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# REPORT

## WORKSHOP ON THE PREVENTION OF INTRODUCTION OF SOUTH AMERICAN LEAF BLIGHT (SALB) OF RUBBER



13 – 17 December 2010  
Crowne Plaza Mutiara, Kuala Lumpur, Malaysia

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**REPORT**

**1.0 INTRODUCTION**

**1.1 Background**

APPP agreement in principle emphasizes regional cooperation to control the entry of quarantine pests through trade with special focus on the control of South American Leaf Blight - SALB disease of rubber in South America. The 20<sup>th</sup> Meeting of APPPC in Chiang Mai, Thailand in 1997, had decided to review the APPP agreement based on the recent developments and in accordance with the acceptance of the new revised text of the agreement in International Plant Protection Convention (IPPC) of FAO in the 29<sup>th</sup> session in November 1997 and the WTO Agreement on Sanitary and Phytosanitary Measures.

As a result, the revised text was presented at the 21<sup>st</sup> APPPC Meeting in Bandung, Indonesia in 1999 and it was agreed that the revised agreement be divided into two sets. The first set of the agreement is related to all matters (except matters affecting the SALB), be adopted by member countries. The second set of agreement is related to the repeal of the provisions on measures to prevent SALB disease contained in Article 4 and Appendix B in the existing agreement.

Following the decisions, two series of workshops on Pest Risk Analysis (PRA) on SALB was held in Malaysia in 2003 in collaboration with FAO. A draft PRA on SALB was produced from these workshops. In addition, another workshop was organized to update the draft PRA on SALB, as a result of new information from findings by the Malaysian expert on SALB during his study visit to Brazil in 2005. The workshop was held in Malaysia in December 2006.

Pest Risk Analysis on SALB was adopted by the 25<sup>th</sup> session of the Asia Pacific Plant Protection Commission in Beijing, China in 2007. During the session, Malaysia, with assistance from Thailand, China and New Zealand were requested to draft the regional standard on SALB. Malaysia's National Plant Protection Organization – NPPO (DOA Malaysia) with assistance from internal experts had completed the draft standard in 2008 which was then circulated for country comment in the same year. The Guidelines for Protection against South American Leaf Blight of Hevea were then adopted at the 26<sup>th</sup> session in September 2009 in New Delhi, India.

During the 26<sup>th</sup> session of the APPPC, it was suggested that the Commission set up a working group on SALB to develop detailed guidelines to support the SALB Regional Standard. The working group would arrange for a workshop to develop harmonized plant import requirements, inspection procedures, diagnostics and disinfection of plants from SALB

endemic countries. The SALB Working Group would be led by Malaysia with participation of China, India, Indonesia, Philippines, Thailand and Vietnam.

A "Workshop on the Prevention of Introduction of South American Leaf Blight (SALB)" was held in Kuala Lumpur, Malaysia from 13-17 December 2010, to develop detailed guidelines to support the APPPC SALB standard.

## **1.2 Workshop Objectives, Programme and Activities**

The objectives of this workshop are as follows:

- to produce a draft of harmonized import requirements for the importation of rubber and other commodities from the South American Leaf Blight (SALB) endemic countries;
- to formulate phytosanitary measures based on scientific evidence to address the risks of introducing SALB into the region to be in line with SPS Agreement;
- to promote awareness and develop understanding and expertise among officers involved in making decisions on phytosanitary measures for importation of agricultural products;
- to provide opportunities for officers to exchange knowledge and expertise in the field of quarantine and SALB disease.

## **1.3 Participation**

A total of thirty nine (39) participants from ten countries namely from Australia (1), China (2), India (2), Indonesia (1), Malaysia (3), Sri Lanka (1), Philippines (2), Singapore (2), Thailand (3), Vietnam (2), FAO-RAP (1), APPPC Secretariat (1), Resource persons (3) and observers (15) attended the workshop. Several Brazil experts were invited and 3 experts were nominated by Brazil NPPO, who were supposed to provide inputs of their expertise including identification, diagnostic, surveillance and treatments of SALB. However at the last minute due to unforeseen administrative reason from Brazil they were not able to participate in the workshop. Instead a Brazil Embassy official from Malaysia attended the meeting to take note of the discussions. The list of participants appears as Annex 1.

## **1.4 Workshop Programme**

The Workshop Programme is as in Annex 2.

## **2.0 OPENING SESSION**

### **2.1 Opening Remarks**

Mr. Piao Yongfan, Secretary of APPPC thanked Malaysia for hosting this workshop. In his speech, Mr. Piao mentioned that the various guidelines developed by the workshop would provide an operational advice and recommendations on detailed aspects of phytosanitary measures for SALB. He hoped that the output would provide a follow up activities of APPPC for the prevention against SALB including development of RSPMs.

Mr. Piao also mentioned that this workshop was one of the prioritized work plans of APPPC, which was adopted by the 26<sup>th</sup> Session in 2009. SALB is a historical concern of this region and was a significant issue to be dealt with under the framework of IPPC, which provides various ISPMs for phytosanitary measures.

The outputs of the workshop were expected to contribute to the way-forward of the application of the PRA and implementation of the RSPM No. 7. It would be a milestone for the evolution of APPPC to facilitate potential endorsement of the 1999 amendment of the Asia and Pacific Plant Protection Agreement.

## **2.2 Inaugural Speech and Workshop Opening**

Y. Bhg. Dato' Sulaiman Md. Zain, the Deputy Director General of Agriculture, Malaysia on behalf of the Government of Malaysia extended his warmest welcome to the distinguished facilitators and participants to the workshop. Dato' Sulaiman mentioned that the current total production of natural rubber is about 9 million tonnes and over 90% of it is produced from this region, mainly in Thailand, Indonesia and Malaysia. Other major producing countries are India, China, Vietnam and Sri Lanka. Dato' Sulaiman stressed that currently in Malaysia, rubber producers be it smallholders or plantations are enjoying economic boom due to the high price of rubber. This had resulted in the revival of the industry which was once considered as sunset to the Malaysian economy. Dato' Sulaiman stressed that, while APPPC member countries have put some measure of protection against incursion of SALB, not all countries have the same level of capacity and prioritization – technically and financially, in implementing SPS measures in agricultural trade with high risk countries. Hence this workshop will serve as a platform to discuss guidelines on import requirements, so that all countries in this region could impose the required level of protection against SALB. In order to implement the newly developed phytosanitary measures effectively, all plant quarantine personnel in the region should have all the relevant skills and knowledge, while the entry points be well equipped with modern and sound facilities and equipments to detect the quarantine pest.

Y. Bhg. Dato' Sulaiman lastly wished all participants a stimulating and successful Workshop and pleasant stay in Malaysia for oversea participants. The full text of his speech appears in Annex 3.

## **3.0 SESSION 2: INTRODUCTION**

### **3.1 Purpose and Scope of the Workshop**

Ms Wan Normah Wan Ismail the chairperson of session 2 of the workshop briefed on the background, objectives and expected output of this workshop. The workshop was organized as a result of the last APPPC session held in New Delhi, India and other previous related workshops. The purposes of the workshop were:

- To develop guidelines for importation requirements;
- To harmonize guidelines between countries, but the implementation depends on each country situations and needs;
- To formulate phytosanitary measures for SALB;

- To develop awareness and capacity building for pathologists, plant quarantine officers and other stakeholders.

The expected outcomes were:

- Draft of Plant Quarantine Import requirements;
- Point of entry inspection and pre-clearance system;
- Laboratory diagnostics system;
- Surveillance system and eradication programme;
- Capacity-building – awareness, diagnostics and surveillance.

### **3.2 Overview of Quarantine Development Pertaining to SALB in APPPC Agreement**

An overview of Quarantine development pertaining to SALB in APPPC agreement was delivered by Mr. Piao Yongfan. Mr. Piao briefed the participants on the background of SALB issues and the development of agreements related to SALB of rubber which includes control measures, pre-condition of importation of SALB host and non-host materials and development of RSPM for SALB (RSPM No. 7). He also mentioned that a Workshop was held in Korea to discuss and develop a contingency plan for the management of SALB incursion. The report of the workshop will be used as a guideline or complement the activities of this workshop.

### **3.3 Presentation on APPPC Pest Risk Analysis for South American Leaf Blight**

Pest Risk Analysis for South American Leaf Blight was presented by Dr. Chee Kheng Hoy. He highlighted that PRA on SALB of rubber was drafted by him with assistance from a few PRA experts in New Zealand. Dr Chee explained the content of the PRA report and highlighted the following parts:

- The biology and pathways of the disease – very essentials to know on the dispersal of the spores and biology of the disease as guideline for implementation on exportation/importation of plant material, surveillance systems and eradication programme for the country;
- Risk Analysis table describing the level of risk associated with the pathways;
- The dispersal and pathways of infestation of SALB;
- Recommendations on treatment for planting materials imported from SALB endemic countries.

### **3.4 Presentation on RSPM No. 7 – Guidelines for Protection against South American Leaf Blight of Rubber**

Dr Ismail Hashim presented the RSPM No. 7 – Guidelines for Protection Against South American Leaf Blight of Rubber. The guideline was prepared to assist APPPC member countries to improve and develop their phytosanitary measures, including prevention, eradication and control against SALB. Dr. Ismail also mentioned that introduction of SALB into the rubber growing countries could be prevented by implementing phytosanitary import requirements, as stated in the RSPM No. 7 and PRA document including inspection system at point of entry, diagnostic system and surveillance system. APPPC countries were advised to consider developing capacity building for their personnel and upgrading their quarantine facilities such

as training of personnel as pathologist in SALB, establishment or upgrading of laboratory for diagnostic and quarantine purposes.

#### **4.0 SESSION 3: COUNTRY PRESENTATION**

Each country presented a short summary of current import requirement imposed on items from high risk countries, type of products imported, current phytosanitary measures imposed on planting materials of rubber from any country. The details of each country presentation are in Annex 4.

#### **5.0 BREAKOUT SESSION**

Introduction to the breakout session was chaired by Dr. Rob Schwartz. Four papers were presented i.e. Requirements to Reduce Risk by Dr. Ismail Hashim; Inspection System at Points of Entry to Ensure Compliance with Import Requirements by Dr. Rob Schwartz; Diagnostic System by Dr Ismail Hashim; Surveillance System and Eradication by Mr. Yusof Othman. In the breakout session participants were divided into 4 groups. The first group discussed on Import requirements, the second group on Points of Entry Inspection System, third group on Laboratory Diagnostic System and fourth group on Surveillance System and Eradication Programme. The workshop session was facilitated by Dr Chee Kheng Hoy, Dr. Rob Schwartz, Dr. Ismail Hashim and Mr. Yusof Othman. The report of the workshop group is consolidated into 4 chapters:

- (i) Import Requirements and Inspection System
- (ii) Diagnostic System
- (iii) Surveillance System and Eradication
- (iv) Capacity Building

#### **5.1 IMPORT REQUIREMENTS AND INSPECTION SYSTEM**

##### **5.1.1 Introduction**

The PRA document on South American Leaf Blight of Rubber (SALB) and RSPM 7, had listed a number of risk management options for viable host material and non-viable host material. Drawing from the experience of member countries and the science behind the risk analysis of the disease, the import requirements and inspection system for plants, planting materials and agriculture products from the SALB endemic countries was developed.

The import requirements and points of entry inspection system was developed for the following categories of materials:

- (i) Host Materials
- (ii) Non-Host Materials For Planting Purposes
- (iii) Passengers
- (iv) Non Host Materials (not for planting)

## 5.1.2 Host Material

### (i) Budded Stumps or Budwood

#### **Pre-export Inspection and Treatment**

(At the country of origin)

- Mother plants must be inspected by qualified plant pathologist for presence of SALB before harvesting of budwood and during the optimal period for disease expression.
- Recognizing that SALB is widespread in South America, it was agreed that propagating material must only be taken from mother trees which show no evidence or very low incidence of symptoms of SALB.
- Budwood should be grafted in a nursery registered by the NPPO of the country of origin.
- Nurseries should be located away from SALB area and should be monitored by the plant pathologist.
- Harvesting of budwood should only occur when the bark has been hardened and during low disease season.
- Budwood or budded stumps must be dipped in surface sterilant (sodium hypochlorite at 200ppm) and a specified systemic fungicide or approved equivalent and dusted with thiabendazole at 0.5% or equivalent.
- Supervision of the treatment by plant pathologist of the importing country, or if there is no pathologist from the importing country, certification from the NPPO is required.
- Propagating material must be free from soil.
- Packaged to limit re-infestation after treatment during export.
- Treatment and packaging should be done in an enclosed and secure facility to prevent from contamination.
- Clean packaging material should be used.
- NPPO should supervise packaging.
- Commodity should be shipped immediately (24 hours) after packaging.
- Based on the inspection by plant pathologist, NPPO will issue certificate that the harvested budwood does not show symptoms of SALB, and the mother trees have a low level of infection.

#### **Intermediate Post-Entry Quarantine**

- Intermediate Post-entry quarantine can be undertaken in another country (free from SALB) if the risk of PEQ in importing country is considered to be too high.
- Inspection of facility should be done by accredited plant pathologist from the NPPO.
- Budding is done on pre-planted rootstocks.
- Leaf disease is monitored by a qualified plant pathologist. If no SALB is observed during the 12-18 months observation period, budwood is harvested and treated as prescribed in Import Permit, packed and sent to the receiving country.
- Verification of documents: Planting materials should be consigned to Director of the NPPO.

- Dipping in surface sterilant and systemic fungicide.
- General inspection for signs of disease or regulated pests.
- In case of non-compliance, destroy the whole consignment or re-export at the importer's expense.
- Plants maybe released from facility under quarantine supervision after no signs of SALB for at least a year or until the seedling has produced new foliage at least 6 times.
- Re-export PC to be issued by the intermediate country.
- Importation of any stump is discouraged.

### **Post-Entry Quarantine**

#### **(At the country of import)**

- At Post-Entry Quarantine Station, budwood is to be grafted to healthy stock and grown in an insect-proof glasshouse.
- Prior to release from the glasshouse, all plants should be inspected by a qualified plant pathologist for sign of SALB infection.
- Plants maybe released only after having been inspected free from any sign of SALB for at least one year or after new foliage has been produced at least 6 times.
- Inspection of facility should be done by accredited plant pathologist from the NPPO (Work experience with SALB should be considered)
- It is recommended that the PEQ facility should be located at least 3kms away from host material, however this maybe varied by the importing country.

### **General requirements**

Imported budwood should be no longer than 1 meter and in the form of brown budwood. The quantity allowed to be imported is as follows:

- a) Number of genotype or clone depends on space availability of the contained glasshouse facility.
- b) Number of genotype or clone to be imported should be as small as possible to reduce risk of introduction of SALB.

A model work plan for importation of Budded Stump or Budwood proposed by Australia was accepted. The work plan is in Annex 5.

#### **(ii) Seeds, Flowers and Fruits**

- All importations of seeds, flowers and fruits must obtain an import permit prior to importation. All consignments without IP shall be destroyed.
- Imports of seeds other than by the Government will be allowed in limited quantities only to reduce risk.
- Specify quantity: Volume to be decided by importing country.



### 5.1.3 Non Host Material for Planting Purposes

- Import Permit and Phytosanitary Certificate required.
- Additional Declaration in PC that phytosanitary measures if any prescribed in Import Permit are carried out.
- Post Entry Quarantine.

### 5.1.4 Passengers

- Target passengers arriving from SALB countries within the last 21 days.
- Passenger Card/Declaration (greater cooperation with Customs official needed).
- Inspection of baggage of persons targeted.
- Destroy any contamination by *Hevea spp.* leaf material if detected.
- Plants and any parts of plants intercepted from passengers arriving from SALB endemic countries will be seized/treated.
- Detector dogs and/or X-ray machines could aid with screening.

### 5.1.5 Non-Host Materials (agriculture produce) e.g. Fruits, vegetables, cut flowers

- Import Permit and Phytosanitary Certificate as required by importing country.

### 5.1.6 Non- Host Material Not For Planting

- Inspection of shipments upon arrival.
- Cleaning of commodities (steam/washing): facilities should be available.
- Identify list of potentially contaminated non-viable host material.
- Arrange with Customs Authorities on how to address these consignments such as tagging consignments from SALB countries.

## 5.2 LABORATORY DIAGNOSTIC SYSTEMS

### 5.2.1 Introduction

An important component in the chains of activities to reduce the chance of introduction of SALB is to intercept the entry of the causal pathogen, *M. ulei* infecting host plant materials and also through contamination of other goods at the points of entry of these pathways. Upon detection of suspected host materials infected with *M. ulei* or contaminating other goods it is pertinent that the suspect causal pathogen be identified. 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. In addition, the laboratory should perform similar objectives for other pests and diseases of other crops. The laboratory should follow standard procedures for diagnosing pathogens.

### 5.2.2 Location

In most countries, importation of goods is through several entry points either airports, seaports or through land borders. It is noted that at all these entry points there exists quarantine facilities of variable sizes in tandem with the volume of imports and trading

activities handled by these points of entry. In addition only few of the major points of entry are involved with international trade especially with SALB endemic countries. Bearing in mind the existence of various entry points in most member countries, it is suggested that each member country should have a Central Diagnostic Laboratory and Satellite Quarantine Laboratories.

**(i) Central Diagnostic Laboratory**

The Central Diagnostic Laboratory is to be sited at or nearby the major entry points. Where possible, it should be located far from rubber growing areas, preferably near the capital city. The Central Diagnostic Laboratory should be well equipped to carry out the various functions stipulated. This laboratory should also have sufficient well trained personnel.

**(ii) Satellite Quarantine Laboratories**

Each country should have Satellite Quarantine Laboratory (SQL) and should be located near or at every or selected entry point involved in importation of goods from high risk country. The number and locations of Satellite Quarantine Laboratory is the prerogative of each country to decide. Nevertheless, it is recommended that SQL be located at entry points involved in importation of goods from SALB endemic countries. While the Central Diagnostic Laboratory is well equipped and staffed with large number of trained personnel, the Satellite Laboratory is equipped with basic equipment only and staffed with smaller number of personnel.

### **5.2.3 Functions**

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

**(i) Central Diagnostic Laboratory**

- Diagnostic and Confirmation;
- Treatment;
- Disposal;
- Reporting;
- Major databases;
- National, regional and international linkages.

**(ii) Satellite Quarantine Laboratory**

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

### **5.2.4 Requirements**

The following are the requirements of Central Laboratory and Satellite Laboratory. Generally the Central Laboratory will have more equipment compared to the Satellite Laboratory.

### **Central Diagnostic Laboratory**

- Infrastructure and utilities;
- Equipment and Apparatus;
- Chemicals and consumables;
- Information and storage facilities;
- Human Resource - Qualified plant pathologist with good knowledge of SALB, and trained supporting staff;
- Funds for operating expenditure.

### **Satellite Quarantine Laboratory**

- Basic infrastructure and utilities;
- Basic equipment and Apparatus;
- Basic chemicals and consumables;
- Basic information and storage facilities;
- Human Resource – Qualified quarantine officer and trained supporting personnel;
- Funds for operating expenditure.

### **Infrastructure**

- General laboratory (isolation of pathogens);
- Sterilization rooms;
- Incubation rooms;
- Microscope rooms;
- Information and storage room;
- Glasshouse/humidity and temperature control room/chamber;
- Disinfection and treatment rooms;
- Disposal room;
- Internet facilities/infrastructure.

### **Equipment and Apparatus**

The details of equipment and apparatus for the following purposes are listed in Annex 6.

- General pathology equipment
- Disease treatment and disposing equipment
- Chemicals and consumables
- Containers for sampling & transportation of samples
- Information and storage facilities

## **5.2.5 Diagnostic Methodology**

### **Visual Assessment:**

#### ***Symptoms***

The first visible symptoms are the distortion of leaves. These 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).

SALB also infect other parts of plants such as the young stems, young fruits, young shoots petioles and flower stalk.

### **The spores**

*Microcyclus ulei* is an ascomycete with sexual and asexual stages. Sexual spores are produced in special sacs: perithecia (pycnidiospores) and ascus (ascospores). *M. ulei* produces three types of spores: The asexual spores are the conidia and the sexual spores are the pycnidiospores (non functional in disease development) and the ascospores.

The conidia are mostly septate (two cells) with small proportion 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 and are characteristically twisted. They are hyaline when young and turn grayish with age. The size of septate conidia varies from 23-65 x 5-10 µm (Chee); 23-62 x 5-10 µm (Holliday); 12-30 x 5-8 µm (Langford). The size varies with locations and season.

The pycnidia are small (120-160 µm diameters) dark bodies forming on the upper leaf surface along the periphery of lesions on the lower leaf surface. The pycnidia produce the pycnospores. The pycnospores are dumb bell shaped, one end larger (12-25 µm) than the other end (2-5 µm). They are 6 -10 µm in length and are borne on hyphal elements.

At the site of the pycnidia, large (200-400 µm diameter) raised black bodies (perithecia) developed. The perithecia produced the ascospores that are septate with constriction at the septum. The cells are unequal in size and various sizes were reported (12-20 x 2-5 µm (Chee); 10-15 x 3-5 µm (Holliday); 3-5 x 10-15 µm (Langford)).

Other plant parts infected.

Disease symptoms may occur on young stems, leaf petioles and also on inflorescence.

### **Mycological Method (Microscopic assessment, isolation, inoculation, incubation)**

For Hevea and inanimate products from SALB countries – Refer to Annex 7.

### **Molecular and Serological Detection Technique (e.g. PCR, ELISA)**

For detection of large number of samples e.g. in status or detection of SALB in the country.

#### **5.2.6 Recommendations**

In order to increase the effectiveness of the diagnostic laboratory, the following suggestions are made:

- Need an easy, quick and reliable method of identification of SALB that are “friendly” to front line officers.
- Develop rapid test kits.
- Explore other potential procedures or techniques.

- Establish a Reference Laboratory for consultation and providing technical assistance especially to countries lacking in laboratory facilities and expertise. The Reference Laboratory may also provide manpower training.
- Financial assistance is required for capacity building and also for setting up laboratories in some countries.

### **5.3 SURVEILLANCE SYSTEM AND ERADICATION PROGRAMME**

#### **5.3.1 Introduction**

Early detection of incursion of SALB in any of the APPPC member countries will ensure that the disease will not be established and if incursion occurred, early detection will reduce the cost of eradication. Hence a sound surveillance system is crucial to enable early detection. Meanwhile eradication programme is the most sensible option in controlling limited incursion of invasive exotic plant pest. Several eradication programmes have been successful in controlling pest problem and stop further crop loss. Since SALB is one of the most destructive diseases of rubber, eradication needs to be carried out as early as possible.

#### **5.3.2 Surveillance System**

Purpose of surveillance system:

- Early detection of incursion or quarantine breach for possible introduction of SALB to enable effective response and implementation of eradication programme.

##### **5.3.2.1 Organization**

- (i) Need to establish a coordinating agency/authority for implementing the SALB detection surveillance – the choice of agency/authority will depend on administrative structure of each member country.
- (ii) To establish a competent surveillance team. The surveillance team could be led by any of the agency/authority e.g. NPPO, Rubber Research and Development Authority or other Research Institutions depending on the country's organization and administrative structure.
- (iii) Ensure available competent personnel in the surveillance team especially core groups that can verify suspected incursion of SALB.
- (iv) Organize training for surveillance team members such as extension workers, nursery owners, small holders, estate owners and the public.
- (v) Seek adequate funds to conduct the surveillance programme yearly.
- (vi) Prepare comprehensive work plan for surveillance programme.
- (vii) Collate basic information of the rubber industry e.g.: acreage, distribution, GIS, growth phenology etc.
- (viii) Conduct air passengers profile arriving from SALB infested country e.g.: their destination, peak arrival time etc.
- (ix) Collate and analyze surveillance report for the country.

### 5.3.2.2 Survey Work Plan

- (i) Classify rubber planting areas into high risk and low risk areas based on the probability of the SALB spore introduction and establishment.
- (ii) The high risk rubber holding areas are plantations within 5 km radius surrounding the international airport and 200 meters along the route and destination of air passengers from SALB infested countries which practice minimum plant quarantine mitigation at the entry point.
- (iii) Other high risk areas are plantations within 5 km radius surrounding PEQ station where spores or other rubber diseases are detected in the imported rubber consignment from SALB infested countries.
- (iv) Low risk areas are rubber plantations near seaports where non-hosts products and tools imported from SALB infested countries arrived more than 21 days after departure.
- (v) Involvement of all stakeholders especially the owner of rubber plantations, agriculture extension workers and the public in providing detection data within their own plantation or locality. Information channel should be provided for these stakeholders to send their reports.
- (vi) To spell out the responsibilities of the surveillance team especially their coverage, intensity and frequency of reporting to the coordinating agency/authority.

### 5.3.2.3 Detection Survey

- (i) Specific detection survey for high risk areas
  - Nurseries and immature plants (< 5years)- survey should be conducted every month
  - Matured plantations - survey should be conducted every 2 months or periodically especially during the onset of disease incidence where probability of SALB establishment is high during the leaf flushing
- (ii) General disease detection survey for low risk areas
  - Survey should be conducted at least once in 2 years.
- (iii) The number of plants inspected in the high risk area should fulfil the 95% confidence limit. The number of samples can be based on the number of rubber holdings and acreage.
- (iv) Samples of diseased part from suspected SALB infested plants should be collected following the sampling procedure and sanitation standard to avoid spread to other areas. Samples should be sent to competent laboratory for identification.

### 5.3.2.4 Requirements

Competent surveillance personnel and survey planners:

- (i) Awareness campaign for rubber growers and the public to carry out visual inspection of rubber plants in their holding or locality.
- (ii) Training to agriculture extension workers on surveillance technique.
- (iii) Spore traps.

- (iv) Survey equipments/tools e.g. transportation, specimen collection tools, binoculars, GPS.
- (v) Communication and information dissemination through mass media, SMS.
- (vi) Baseline information for early detection e.g. distribution of rubber plantation, acreage, mapping, GIS etc.

#### **5.3.2.5 Reporting**

- (i) To be submitted to the authority – for decision making.
- (ii) Compiling surveillance report (analysis/recommendations) for measures to be taken.
- (iii) Provide information to the APPPC secretariat for members' reference.

### **5.3.3 Eradication Programme**

#### **5.3.3.1 Legislation**

APPPC member countries should ensure their laws/regulations contain provisions on:

- (i) Powers to enter holdings.
- (ii) Authorized officers to execute eradication procedures.
- (iii) Powers to collect specimens from infested plants.
- (iv) Powers to direct owners to implement eradication measures.

#### **5.3.3.2 Organization**

- (i) Assign agency/authority to manage eradication programme.
- (ii) Prepare response plan based on established SALB contingency plan.
- (iii) Consult stakeholders involved in the programme e.g. local government, Plant Quarantine stations, rubber owners/growers, the public.
- (iv) Establish agreements with the local government or rubber producer's association for sharing of fund and personnel in eradicating programme.
- (v) Document all activities e.g. record of progress, status of pest incidence and GIS information systems etc.

#### **5.3.3.3 Determination of Treatment Area/Zoning**

- (i) Conduct delimiting survey from the identified infested plants. Every single plant within 100m radius will be surveyed and specimens from suspected SALB infested plants will be collected for laboratory confirmation. The delimiting areas will be expanded if new SALB infested plant is detected within a 100m zone with additional 100m radius from the fringe of the infested zone.
- (ii) The size and shape of each zoning area is determined by a number of factors e.g. location of the incursions, surrounding host and non-host plants, topography etc.
- (iii) The zoning can be divided into:

- Infested zone - within 100m of the infected area as indicated by the distance of spores dispersal carried by wind
- Quarantine zone - 200m radius surrounding the infested zone
- Buffer zone - 3km radius surrounding the quarantine zone
- Restricted zone – the whole planting block of the infested areas and isolated from other rubber growing block by a minimum of 5 km

#### **5.3.3.4 Eradication measures**

##### **Infested zone**

- (i) All rubber plants in the infested zone will be culled and disposed by burning;
- (ii) Movement of machinery, rubber products, non rubber products, implements and tools to the other zones is prohibited;
- (iii) Personnel entering the infested zone have to be sanitized before leaving the area;
- (iv) Non-sanitized items or peripherals should be disposed by burning.

##### **Quarantine zone**

- (i) All rubber plants in this zone will be sprayed with recommended fungicide starting from the fringe towards the center of the zone at least twice a month;
- (ii) Movement of machinery, rubber products, non rubber products, implements and tools to the buffer and restricted zone is prohibited;
- (iii) Personnel entering the quarantine zone have to be sanitized before leaving the area;
- (iv) Non-sanitized items or peripherals should be disposed by burning.

##### **Buffer Zone**

- (i) All rubber plants in this zone will be aerial sprayed with recommended fungicide. (Seek approval from all relevant authorities before carrying out aerial spraying activities);
- (ii) Movement of machinery, rubber products, non rubber products, implement and tools to the restricted zone is prohibited;
- (iii) Personnel entering the infested zone have to be sanitized before leaving the area;
- (iv) Non-sanitized items and peripherals should be disposed by burning.

##### **Restricted zone**

- (i) No movement of machinery, rubber products, non rubber products, implement and tools to the others rubber growing blocks;
- (ii) Strategic check point should be setup within this zone to ensure compliance to the above measures.



### 5.3.3.5 Evaluation of Eradication Programme

- (i) Conduct spore trapping in the infested area weekly for a year;
- (ii) Suspected infested specimen should be sampled from the quarantine zone every 3 months for laboratory analysis;
- (iii) Suspected infested specimen should be sampled from the buffer zone every 6 months for laboratory analysis.

Visual inspection of rubber plants in the quarantine zone and the number of plants inspected should fulfil the 95% confidence limit.

## 5.4 CAPACITY BUILDING

### 5.4.1 Introduction

Capacity building is very crucial to ensure sustainability of expertise in the SALB disease detection as well as to develop awareness among stakeholders on the risk, identification and management of the disease. The capacity building programme has been prioritized as follows:

- a) National awareness programme for stake holders e.g. policy makers, extension officers, rubber growers, plant quarantine personnel, embassies and travelers to SALB endemic countries.
  - Training seminars and workshops for identification and treatment of SALB including updates on new and relevant SALB information.
- b) Publication of Reference Materials on disease identification.
  - Reference materials for inspectors and other stake holders to be able to identify symptoms of SALB and plant products e.g. *Hevea* plant materials such as seeds, flowers, foliage, leaf pieces, budwood and budded stumps.
  - In the form of leaflets, laminated pictures, posters, preserved specimens (e.g. inert specimens of leaf symptoms) and other references from rubber institutes, industries or other sources.
- c) Regional pilot training.
  - SALB working group to compile and prepare draft materials for publication.
  - Organize pilot training workshop
- d) Building capacity for pathologists
  - Short courses for disease identification in Brazil.
  - Attachment with rubber research organizations in Brazil e.g. Michelin.
  - Collaboration with IRRDB on capacity building activities.
  - Setting-up linkages between experts at national, regional or international level.
  - Periodic hands-on training on SALB and other rubber diseases, operational laboratory techniques, molecular techniques, diagnostic procedures, treatments and disposal protocol, specimen collection and sending of specimens, sampling procedures, data base and laboratory information management.

- e) Development of national work plan for SALB Surveillance System.
- f) Development of national work plan for SALB Eradication Programme based on the APPPC SALB Contingency Plan.
  - Mock exercise (simulation) of SALB eradication activities.

## **6.0 RECOMMENDATIONS**

Based on the workshop discussions and output, recommendations were made as a way forward for future activities on prevention of SALB into the region.

- a) Malaysia to continue to lead the Working Group on SALB to prepare a draft frame programme for the publication of reference materials including:
  - (i) Collect and edit reference materials for publication
  - (ii) Organize pilot training and finalise reference materials for publication
- b) Each country to develop national work plan for:
  - (i) Surveillance system and eradication programme
  - (ii) Training programme
  - (iii) Awareness programme
- c) To report the output of the workshop to the next session of the APPPC
- d) To recommend Model Work Plan (proposed by Australia) for the Importation of Budded Stumps and Budwood to be adopted by APPPC as Annex to RSPM No.7
- e) To propose the Working Group on SALB to prioritize regional activities on SALB for submission to the next APPPC session for inclusion in the work plan for 2012 – 13 (e.g. development of a diagnostic protocol for SALB).

## **7.0 CLOSING**

The workshop was officially closed by Dato' Sabtu Slamet, Deputy Director General of Agriculture (Development) Malaysia. In his closing speech he hoped that the guidelines and recommendations by the workshop will be used by participants to improve their phytosanitary measures in preventing the introduction of SALB into their countries. All participants received their certificate of attendance from the Deputy Director General.

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**WORKSHOP ON THE PREVENTION OF INTRODUCTION OF  
SOUTH AMERICAN LEAF BLIGHT (SALB) OF RUBBER  
13<sup>TH</sup> – 17<sup>TH</sup> OF DECEMBER 2010  
CROWNE PLAZA MUTIARA, KUALA LUMPUR, MALAYSIA**

**WORKSHOP PROGRAMME**

Day 1		
<b>Monday, 13 December 2010</b>	<b>Session 1: Opening Ceremony</b>	
	<b>Time</b>	
	<b>Event</b>	
	8.30 – 9.00	Registration
	9.00	Arrival of Director General, Department of Agriculture, Malaysia
	9.15 – 9.30	<ul style="list-style-type: none"> <li>• Welcome Remarks by Secretary, APPPC</li> </ul>
	9.30 – 9.45	<ul style="list-style-type: none"> <li>• Opening Remarks by Director General, Department of Agriculture, Malaysia</li> </ul>
	10.00	Group Photograph Session
	10.15 – 10.45	Coffee Break
	<b>Session 2: Introduction</b> <b>Chairperson: Ms. Wan Normah</b>	
	11.00 – 11.15	Introduction of Participants
	11.15 – 11.30	Purpose and Scope of Workshop (workshop objectives and expected output)
	11.30 – 12.00	Overview on quarantine development pertaining to SALB in APPPC agreement – <i>Dr. Piao Yongfan</i>
	12.00 – 12.30	APPPC Pest Risk Analysis for South American Leaf Blight – <i>Dr. Chee Kheng Hoy</i>
12.30 – 14.00	Lunch	
14.00 – 14.30	RSPM No. 7 – Guidelines for Protection against South American Leaf Blight of rubber – <i>Dr. Ismail Hashim</i>	

<b>Session 3: Country Presentation</b> <b>Chairperson: Ms. Wan Normah</b>	
14.30 – 15.00	Update on phytosanitary measures in place in Brazil for agriculture produce exported to rubber growing countries in the APPPC region – Brazil Participant  Information on Brazilian systems for surveillance, diagnostics and pest control – Brazil Participant
15.00 – 17.00	Present import requirements of each country (Short summary of current import requirement imposed on items from high risk countries, type of products imported from SALB countries, current phytosanitary measures imposed on planting materials of rubber from any country) <b>-China</b> <b>-Indonesia</b> <b>-India</b> <b>-Philippines</b> <b>-Singapore</b> <b>-Sri Lanka</b> <b>-Thailand</b> <b>-Vietnam</b> <b>-Malaysia</b>
17.00 – 17.30	Tea Break
19.30 – 21.30	Official Dinner

## Day 2

<b>Tuesday, 14 December 2010</b>	<b>Session 4: Prevention of the Introduction of SALB into the Region – Breakout Session</b> <b>Chairperson: Dr. Rob Schwartz/Dr. Piao Yongfan</b>	
	<b>Time</b>	<b>Event</b>
	8.30 – 10.30	Requirements to reduce risk – <i>Dr. Chee Kheng Hoy/ Dr. Ismail Hashim</i> Inspection system at points of entry to ensure compliance with import requirements – <i>Dr. Rob Schwartz</i> Diagnostic System – <i>Dr. Ismail Hashim</i> Surveillance System and Eradication – <i>Mr Yusof Othman</i> Capacity Building – <i>Mr. Masahiro Sai</i>
	10.30 – 11.00	Coffee Break
	11.00 – 12.30	Breakout Group Discussions <b>Group 1 – Import Requirements</b> Facilitator – <i>Dr. Chee Kheng Hoy</i> <ul style="list-style-type: none"> <li>▪ Host material</li> <li>▪ Non Host material (SALB endemic countries)</li> <li>▪ Passenger</li> <li>▪ Other Items</li> </ul> <b>Group 2 – Points of Entry Inspection System</b> Facilitator – <i>Dr. Rob Schwartz</i> <b>Group 3 – Laboratory Diagnostic System</b> Facilitator – <i>Dr. Ismail Hashim</i> <b>Group 4 – Surveillance System and Eradication Program</b> Facilitator – <i>Mr. Yusof Othman</i> <b>Group 5 – Capacity Building and Technical Requirement</b> Facilitator – <i>Mr. Masahiro Sai</i> (Background information will be provided by resource persons)
	12.30 – 14.00	Lunch
	14.00 – 17.00	Continuation of Group Discussions
	17.00 – 17.30	Tea Break

Day 3		
Wednesday, 15 December 2010	<b>Session 5: Group Presentation and Panel Discussion</b> <b>Chairperson – Dr. Rob Schwartz (assisted by Dr. Chee, Dr. Ismail, Ms. Wan Normah and Mr. Masahiro Sai)</b>	
	Time	Event
	9.00 – 10.00	Group 1 Import Requirements
	10.00 – 10.30	Coffee Break
	10.30 – 11.30 11.30 – 12.30	Group 2 Points of Entry Inspection System Group 3 Laboratory Diagnostic System
	12.30 – 14.00	Lunch
	14.00 – 15.00 15.00 – 16.00 16.00 – 17.00	Group 4 Surveillance System and Eradication Program Group 5 Capacity Building and Technical Requirement Preparation of Final Guidelines
	17.00 – 17.30	Tea Break

Day 4		
Thursday, 16 December 2010	<b>Field Trip</b>	
	Time	Event
	8.00 – 11.30	Visit to Rubber Research Institute Malaysia
	12.45 – 14.00	Lunch
	14.30 – 17.00	Continue Preparing Final Guidelines

Day 5		
Friday, 17 December 2010	<b>Session 6: Wrap Up</b> <b>Chairperson: Ms. Wan Normah and Dr. Rob Schwartz</b>	
	Time	Event
	8.30 – 10.30	Presentation of Final Guidelines by each Group
	10.30– 10.45	Coffee Break
	10.45 – 11.15	Wrap Up by Chairperson
	<b>Session 7: Closing</b>	
	11.15 – 12.00	Presentation of Certificates and Official Closing
	12.00 – 13.00	Lunch



**OFFICIAL SPEECH BY  
THE DEPUTY DIRECTOR GENERAL (OPERATION),  
DEPARTMENT OF AGRICULTURE, MALAYSIA**

**IN CONJUNCTION WITH**

**THE OPENING OF THE WORKSHOP ON  
“THE PREVENTION OF INTRODUCTION OF SOUTH AMERICAN  
LEAF BLIGHT (SALB) OF RUBBER”**

**CROWNE PLAZA MUTIARA, KUALA LUMPUR,  
13 DEC 2010**

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Bismillahir Rahmanir Rahim  
Assalamualaikum Warahmatullahi Wabarokatuh  
and a Very Good Morning

Dr. Piao Yongfan, Secretary for the Asia Pacific Plant Protection Commission,  
Ms Wan Normah Wan Ismail, Director, Crop Protection and Plant Quarantine Division,  
Department of Agriculture, Malaysia,  
Distinguished speakers, guests, participants

**Ladies and Gentleman,**

Before I proceed, I would like to convey deepest regrets from the Director General of Agriculture for not being able to be with us today because he had to fly overseas yesterday to attend a very important meeting. Therefore, I will read the opening text on his behalf.

It is indeed a great honour for me to be invited to officiate the opening of this Workshop on the Prevention of Introduction of South American Leaf Blight (SALB) of Rubber. I would like to take this opportunity to welcome all of you, especially those from abroad – ‘Selamat Datang’ to Malaysia.

On behalf of the Government of Malaysia, I wish to express our gratitude to the Asia and Pacific Plant Protection Commission (APPPC) for choosing the Department of Agriculture Malaysia as the host for this Workshop. As far as I can remember, Malaysia had hosted a series of workshops dealing with SALB since 2003. This clearly demonstrates our strong commitment to resolve the issue of SALB in international trade, whether within the region or outside.

I am very pleased to note the presence of many senior crop protections and plant quarantine officers from relevant organizations participating in this Workshop. I was informed that participants are from 11 countries, namely Australia, Brazil, China, India, Indonesia, Philippines, Singapore, Sri Lanka, Thailand, Vietnam and Malaysia. This reflects the full commitment of

member countries in their endeavour to ensure the security of the rubber industry, which is without a doubt, one of the most valuable assets for this region.

**Ladies and Gentleman,**

To share some statistics, current total production of natural rubber is about 9 million tonnes and over 90% of it are produced from this region, mainly in Thailand, Indonesia and Malaysia. Other major producing countries are India, China, Vietnam and Sri Lanka. Currently rubber producers be it smallholders or plantations are enjoying economic boom due to the high price of rubber. This has resulted in the revival of the industry which was once considered as sunset to the Malaysian economy and may be to some other rubber producing countries. At present there are about 1.2 million hectares of rubber planted in Malaysia with over 410,000 households dependent on the crop for their livelihood and I am sure the number of households involved in the industry in countries such as Thailand and Indonesia are much more. Therefore, it is imperative that sound measures be in place to protect this region from the incursion of SALB, a very serious quarantine disease for this socio-economically important crop. The entry of this disease into the region will have devastating effect on our rubber industry.

Let me share with you Malaysia's bitter experience on the incursion of a quarantine pest on cocoa some 20 years ago. In the 1980's cocoa was Malaysia's third most important crop and we were one of the world's leading producers. The unintended introduction of cocoa pod borer had virtually destroyed the entire cocoa industry in Peninsula Malaysia in 1987. Despite the government's efforts by spending millions of Ringgit to help the smallholders to control and eradicate this pest, cocoa industry in Malaysia had never recovered and we are now a net importer of cocoa products. This signifies the important role of plant quarantine and biosecurity in order to prevent the entry of any exotic pest by regulating the import of plants or any article which poses high risk to the country.

**Ladies and Gentleman,**

The world is facing many issues and challenges as a result of globalization. In the light of the pressure emanating from increasing trade liberalization, trans boundary movement and international obligations, the risk of pest incursion into a region or country will definitely increase.

As you are all aware, the Sanitary and Phytosanitary (SPS) Agreement under the World Trade Organizations (WTO) has allowed each of its members to implement measures that would allow an appropriate level of protection for human, animal and plants against quarantine pests and diseases. Although this WTO-SPS Agreement has given rights for nations to build 'trade barrier' by formulating an appropriate trade policy in Plant Biosecurity, any barrier must be scientifically justified. It is no longer acceptable for a country to ban importation from any country which harbours quarantine pests. Guidelines had been developed in the form of standards both at the regional (RSPM) or international levels (ISPM) for countries to use in order to put in place biosecurity policy and impose phytosanitary measures for safe trade.

In the case of rubber, APPPC member countries had been united until now in protecting the region from the incursion of SALB, although some member countries are not rubber producers. Following the events in the last few years to respond to the proposed deletion of Article IV and Appendix B of the Asia Pacific Plant Protection Agreement dealing with SALB, which was

deemed to go against the WTO Agreement, hopefully this workshop will be the final stage towards compliance to the Agreement.

While APPPC member countries has put some measure of protection against incursion of SALB, It is noted that not all countries has the same level of capacity and prioritization – technically and financially, in implementing SPS measures in agricultural trade with risk countries. Hence this workshop will serve as a platform to discuss guidelines on import requirements, so that all countries in this region could impose the required level of protection against SALB. In addition, it is imperative that inspection, surveillance and diagnostic systems be developed and strengthened, while capacity building programmes identified and planned to further assist countries' efforts to safeguard against the incursion of SALB into this region.

In order to implement the newly developed phytosanitary measures effectively, all plant quarantine personnel in the region should have all the relevant skills and knowledge, while the entry points should be well equipped with modern and sound facilities and equipments to detect the quarantine pest. I hope this workshop will discuss and come up with a comprehensive guideline on import requirements, surveillance system, diagnostic system, inspection as well as capacity building to secure this region from the threat of SALB, while facilitating smooth trade within the region and with potential trade partners from risk areas.

**Ladies and Gentleman,**

To the APPPC or any other donor country, Malaysia would like to offer herself, with your assistance, to lead further projects for enhancing/strengthening capacity in order to prevent the introduction of SALB or any quarantine pest to this region.

At this juncture, I would like to congratulate the Asia and Pacific Plant Protection Commission, the FAO office of the Asia and Pacific Region, Bangkok and the Department of Agriculture Malaysia for organizing this important workshop. I would also like to thank APPPC for the financial support and the Organizing Committee for the hard work they have put in to ensure the success of this workshop.

Lastly, I wish you all a stimulating and successful Workshop and for those who have come from abroad, I hope you will have a pleasant stay in Malaysia. Kuala Lumpur is known as the 'Garden City of Lights' with two iconic monuments, the Kuala Lumpur Tower and the Petronas Twin Towers gracing its skyline. Do take some time off to explore Kuala Lumpur. I am sure the secretariat will be able to provide some guide to visit these places and other places of interest. Additionally, don't forget to sample the rich array of local delicacies representing the various ethnic groups making Malaysia, Truly Asia.

Once again, it is my sincere hope that this Workshop here in Kuala Lumpur will be a successful and fruitful event. On that note and with the recitation of Bismillahir Rahmanir Rahim, it is now my privilege and pleasure to officially declare open this Workshop on The Prevention of Introduction of South American Leaf Blight (SALB) of Rubber"

**THANK YOU**

## COUNTRY PRESENTATION

### 3.1 Indonesia

3.1.1 Any importation of plant propagating materials of Hevea from the countries where SALB is known to be present is prohibited.

3.1.2 Any importation of plant propagating materials of Hevea from the countries where SALB is known to be present is prohibited. Importation:

- Can only be done in the form of seed for breeding purposes by a government research institute;
- From production area or place free from SALB;
- Authorized under import permits issued by Minister of Agriculture;
- Treated in the country of origin or upon arrival in the territory of the Republic of Indonesia;
- Has been freed from soil at the country of origin;
- Accompanied by a Phytosanitary Certificate from the country of origin or transit country;
- Through the designated place of entry;
- Reported and submitted to the plant quarantine officials;
- Upon arrival shall undergo post entry quarantine.

3.1.3 Treatment of seeds or plant seedlings.

- Irradiation / radiation using ultraviolet light (253-257) nm for 60 minutes, with a specification for 40 Watt UV lamp with a volume of 1.5 m<sup>3</sup>, or
- Treatment with steam heat at a temperature of 56 ° C for 30 minutes, or
- Treatment with hot air at a temperature of 75 ° C for 30 minutes, or
- Soaking in a solution of sodium hypochlorite disinfectant at a concentration of 200 ppm or more, followed by drying.

3.1.4 Fresh Plant Products

- Irradiation / radiation using ultraviolet light (253-257) nm for 60 minutes, with a specification for a 40 Watt UV lamp with a volume of 1.5 M<sup>3</sup>, or
- Vapor heat treatment at 56° c for 30 minutes, or
- Hot air Treatment at 75 ° C for 30 minutes, or
- Disinfectant Soaking in a solution of 200 ppm (sodium hypochlorite), followed by drying.

3.1.5 Dried Plant Products

- Irradiation / radiation using ultraviolet light (253-257) nm for 60 minutes, with specifications for 40 Watt UV lamp with a volume of 1.5 M<sup>3</sup>, or
- Vapor heat treatment at 56° c or more for 30 minutes, or

- Hot air Treatment at 75 ° C or more for 30 minutes.
- 3.1.6 For wood or timber products
- Vapor heat treatment at 56° c for 30 minutes, or heat treatment 75 ° C or more for 30 minutes.
- 3.1.7 Cereals
- Irradiation / radiation using ultraviolet light (253-257) nm for 60 minutes, with a specification for a 40 Watt UV lamp with a volume of 1.5 M3, or
  - Vapor heat treatment at 56° c or more for 30 minutes, or
  - Hot air Treatment at 75 ° C for 30 minutes.
- 3.1.8 Fibre Products
- Irradiation / radiation using ultraviolet light (253-257) nm for 60 minutes, with a specification for a 40 Watt UV lamp with a volume of 1.5 M3, or
  - Vapor heat treatment at 56° c or more for 30 minutes, or
  - Hot air Treatment at 75 ° C for 30 minutes.
- 3.1.9 Passenger luggage
- Suitcases, shoes or footwear, and other hand carried luggage (spraying with sodium hypochlorite 200 ppm or more), or
  - Irradiation / radiation using ultraviolet light (253-257) nm for 60 minutes, with specifications for 40 Watt UV lamp with a volume of 1.5 M3
- 3.1.10 Vehicles and Containers
- Washed with detergent and sprayed with pressurized water.
- 3.1.11 Equipments
- Spraying disinfectants (sodium hypochlorite) 200 ppm or more, or
  - Irradiation / radiation using ultraviolet light (253-257) nm for 60 minutes, with specifications for 40 Watt UV lamp with a volume of 1.5 M3.
  - For equipments made of metal, wash with detergent and sprayed with pressurized water.
- 3.1.12 Packing Materials
- Spraying with disinfectants (sodium hypochlorite) 200 ppm or more, or
  - Irradiation / radiation using ultraviolet light (253-257) nm for 60 minutes, with specifications for 40 Watt UV lamp with a volume of 1.5 M3.

## **3.2 India**

3.2.1 Plant Quarantine Order (Regulation of Import into India) Order 2003 effective from 1 January 2004.

3.2.2 Hevea planting material

- Prohibited from American continent and West Indies. For remaining countries require Import Permit, Phytosanitary Certificate, Intermediary Quarantine station and Post Entry Quarantine.
- Special permission from export and import committee of Department of Agriculture and Cooperation.
- Can only be imported through/by Director of Rubber Institute, Kottayam (Kerela).

3.2.3 Hevea plant product

- Require Import Permit, Phytosanitary Certificate and inspection upon entry.

3.2.4 Non Hevea planting material

- Require Import Permit, Phytosanitary Certificate, inspection upon entry and post-entry quarantined.

3.2.5 Non Hevea plant products.

- Require Import Permit, Phytosanitary Certificate and inspection upon entry

3.2.6 Passengers from SALB area/Container

- No requirement

## **3.3 Philippines**

3.3.1 Application - Any person/company intending to import plants/plant products shall file an application for Permit to Import (BPI Q Form No. 1) with the PQS prior to importation.

3.3.2 Importations which require Permit to Import and are subject to inspection:

- Living plants
- Nursery stocks, including vegetative parts thereof used as propagating materials
- Seed nut for planting
- Fresh fruits, vegetables, and other plant products, which have been declared as prohibited/restricted imports under Special Quarantine Orders by virtue of their being known as hosts of certain plant pests, or because they originate from restricted areas.

3.3.3 Evaluation of Application - Evaluation and pest risk analysis (PRA) shall be conducted prior to the issuance of an import permit. Conditions for entry shall be based on the pest present in the country of origin and other phytosanitary measures. For commodities that will requires inspection and evaluation of field/growing area the following are conducted:

- Inspection of the field where the materials will be planted;
- Make the necessary phytosanitary measures/recommendations required by the PQS;
- Approval of the area by the PQS;
- Sketch location of the growing area to guide the post entry team in monitoring and observing the materials for presence of pests during the growing period.

3.3.4 Approval of application for Issuance of Permit and Import - Upon approval of the application, documentary evaluation and payment of regulatory fee, the Permit to Import is issued to the applicant/importer . This should be presented to the Plant Quarantine Officer at the port of entry upon lodging of application for inspection.

3.3.5 Inspection upon Arrival of Commodities - The importer shall file with the PQS a duly accomplished Application for Inspection at least twenty-four (24) hours prior to arrival of the consignment. The following documents shall be submitted:

- Phytosanitary Certificate (original FAO/IPPC Model) issued by the PQ of the country of origin;
- Plant Quarantine Clearance (IP) issued by PQS
- Customs Documents

3.3.6 Inspection at the Designated Inspection Area

3.3.7 Sampling

3.3.8 Phytosanitary Treatment (if necessary)

3.3.9 Post-entry Quarantine (if necessary)

3.3.10 Clearance

## **3.4 Singapore**

3.4.1 Hevea Planting Materials

- Import Risk Assessment
- For Scientific & Research Purposes
  - Approval from the Director General of AVA
  - Post-entry Quarantine
  - Phytosanitary Certificate

3.4.2 Non-Hevea Planting Materials

- Import Permit from AVA
- Phytosanitary certificate with additional certification of SALB freedom or sourced from SALB-free areas
- Certified pre-departure treatment

- Chemical : 0.1% a.i. fenamiphos + 0.1% a.i. benomyl + 0.1% a.i. chlorothalonil
- Post-entry Inspection – at premises pre-approved by AVA

#### 3.4.3 Fresh Fruits, Vegetables & Cut Flowers

- Import permit from AVA
- Phytosanitary certificate with additional certification of freedom of SALB
- Post-entry Inspection

#### 3.4.4 Cut Foliages And Grass (For Bouquets And Ornamental Purposes)

- Import permit from AVA. Sourced from SALB free areas
- Phytosanitary certificate with additional certification of freedom of SALB
- Post-entry Inspection

#### 3.4.5 Herbarium Specimens of Hevea

- Import Risk Analysis

#### 3.4.6 Herbarium Specimens of non-Hevea spp

- Phytosanitary certificate - AD from a SALB free area or tested free from the regulated pest
- Certified pre-departure treatment with heat or equivalent
- Post-entry inspection

### 3.5 Sri Lanka

#### 3.5.1 Regulatory Position on Importation of Rubber

- Import of rubber planting material is allowed only for scientific purposes.
- Import permit from Director General of Agriculture (DGA) is necessary.
- Permission from Rubber Controller is necessary.
- DGA will issue the permit only for the Director of The Rubber Research Institute Sri Lanka (RRISL).
- Import conditions specified.
- Soil, packing material of plant origin and planting media are not allowed.
- Phytosanitary certificate required.
- Inspection at port of entry.
- Mandatory PEQ for a considerable period.

#### 3.5.2 Import from SALB Endemic Area.

- All conditions given in previous case will apply.
- Collection of the material preferably by a senior researcher of RRISL.
- Intermediate Plant Quarantine is mandatory.
- Phytosanitary certificate for re-export is required.
- Inspection at port of entry.
- Packing material and packaging material should be destroyed.



- 3.5.3 Other (non Hevea) planting material from SALB endemic area for scientific studies.
- Import permit of DGA is required.
  - No soil and planting media.
  - Intermediate Plant Quarantine Station.
  - Phytosanitary certificate (PC) and PC for re-export are required.
  - Inspection at port of entry.
  - Mandatory PEQ for a period less than 2 years.
  - RRISL should have a contingency plan to combat the disease, in case of accidental entry.

### 3.6 Thailand

- 3.6.1 Importation of Hevea spp. and carriers for research purpose
- be approved by PQ sub-committee
- 3.6.2 Importation for commercial purpose
- be approved by PQ sub committee
  - and PQ committee
- 3.6.3 Importation of Hevea spp. and carriers for research purpose
- importer must have:
    - an import permit
    - a phytosanitary certificate
    - IPQS
  - only through the of following PQ Station:
    - Bangkok Sea port PQS
    - Suvarnabhumi Int. Airport PQS
    - Bangkok Post Office PQS
  - thorough inspection
  - if pest is detected:
    - consignment shall be ordered to destroyed or send out PEQ
- 3.6.4 Importation of Hevea spp. and carriers for commercial purpose
- must be subjected to PRA

### 3.7 Vietnam

- 3.7.1 Basic Law for Doing Pest Risk Analysis (PRA)
- **Decree on Plant Quarantine** (revised 2007) included:
  - **Ordinance on Plant Protection and Quarantine** (revised 2001);
    - Item 16, Article 3, Chapter 1; Item b), Article 7, Chapter I; Item 3, Article 9, Chapter II; Item 3, Article 10, Chapter II; etc.

- **Decision of Minister of MARD number 4096/QD/BNN-KHCN** dated 29/12/2006 on the establishment of national standard of Pest Risk Analysis Procedure for import of plants and plant products to Vietnam;
- **Decision of Minister of MARD number 34/2007/QD-BNN** dated 23/4/2007 on the list of import commodities which should be done PRA before importing to Vietnam;
- **Decision of Minister of MARD number 48/2007/QD-BNN** dated 29/5/2007 on procedure for issuing the import permit of import commodities which should be done PRA before issuing the import permit;
- **Decision of DDG of PPD number 1477/QD/BVTV-HCTC** dated 02/10/2007 on authorization for PQDC to preside and conduct PRA for import plants and plant products into Vietnam.

3.7.2 Issuing the IP should be based on:

- Result from PRA;
- Regulating specific conditions for importation of consignment and commodity;
- Do not issue the IP for import commodities when posed high risks of plant pests.

3.7.3 Did not import plant materials for planting (including of rubber plants) from high risk countries into Vietnam for the last two years.

### 3.8 Malaysia

3.8.1 Restriction on the importation of Hevea planting materials

- Only for research
- Consigned to the Director of DOA
- Refer to Director General of Rubber Research Board before importation
- Subject to PEQ upon arrival

3.8.2 Hevea from area where SALB is present

- PEQ outside SEA and Pacific Region for a period of time
- Free from pests
- Accompanied by phytosanitary certificate
- Seed of any Hevea species from SALB area is prohibited unless inspected and treated outside the area where SALB is not found:
- Outside Pacific and South East Asia
- Repacked with new packing / containers
- Accompanied by phytosanitary certificate

3.8.3 Vessels

- If more than 30 days, allow entry (spores of SALB will not survive).
- Consignment can only be released for inspection after 30 days from the date of export from country of origin.

#### 3.8.4 Plants (for consumption, planting, etc.) other than *Hevea*.

- For research purpose only.
- Consigned to the Director of DOA
- Plant has been subjected to quarantine or treatment at a place approved by the Director for this purpose, situated outside the South East Asia and Pacific Region, and the American Tropics or any other region or country where the SALB is present or believed to be present, and has been found to be free from pests and is accompanied by a certificate to that effect and signed by the officer-in charge of the place of such quarantine or treatment.

#### 3.8.5 Seeds of *Hevea* for propagation.

- Seeds of any *Hevea* species from SALB countries is prohibited unless inspected and treated outside the area where SALB is not found:
  - Outside of South East Asia and Pacific region.
  - Repacked with new packing / container at the intermediate country.
  - Accompanied by phytosanitary certificate.

#### 3.8.6 Plants of *Hevea* not capable for further growth / propagation.

- Restricted except for research purposes or any other purposes approved by the Director (Example: fresh or dried herbarium specimens), and materials has been sterilized in the country of origin

#### 3.8.7 Direct flight

- All airlines are requested to tag with “Q” tag for all baggage from high risk countries.
- All baggage from high risk countries after tagging with “Q” tag must be put in separate containers labeled “Q”.
- Passengers from high risk countries are encouraged to transit in a temperate country at least over night
- All passenger must fill in the Quarantine Declaration Card (known as IMM 26) prior to disembarkation in Malaysia.
- All aircrafts arriving from high risk countries must use gate C12 and C32 at satellite building A at KL International Airport.
- Passengers to proceed through floor mat (disinfested with dettol at 0.5%) and air tunnel (blowing at the speed of 27m/sec) at gate C12 & C32 at satellite A building.
- Passengers to submit Quarantine Declaration Card at Plant Quarantine Office behind Immigration Counter No.58.
- Passengers collect baggage and report to PQ Office.
- PQ Inspector to interview passenger (ask their origin city, date of departure from that particular city, and any place they had stopped over etc.)
- In case passenger comes from rubber growing area :
  - If pax depart less than 30 days, baggage to be opened by pax for preparation of treatment.
  - Customs Officer to check for dutiable goods.

- If passenger bring plants.
- Plants to be seized unless comply with special procedure specified by Director of Agriculture, Malaysia.
- Baggage to be put inside Ultra Violet Chamber/Room for treatment at least 30 minutes exposure time.

### **3.9 China**

SALB is listed on the Quarantine Pest List (A1)

#### **3.9.1 Hevea Propagation Material**

- Prohibited from SALB endemic countries, except Research Materials (eg, Tissue Culture Material)

#### **3.9.2 Non-Hevea Propagation Material**

- Restricted from SALB endemic countries, based on PRA

#### **3.9.3 Permit for Research Hevea Materials :**

- (e.g. Tissue Culture Material\Seeds)
- Post-entry Quarantine Stations

#### **3.9.4 Soils**

- Prohibited from SALB endemic countries, except Sterilized

#### **3.9.5 Cargo and Containers from SALB endemic countries**

- Quarantine treatments such as fumigations

#### **3.9.6 Passengers from SALB endemic countries**

- Plant Quarantine Declaration Card,
- Dog, Inspection

#### **3.9.7 Monitoring of SALB:**

- Hevea Field Inspection

#### **3.9.8 Research**

- Molecular Diagnosis Method,
- Air-Monitoring,
- Seed-borne.

(Importing country NPPO name)

## ***MODEL***

**Work Plan for the Importation  
of Budded Stumps or Budwood  
of *Hevea spp.***

**from  
(*exporting country*) into  
(*importing country*)**

## 1 Scope

This Work Plan describes the mandatory operational requirements and the phytosanitary procedures, for the importation of budded stumps or budwood of *Hevea spp* from (exporting country) into (importing country) in order to address the risk of South American Leaf Blight and other regulated pests.

The measures and requirements detailed in this document meet the management measures described in the '*Pest Risk Analysis for South American Leaf Blight (SALB) of Rubber (Hevea)*, and the phytosanitary import requirements for other potential pests of concern to (*importing country*).

## 2 Definitions

Treatment	Official procedure for the killing, inactivation or removal of pests, or for rendering pests infertile or for devitalization [FAO, 1990, revised FAO, 1995; ISPM No. 15, 2002; ISPM No. 18, 2003; ICPM, 2005]
Intermediate Quarantine	Quarantine in a country other than the country of origin or destination [CEPM, 1996]
Inspection	Official visual examination of plants, plant products or other regulated articles to determine if pests are present and/or to determine compliance with phytosanitary regulations [FAO, 1990; revised FAO, 1995; formerly inspect]
Packaging	Material used in supporting, protecting or carrying a commodity [ISPM No. 20, 2004]
Phytosanitary Certificate	Certificate patterned after the model certificates of the IPPC [FAO, 1990]
post-entry quarantine	Quarantine applied to a consignment after entry [FAO, 1995]
IP	Import Permit
NPPO	National Plant Protection Organisation
IPPC	International Plant Protection Convention
PC	Phytosanitary Certificate
Verification	The process of reconciling export certification and product description with respective consignments and labelling requirements
Mother plants	Plants from which exported budded stumps or budwood is obtained

### 3 Product Being Exported

- 3.1. *Hevea* spp budded stumps or budwood produced in compliance with this work plan may enter (*importing country*), subject to on-arrival inspection and treatment and disease screening in a post-entry quarantine facility.
- 3.2 Post-entry quarantine may occur in (*importing country*) or in another country which is free of SALB.
- 3.3 Imported budded stumps or budwood must not be longer than 1m, and must be free of soil and other regulated articles, such as trash and quarantine pests.
- 3.4 Consignments must be packaged using clean, new materials and be accompanied by a PC issued by the NPPO of the exporting country, confirming that the consignment meets the management measures described in this work plan.

### 4 Responsibilities

- 4.1. Responsibilities of the NPPO of the exporting country
  - 4.1.1 The NPPO shall follow all requirements of the work plan, the import permit issued by the importing country and any applicable regulations.
  - 4.1.2 The NPPO will advise the grower/exporter of the export conditions.
  - 4.1.3 The NPPO will ensure that a plant pathologist accredited by the importing country conducts inspections of mother plants for symptoms of SALB and any other diseases of concern to the exporting country in accordance with this work plan. Any mother plants showing signs of disease of SALB or other diseases of concern to the importing country shall not be used to propagate planting material for export to (*importing country*).
  - 4.1.4 Propagating material must only be taken from mother trees which show no evidence or very low incidence of the symptoms of SALB.
  - 4.1.5 Any propagating material showing signs of disease of SALB or other diseases of concern to the importing country shall not be exported to (*importing country*).
  - 4.1.6 The NPPO will ensure through direct supervision that budded stumps or budwood for export are harvested at the brown bark stage of development and during the low-disease season, and are no longer than 1m.
  - 4.1.7 Where nurseries are used to establish budded stumps, the NPPO will register these nurseries and ensure that budded stumps for export are segregated from other product..

- 4.1.8 The NPPO will ensure that budded stumps or budwood for export have been dipped in the specified surface sterilant and systemic fungicide and shall supervise the application of the treatments.
- 4.1.9 The NPPO will inspect the consignment and issue a PC certifying that the requirements of the work plan have been met and that the consignment conforms to the import requirements of the importing country.
- 4.2. Grower/Exporter Responsibilities
  - 4.2.1 The grower/exporter will contact the exporting country's NPPO when it receives an import permit for budded stumps or budwood and request that the NPPO provide inspection and certification services in accordance with the IP and this work plan.
  - 4.2.2 The grower/exporter will provide the location of mother plants, exporting nurseries and packing houses to the NPPO and any other relevant information required by the NPPO to conduct inspection and the certification in accordance with this work plan.
  - 4.2.3 The grower/exporter will abide by all requirements of this work plan and applicable regulations.
  - 4.2.4 The grower/exporter will maintain accurate records of all activities, identify the growing sites, assign lot numbers to each of the plantings and identify the plant material for export.
    - 4.2.4.1 The identification of product for export must allow product to be traced back to mother plant blocks, nurseries and packing houses.
  - 4.2.5 Where the grower/exporter has a quality management system in place, the requirements of this work plan will be referenced in the quality manual.
  - 4.2.6 The grower/exporter will abide by the recommendations of the accredited plant pathologist.
- 4.3. Importing Country Responsibilities
  - 4.3.1 The NPPO of the importing country will issue an import permit for the importation of budded stumps or budwood specifying the import conditions and referencing this work plan.
  - 4.3.2 The NPPO of the importing country may conduct audits and verification checks to ensure compliance with this work plan.



- 4.3.3 The NPPO of the importing country may elect to send a plant pathologist to the exporting country to conduct inspections of plant material for export and to supervise any other elements of this work plan.
- 4.3.4 The NPPO of the importing country will conduct an on-arrival inspection of the imported consignments to verify freedom from pests and other regulated items.
- 4.3.5 The NPPO of the importing country will dip budded stumps or budwood in an appropriate surface sterilant and systemic fungicide on arrival.
- 4.3.6 The NPPO of the importing country will ensure that all original packing material is destroyed or sterilised.
- 4.3.7 The NPPO of the importing country will transfer the imported consignment to a suitable post entry quarantine facility for growth and disease screening.
- 4.3.8 Alternatively, the NPPO of the importing country will arrange for intermediate post entry quarantine in another country.

## **5 Quarantine pests**

*M.ulei* is the principle pest of concern for the importation of *Hevea spp.*, however the consignment must comply with the general import conditions of the importing country for plant propagating material. These requirements are set out in K.H. Chee, W.I. Wan Normah, A.K.J. Atikah, K.C Gupta, K.H. Ong, Budiman, Y. Surapol, T.D. Phan, C.M.Barron, 2010, “*Import Requirements of Plants, Plant Materials and Agriculture Products from the SALB Endermic Countries*”, Workshop on the Prevention of Introduction of South American Leaf Blight (SALB) of Rubber, Kuala Lumpur, Malaysia p5-8.

## **6 Mother Stock and Growth Requirements**

- 6.1 Plants must be developed from mother stock that was inspected by an accredited plant pathologist and found free from evidence of diseases and pests of concern to the importing country, in particular SALB.
  - 6.1.1 The NPPO of the exporting country will recommend a suitably qualified plant pathologist to the importing country’s NPPO to conduct inspections of mother plants for symptoms of SALB and any other diseases of concern to the exporting country. The experience and qualifications of the plant pathologist will be sent to the importing country NPPO for consideration.
  - 6.1.2 In consultation with appropriate research institutes, the NPPO of the importing country will consider the recommended plant pathologist and

accredit that plant pathologist if it is satisfied with the qualifications and experience.

6.1.3 Alternatively, the importing country NPPO may send its own plant pathologist to perform inspections.

6.1.4 The NPPO shall verify that the inspections occur immediately before the harvesting of budded stumps or budwood for export, and during a period considered optimal for SALB disease expression.

6.1.5 Any plants showing signs of SALB or other diseases of concern to the importing country shall not be used to propagate budded stumps or budwood for export to the importing country.

6.2 Where nurseries are used to establish budded stumps, they shall be accredited by the NPPO of the exporting country and the importing country NPPO informed.

6.2.1 An accredited plant pathologist shall inspect the budded stumps prior to export for signs of disease.

## **7 Harvesting of Budded Stumps or Budwood**

7.1 The NPPO of the exporting country will verify by supervision that budded stumps or budwood for export are harvested at the brown bark stage of development and during the low-disease season, and are no longer than 1m.

7.2 The NPPO of the exporting country will ensure that harvested budded stumps or budwood is identified to allow trace back to specific mother tree blocks.

## **8 Pre-export Treatments**

8.1 The NPPO will ensure that budded stumps or budwood for export have been dipped in a 200ppm solution of sodium hypochlorite and a solution of thiophanate methyl or alternative chemicals approved by the importing country NPPO.

8.1.1 The NPPO will supervise the application of the above treatments.

8.2 The treatments will be applied in an enclosed secure facility, and the budded stumps or budwood will be packed immediately following treatment to guard against re-infestation.

## **9 Pre-export Packaging and Inspection**

- 9.1 The NPPO will ensure that budded stumps or budwood for export are segregated from budded stumps or budwood which are not for export and that the packing premises are enclosed, clean and not subject to contamination from *M. ulei*.
- 9.2 The budded stumps or budwood will be inspected immediately prior to export by the NPPO and found free from evidence of plant pests and diseases
  - 9.2.1 The NPPO will issue a PC certifying that the requirements of the work plan have been met and that the consignment conforms to the import requirements of the importing country.
- 9.3 The NPPO will ensure that budded stumps or budwood for export are packed in clean, new packaging materials and secured to ensure that they are not exposed to contamination by spores of *M. ulei* after treatment.
- 9.4 The NPPO will supervise the packing of the budded stumps or budwood for export, and ensure that the consignment can be traced back to specific mother tree blocks.
- 9.5 The NPPO will ensure that the consignment is exported within 24 hours of packaging.

## **10 Intermediate Quarantine**

- 10.1 Where budded stumps or budwood are exported to a third country for intermediate quarantine, the importing country will ensure that appropriate records are retained for trace back purposes and that a PC is issued by the third country for export of the released plants to the importing country.
- 10.2 The on-arrival import conditions above will apply for imported budded stumps or budwood received after intermediate quarantine.
- 10.3 The post-entry quarantine period may be reduced for this material.

## **11 Program Review and Evaluation**

- 11.1 The work plan may be reviewed or amended when necessary by the importing country NPPO and the exporting country NPPO.
- 11.2 The NPPO of the importing country may audit the system for compliance with this work plan or arrange for a third party to audit the system on its behalf.

**DIAGNOSTIC SYSTEM – EQUIPMENT AND APPARATUS****A General pathology equipment****1. Major Equipment**

- Autoclave
- Incubators
- Oven
- Water Distiller
- Bio-safety cabinet/Laminar Flow
- Refrigerators
- weighing balances,
- microscopes,
- cameras
- pH Meter
- Water bath
- Shaker
- Microwave Oven
- Thermometer
- Isolation needles

**2. PCR and related accessories**

- Thermocycler,
- Electrophoresis
- Gel documentation
- PCR Workstation
- Pippete
- Grinder
- Fumehood

**3. ELISA**

- ELISAR Reader
- Grinder

**B Disease treatment and disposing equipments**

- Sprayers,
- Moist Heat Chambers,
- Dipping Tanks,
- Incinerators,
- Hot Water Jets Apparatus
- Waterbath

**C Chemicals and consumables item**

- Media (PSA, PDA, MEA etc.)
- Chemicals for making media (agar, sucrose, glucose, malt etc);
- Chemicals for sterilization -formalin, mercuric chloride, sodium hypochlorite, alcohols etc.;
- Glasswares - Petri plates, test tubes, flasks, beaker, Glass slide/cover etc.
- PCR – Primer
- ELISA – Antiserum

**D For sampling and transportation of samples, examples**

- Cooler Box
- Containment Box

**E Information and storage facilities**

- Computer
- Software for data management
- Reference Books
- Networking for diagnostic
- Diagnostic manual

## TECHNIQUES OF ISOLATION, CULTURING AND INDUCTION OF SPORULATION OF MICROCYCLUS ULEI

*Microcyclus ulei*, the causal agent of South American leaf blight (SALB) is classified as an obligate parasite. Various attempts were made to culture the fungus on artificial medium either liquid or solid media. Special techniques had also been used to isolate *M. ulei*. This paper documents some of the most successful methods.

### ISOLATION METHODS

The fungus infects young rubber leaves forming lesions which produced conidiophores and consequently on more mature leaves ascospores. The pure culture of the fungus had been isolated from fresh lesions on young leaves:

#### **Culturing young fresh disease lesion**

Small sections of fresh young leaves are cut and surface sterilized and transferred onto isolation medium. Holliday (0) found it was better to use sections cut across the lesion than sections cut around disease lesions. Sodium hypochlorite or aqueous mercuric chlorides are used for surface sterilization.

#### **Culturing the spores**

Fresh lesions which are actively producing conidia are selected. Contamination is higher if older lesions are used or from leaves harvested on a rainy day. Various methods can be used to transfer the conidia onto the isolation medium:

- Using a moisten tip and sterile isolation needle, gently touch the upper surface of the lesion and place the spores onto the surface of the isolation medium.
- Cut a small piece of isolation medium and mount it at the tip of an isolation needle. Gently touch the piece of agar to the surface of the lesion and then transfer the agar to the surface of isolation medium.
- Tap the conidia from the fresh lesion onto the surface of agar (water agar). Using a stereo-microscope, pick the conidia and transfer to an isolation medium.

#### **Isolation medium**

Various media used to isolate *M. ulei* include water agar and potato dextrose agar. If water agar is used, the fungal colony must be transferred to richer medium. Antibiotics (chloroamphenicol) had been used to reduce bacterial contamination.

#### **Growth medium**

The first success of culturing *M. ulei* on artificial medium was done in 1945 by Langford who used a medium containing malt and extracts of rubber leaf. Since then extracts of rubber leaves of different ages were tried. Similarly, various vitamins and amino acids were also experimented with inconclusive results. Eventually Chee successfully cultured *M. ulei* on potato sucrose medium. The concentration of sucrose in PSA varied from 2.5 g to 5 g per litre. Panvit, a commercial multivitamin and mineral had also been used to enhance growth.

### **Growth of *M. ulei* on artificial medium**

The growth of *M. ulei* in artificial medium is very slow and it is normal that it is hardly visible within the blackish stroma above the surface of the medium. The diameter of the colony is about 2 mm in 10 days and 2 cm in a month. The appearance of the colony also varies with the physiological races of *M. ulei*. Race 2 tends to grow into the medium while the growth of other races is superficial.

*M. ulei* had also been cultured on liquid medium. Growth in liquid PSA is good. Growth is better if the mycelium is floated on the surface of the medium thickened by adding 0.5 g/l agar. Cultures of *M. ulei* are normally maintained in the dark at 24°C. For longer storage, sporulating colonies are submerged in sterile mineral oil however rejuvenation of these cultures is very slow.

### **Production of spores in vitro**

Poor production of spores in vitro has been one of the limiting factors in the study of *M. ulei*. Several factors influence sporulation:

Generally, sporulation is low in medium supporting good growth of *M. ulei*. Modified PSA and PSA amended with peptone or a brand of dog food increased spore production. The age of culture at the time of initiation of sporulation also influences the amount of spores produced. Maximum spores were produced from 2-3 weeks cultures. Light also influences spore production. Conidia production increases when the cultures were exposed to UV light for 45 min per day for 14 days. Cultures grown in the dark also produce more conidia when exposed to fluorescent light 60-90 min/day for 14 days. Alternatively, the cultures can be grown on PSA in the dark for 12 days and later exposed to fluorescent light for 1 h/day for 2 days.

Nowadays *M. ulei* can be cultured on artificial medium with ease. Rubber growing countries should be aware on the movement of these cultures into their region.