

PROCEDURES FOR INSPECTION, DIAGNOSTICS AND DISINFECTION OF PLANTING MATERIALS



Datin Jati Aliah Timin
Department of Agriculture,
Malaysia



POSSIBLE METHODS OF INTRODUCTION OF SALB

- **Infected *Hevea* Plants**
- **Spores contaminating** travelers, their belongings and imported commodities.

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THE PREVENTION OF INTRODUCTION OF SALB INTO THE REGION

- ❖ **Strict phytosanitary import requirements;**
- ❖ **An inspection system at points of entry;**
- ❖ **A laboratory diagnostic system (central & at points of entry);**
- ❖ **An efficient surveillance system that ensures early detection of disease (at points of entry & in the field).**

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POINTS OF ENTRY INSPECTION SYSTEMS

Inspection of consignments - including travelers and their hand carried items that originate from SALB countries to ensure compliance with import/entry requirements.

SPECIFIC POINTS:

- **Examination of documents** – to ensure that imports have the correct certification;
- **Examination of manifests** – to identify animate goods or non-host organic materials that may be contaminated with spores or host materials;
- **Trained personnel** that can recognize *Hevea* plant materials be stationed at entry points;

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POINTS OF ENTRY INSPECTION SYSTEMS

SPECIFIC POINTS:

- Inspection of consignments including **machineries** originating from SALB countries;
- **Availability of equipment and chemical for disinfecting and disposing** of suspect materials and non compliance consignments - Take phytosanitary action for non-compliance and upon detection of SALB;
- Ensure **availability of security and system for packing and transporting** suspect materials to diagnostic laboratory (central);

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PROCEDURES FOR INSPECTION, DIAGNOSTICS AND DISINFECTION OF PLANTING MATERIALS

INSPECTION AND DIAGNOSTICS

Levels of inspection

Level	Site	Activity
I	Point of entry	Inspection of documents ; Visual inspection of imported consignment .
II	Enclosed quarantine facility	Inspection of seedlings and budded plants for disease and pest symptoms visually or aided by hand lenses, binocular or compound microscopes

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PROCEDURES FOR INSPECTION, DIAGNOSTICS AND DISINFECTION OF PLANTING MATERIALS

INSPECTION AND DIAGNOSTICS

Levels of inspection

Level	Site	Activity
III	Open quarantine facility	Inspection of seedlings for symptoms of diseases and pests visually or aided by hand lenses, binocular or compound microscopes
IV	Laboratory	Identification of spores Isolating and culturing of pathogens

PROCEDURES FOR INSPECTION, DIAGNOSTICS AND DISINFECTION OF PLANTING MATERIALS

OUTLINE OF PRESENTATION

1. Procedures for inspection and clearance

- Inspection of documents and import requirements
- Recognizing *M. ulei* and symptoms of SALB
- Recognition of host plant and plant parts

2. Laboratory diagnostic systems

- Laboratory diagnostic protocols and pathogen identification.

PROCEDURES FOR INSPECTION, DIAGNOSTICS AND DISINFECTION OF PLANTING MATERIALS

OUTLINE OF PRESENTATION

3. Quarantine measures

- Disinfection of planting materials
- Disinfection or destruction of infected or non-compliant materials
- Disposal of waste materials

PROCEDURES FOR INSPECTION, DIAGNOSTICS AND DISINFECTION OF PLANTING MATERIALS

1. Procedures for inspection and clearance



PROCEDURES FOR INSPECTION, DIAGNOSTICS AND DISINFECTION OF PLANTING MATERIALS

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PROCEDURES FOR INSPECTION, DIAGNOSTICS AND DISINFECTION OF PLANTING MATERIALS

1. Procedures for inspection and clearance

- ❖ PRE-EXPORT INSPECTION AND TREATMENT
 - At Pre-Border
- ❖ ON-ARRIVAL INSPECTION AND TREATMENT
- ❖ POST-ENTRY QUARANTINE
- ❖ INTERMEDIATE QUARANTINE (optional)
- ❖ INSPECTION UPON ARRIVAL

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PROCEDURES FOR INSPECTION, DIAGNOSTICS AND DISINFECTION OF PLANTING MATERIALS

Planting Materials

- Budded stumps, budwood
- Seeds, flowers, fruits
- Plants in vitro

The IPPC definition of plants for planting includes **whole plants and cuttings, and plants *in-vitro*** (ISPM 5 2006).

For the purposes of risk analysis only **stumps and budwood** have been considered for measures as they **are the most likely form to be transported** between countries for planting.



1. Procedures for inspection and clearance

Budded stumps or budwood


Pre-export inspection and treatment

- At Pre-Border - **At the country of origin**
- Pre-export inspection **by importing country NPPO**

Imported budwood:

- ✓ **no longer than 1 meter**
- ✓ **in the form of brown budwood.**

General requirements



1. Procedures for inspection and clearance

❖ PRE-EXPORT INSPECTION AND TREATMENT



Budded Stumps Or Budwood

General requirements

The quantity allowed to be imported:

Number of genotype or clone

- depend on **space availability** of the contained glasshouse facility.
- should be **as small as possible** to reduce risk of introduction







❖ PRE-EXPORT INSPECTION AND TREATMENT

Budded Stumps Or Budwood

General requirements

- ✓ **Mother plants**- inspected by qualified plant pathologist for presence of SALB
 - before harvesting of budwood
 - during the optimal period for disease expression.
- ✓ **Budwood** - grafted in a nursery registered by the NPPO of the country of origin.

❖ PRE-EXPORT INSPECTION AND TREATMENT

Budded stumps or budwood

- **Budded stumps**
 - should be sourced from **registered nurseries**
 - **certified by NPPO.**
- **Nurseries should be**
 - located **away from SALB area**
 - monitored** by the plant pathologist







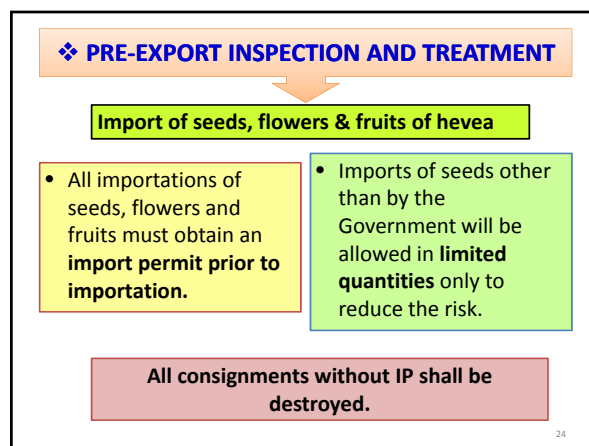
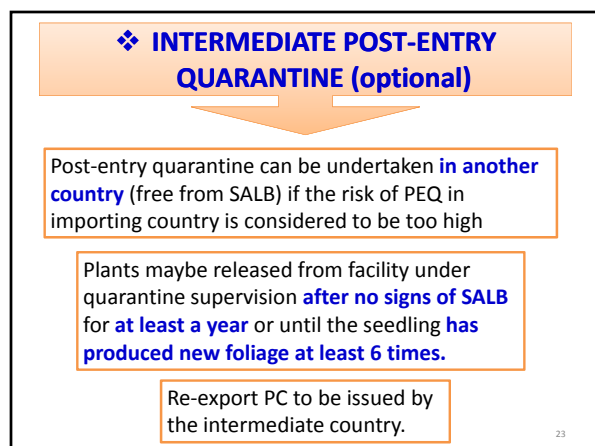
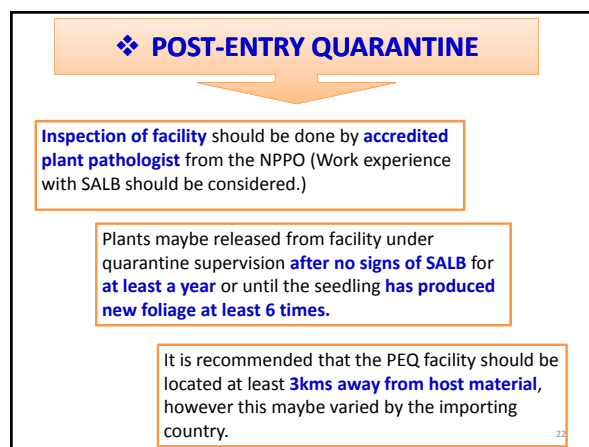
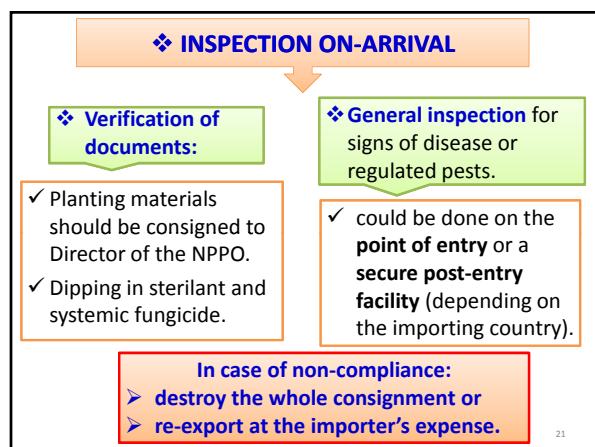
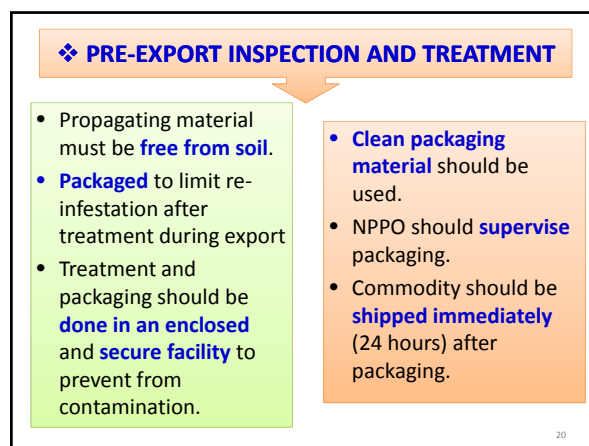
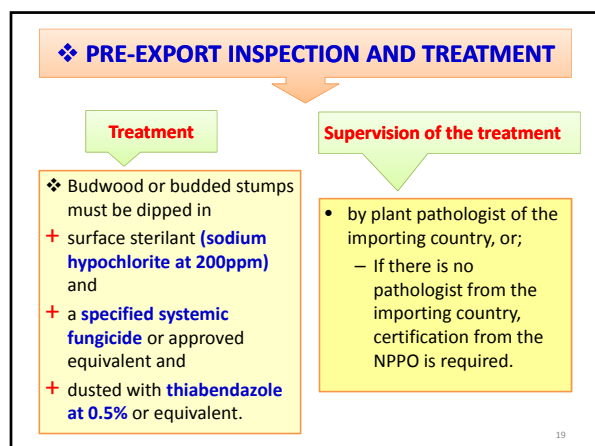
❖ PRE-EXPORT INSPECTION AND TREATMENT

Budded stumps or budwood

Based on the inspection of plant pathologist (approved by or from importing country), NPPO will issue certificate that

- **the harvested budwood does not show symptoms of SALB, and**
- **the mother trees have a low level of infection.**



❖ PRE-EXPORT INSPECTION AND TREATMENT

Import of seeds, flowers & fruits of Hevea

- suggestion to **ban the importation of fruits and flowers?** - high risk. However this would be inconsistent with the PRA
- **Volumes of these products can be regulated** through the issuance of import permits, if required.
- **Specify quantity:** Volume to be decided by importing country

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❖ PRE-EXPORT INSPECTION AND TREATMENT

Import of seeds, flowers & fruits of Hevea

- **PC from exporting country NPPO:**
- **Surface sterilized** (with recommended chemical at dosage rate or the equivalent) and treatment prior to export.
 - ✓ **Sodium hypochlorite at 200 ppm** or approved equivalent (exporting country could suggest equivalent)
- **Additional Declaration for Seeds:** Formalin (or approved equivalent) and fungicide treatment
- A chemical other than formalin may be approved in order to improve germination of seeds.

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❖ INSPECTION UPON ARRIVAL:



HEVEA SEEDS

Re-treatment:

- **Inspection in a secure plant quarantine facility** at entry point or other suitable facility.
- **Destruction of packaging material.**
- Option to re-treat upon arrival.
- It is recommended that following importation, the seeds should be **dusted with thiabendazole.**

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Inspection and diagnosis of pests and diseases

- Planting materials shall be **inspected for disease symptoms**
- Inspection shall concentrate on the lower surface of the young leaves (reddish in colour) of the plants.
- **Record** each inspection (dates, pests detected).

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Inspection and diagnosis of pests and diseases

The identification of pests and diseases shall be based on:

- The **characteristic symptoms** of diseases on young leaves
- The **morphology of the spores** under a microscope;
- The **diagnostic aids**
- In the event of inability to diagnose the disease, a competent authority should be consulted
- Fungal isolation and culturing

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2. LABORATORY DIAGNOSTIC SYSTEMS

The Regional Standards for Phytosanitary Measures – **Guidelines for Protection against SALB of Rubber** recommends that each member country should have or is assessable to a diagnostic laboratory

The main objective of establishing the diagnostic laboratory is to diagnose SALB and identify the causal pathogen.

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2. LABORATORY DIAGNOSTIC SYSTEMS

- Countries should **have or assess to a diagnostic laboratories** that are with the function to identify pathogens and diseases;
- The laboratories should be **at or near the entry points**;
- Should **follow standard procedure for diagnosis of pathogens and diseases**.

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2. LABORATORY DIAGNOSTIC SYSTEMS

MINIMUM REQUIREMENT FOR PERSONNEL AND FACILITIES

Countries should have the following resources and facilities

- Designated **expert on SALB**
- **Trained inspectors** for consignment inspection and surveillance programme
- **Diagnostic capabilities** to detect and identify the pathogen
- PEQ facilities

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2. LABORATORY DIAGNOSTIC SYSTEMS

MINIMUM REQUIREMENT FOR PERSONNEL AND FACILITIES

Countries should have the following resources and facilities

- Assess to aerial spraying companies;
- Disinfection facilities:
 - ✓ Dipping tanks with sodium hypochlorite
 - ✓ Hot water jet system for disinfection
 - ✓ Incinerator
 - ✓ UV chamber
 - ✓ chemicals

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Diagnostic Laboratory

- Will be equipped with **adequate equipment, chemicals** and **consumables** needed to effectively carry out these functions
- The laboratory will also have rooms and **equipment for serological (ELISA) and molecular diagnostic (PCR) methodologies**.
- Will also be allocated with a **suitable insect proof glasshouse**.
- Laboratory, glasshouse and other facilities should follow the **guideline as specified in plant containment facilities requirement** (Note: Malaysia: in draft – Safety in the laboratory code of practice part 3: Bio containment and biosafety in microbiological facility)

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2. LABORATORY DIAGNOSTIC SYSTEMS

- A Central Diagnostic Laboratory
- Satellite Quarantine Laboratories.

FUNCTIONS

Individual laboratory will carry out a **specific function**. The Central Laboratory will undertake greater function compared to the Satellite Laboratory.

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2. LABORATORY DIAGNOSTIC SYSTEMS

- A Central Diagnostic Laboratory
- Satellite Quarantine Laboratories.

Central Quarantine Laboratory

- ✓ Diagnostic & confirmation
- ✓ Treatment
- ✓ Disposal
- ✓ Reporting
- ✓ Major databases
- ✓ National, regional and international linkages

Satellite Quarantine Laboratory

- ✓ Basic diagnostic functions e.g. visual and simple microscopic examination
- ✓ Liaise with Central Quarantine Laboratory
- ✓ Reporting

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2. LABORATORY DIAGNOSTIC SYSTEMS

DIAGNOSTIC METHODOLOGY

- **Visual assessment**
 - ~ Visual assessment of symptoms
 - ~ Other plant parts infected.
- **Mycological Method**
 - ✓ Microscopic assessment, isolation, inoculation, incubation-(microscopic assessment spores)
- **Molecular and serological detection technique** (PCR, ELISA)

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2. LABORATORY DIAGNOSTIC SYSTEMS

DIAGNOSTIC METHODOLOGY

Visual assessment

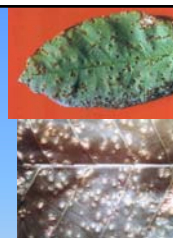
- ✓ Visual assessment of symptoms
- ✓ Other plant parts infected.

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SYMPTOMS

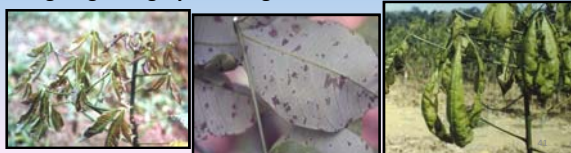
- First visible symptoms are the distortion of leaves.
- Visible symptoms occur 2-3 days after inoculation.
- A few days later irregular shaped lesions developed on lower surface of leaves.
- Lesions appear dark or olive green.
- Size of lesions depends on susceptibility of leaves (age & clone)



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SYMPTOMS OF SALB

- depend on the age of leaves at time of infection;
- The young copper brown leaflets shrivel, curl, turn blackish and drop-off leaving the petioles for several days on the stems;
- The fully expressed conidial lesions are seen on the lower surface of the green leaflets;
- The lesions are covered with powdery masses of conidia giving dark grey to olive green colour.



SYMPTOMS OF SALB

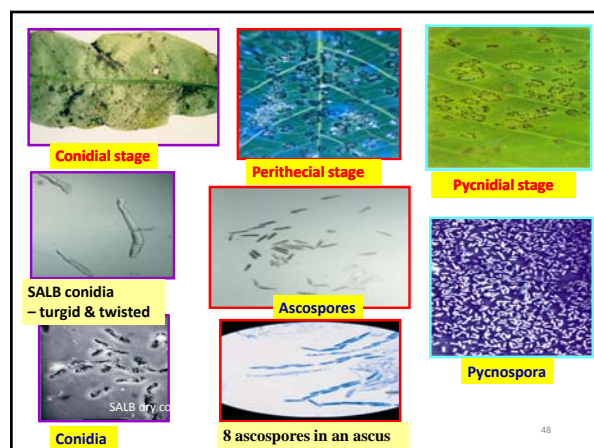
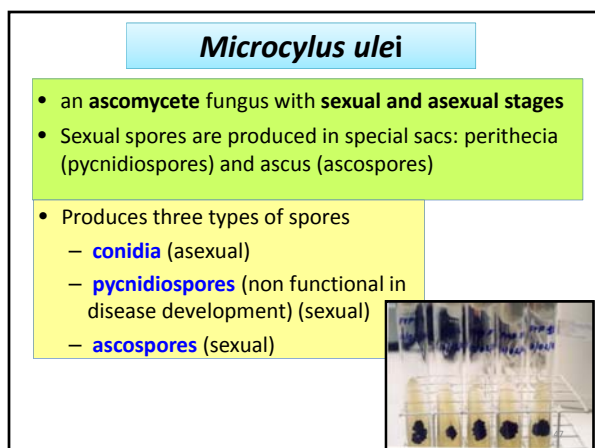
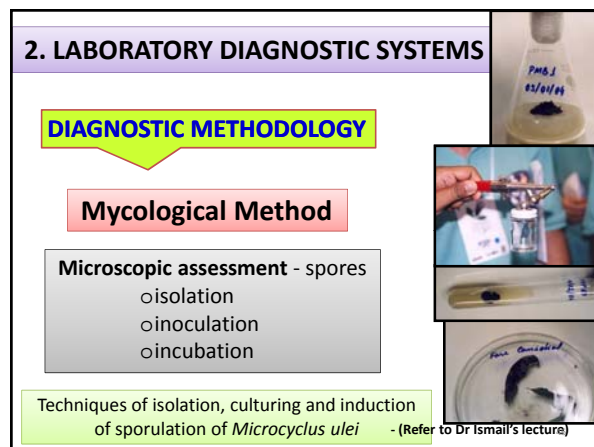
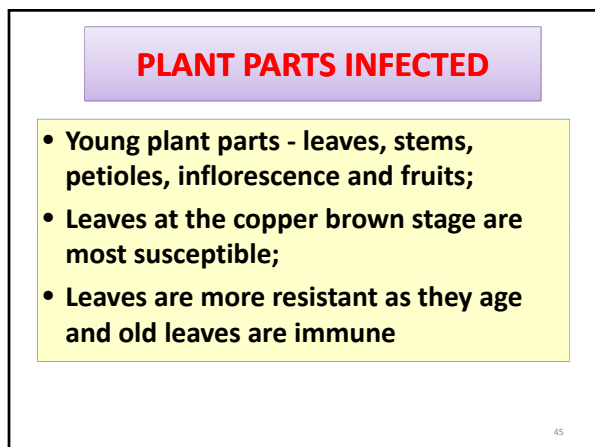
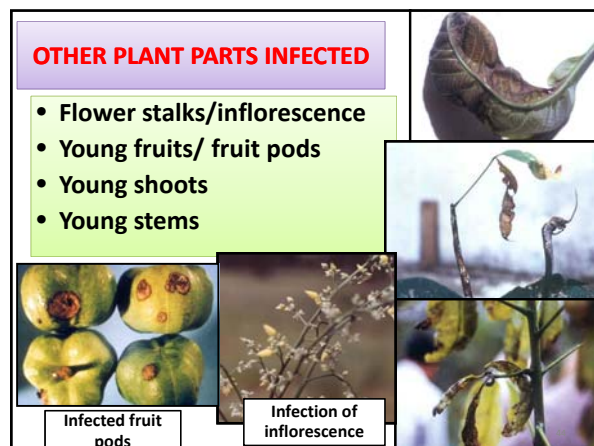
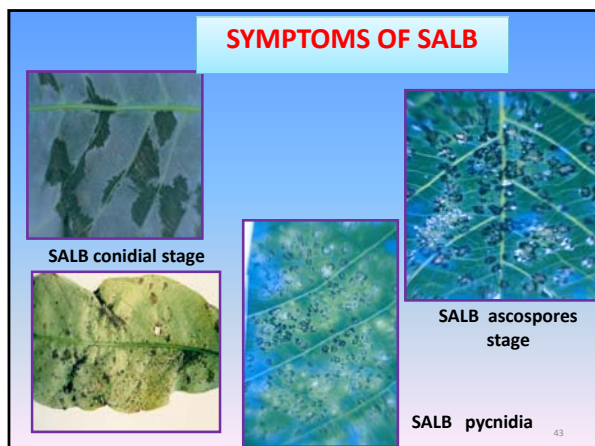


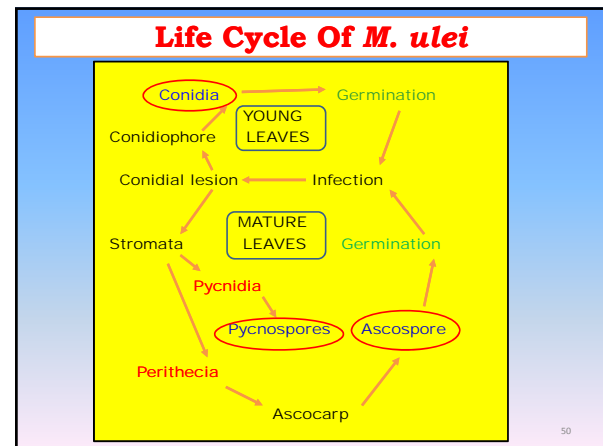
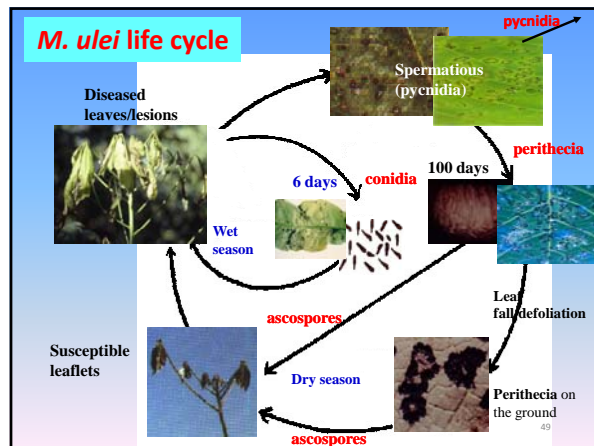
The young copper brown leaflets shrivel, curl, turn blackish and drop-off leaving the petioles for several days on the stems



Petioles remain on the stem for sometimes after the leaflets fall off

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THE PYCNIDIA

- small (120-160 μm diameter) dark bodies forming on the upper leaf surface along the periphery of lesions on the lower leaf surface
- produce the **pycnospores**

THE PERITHECIA

- At the site of the pycnidia large (200-400 μm diameter) raised black bodies (perithecia) developed;
- produced the **ascospores**

Conidial lesion

Conidia

- Either **septate (two cells) or aseptate (one cell)**. Percentage of aseptate conidia increased in drier periods and more common from laboratory cultures.
- Septate conidia are obclavate with broad proximal cell with truncate distal end.
- Possess a **characteristic twist**.
- **Hyaline when young** and turn **grayish with age**.
- Size of septate conidia vary – 23-65 x 5-10 μm (Chee); 23-62 x 5-10 μm (Holliday); 12-30 x 5-8 μm (Langford).
- **Size vary** with locations and season

Microcyclus ulei

Conidia

Shrunken conidia

Microcyclus ulei - pycnospores

- Dumb bell shaped.
- One end larger (12-25 μm) than the other end (2-5 μm).
- 6 -10 μm in length.
- Borne on hyphal elements.
- Germinate but do not cause infection.

Ascospores

- Septate with constriction at septum;
- Cells unequal in size;
- Various sizes reported – 12-20 x 2-5 μm (Chee); 10-15 x 3-5 μm (Holliday); 3-5 x 10-15 μm (Langford);
- Hyaline and ellipsoidal;



Diagnostic protocols & diagnostic aids

- information and guidance on diagnostic procedures that either singly or in combination lead to the identification of the pest
- Diagnostic procedures for quick, presumptive indications of identity
- Detection -method used for identification
- Sampling procedures critical for the detection methods and the diagnostic procedures- Any aspects of the sample that may impact on the diagnostic procedure must be included, for example age of the sample, chemical sprays applied to the crop prior to sampling, sample storage
- Guidance should be provided on resolving possible confusion with similar signs and symptoms due to other causes.

Diagnostic protocols & diagnostic aids

- To assist inspectors, diagnosticians
- Slides, photos on symptoms, conidia/spores - SALB and other rubber diseases



Diagnostic aids

Disease (Pathogen)

1. South American leaf blight (*Microcyclus ulei*)



The conidia of *M. ulei*.

- septate with two cells; the distal cell is narrower than the proximal cell.
- the characteristic twist of the conidia.

Diagnostic aids

1. South American leaf blight (*Microcyclus ulei*)

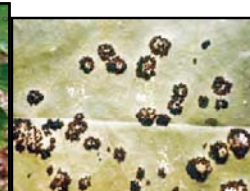


Symptoms:

The initial signs of SALB infection are the crinkling and distortion of the young leaflets (1). Later, dark irregular shaped lesions form on the lower surface of leaflets. The lesions are covered with dark colored spores (2).

Diagnostic aids

1. South American leaf blight (*Microcyclus ulei*)



The perithecia appearing as dark raised bodies occur on the upper leaf surface of mature leaflets.

Diagnostic aids

2. Colletotrichum leaf disease (*Colletotrichum gloeosporioides*)



The necrotic leaftips of leaflets infected by *C. gloeosporioides* and brown lesions are surrounded by yellow region. The lesions are raised and can be felt when touched.

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Diagnostic aids

3. Oidium leaf disease (*Oidium heveae*)



White powdery mycelia on upper surface of leaflet



4. Bird's eye spot (*Drechslera heveae*)



Spots with white centres surrounded by brown region

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Diagnostic aids

5. Corynespora leaf fall (*Corynespora cassiicola*)



The leaf spots (a) and herring bone symptoms (b) and the stick-like spore (c). The herring bone is the characteristic symptoms of the disease. On some leaves the symptoms may appear as spots.



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Diagnostic aids

6. Phytophthora abnormal leaf fall (*Phytophthora palmivora*)



Drops of latex on the darkened lesions on leaf petiole

7. Fusicoccum leaf blight (*Fusicoccum* sp.)



Large lesions with concentric rings

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3. Quarantine Measures

❑ Disinfection of planting materials

✓ surface sterilant (**sodium hypochlorite at 200ppm**) and a **specified systemic fungicide** or approved equivalent

✓ **UV irradiation, X-ray, disinfectant, detergent, formalin, heat**

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QUARANTINE MEASURES

Effect of UV Irradiation On Conidia Germination

Exposure time (min)	Germination (%)	
	6 h incubation	24 h incubation
5	5-10%	30%
10	1-3%	10-15%
15	0%	5-10%

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QUARANTINE MEASURES

Effect of UV Light And Other Treatments On Spore Survival

Treatment	Spore survival
UV irradiation	Some conidia (5-10%) germinated after 15 min however 45 – 60 min exposure totally killed conidia.
X-ray	Killed the conidia
Disinfectant, Detergent, Formalin	Killed the conidia

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QUARANTINE MEASURES

Effect Of Heat On Conidia Germination

Temperature °C	Germination %	
	Dry heat 30 min	Moist heat 30 min
50	-	3-5
55	-	0
70	30	-
75	-	-

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3. Quarantine Measures

- Disinfection or destruction of infected or non-compliant materials
- **Disposal of waste materials**- packaging, packing materials, debris, wastes derived from budwood (remainder of budwood after budding operation), damaged budwood, poor quality seeds, ungerminated seeds.
- Burned , autoclaved or soaked for at least 30 min in 10% formaldehyde/ sodium hypochlorite

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*Terima kasih &
Thank you*



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WHAT DO WE KNOW?

As we know,
There are **known knowns**.
There are things we know we know.
We also know
There are **known unknowns**.
That is to say
We know there are some things
We do not know.
But there are also **unknown unknowns**,
The ones we don't know
We don't know.

D.H. 'Rummy' Rumsfeld

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