M = Moderate risk - adoption of generic risk treatment plans will reduce the risk to suitable levels.

L = Low risk - manage through routine procedures.

N = Negligible risk.

Example:

Estimating the Overall Risk Posed by a Quarantine Pest

Risk Estimation Matrix for Australia

Likelihood of entry, establishment and spread	Consequence of entry, establishment and spread					
	Negligible	Very low	**Low**	Moderate	High	Extreme
High	Negligible	Very low	Low	Moderate	High	Extreme
Moderate	Negligible	Very low	Low	Moderate	High	Extreme
Low	Negligible	Negligible	Very low	Low	Moderate	High
V. Low	Negligible	Negligible	Negligible	Very low	Low	Moderate
E. Low	Negligible	Negligible	Negligible	Negligible	Very low	Low
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Very low

THANK YOU