

**REGIONAL STANDARDS  
FOR PHYTOSANITARY MEASURES**

***GUIDELINES FOR PROTECTION AGAINST  
SOUTH AMERICAN LEAF BLIGHT OF RUBBER***

**APPPC RSPM No. 7**

(including Appendix 5)





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(including Appendix 5)

The Asia and Pacific Plant Protection Commission (APPPC)  
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS  
REGIONAL OFFICE FOR ASIA AND THE PACIFIC  
Bangkok 2012

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## ***Endorsement***

Regional standards for phytosanitary measures are developed and adopted by the Asia and Pacific Plant Protection Commission as part of the plant protection programme of the Commission's contracting parties. This programme makes available to contracting and other interested parties regional standards for phytosanitary measures to support regional harmonization, with the aim to facilitate trade and avoid the use of unjustifiable measures as barriers to trade.

This standard was endorsed by the twenty-sixth session of the Asia and Pacific Plant Protection Commission in September 2009.

The 27<sup>th</sup> Session of APPPC (2011) decided to add the Appendix 5 "Importation Requirements for *Hevea* Plant Material" to this Standard.



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### ***Endorsement***

This Asia and Pacific Plant Protection Commission (APPPC) Regional Standard for Phytosanitary Measures was endorsed by the twenty-sixth session of the APPPC held from 31 August to 4 September 2009 in New Delhi, India. The 27<sup>th</sup> session (20110 decided to add the Appendix 5 (for information) to this standard.

### ***Review***

APPPC Regional Standards for Phytosanitary Measures are subject to periodic review. The next review date for this standard is 2013. The standard may be reviewed earlier if the APPPC decides this is necessary.

### ***Distribution***

APPPC Regional Standards for Phytosanitary Measures are distributed by the Executive Secretariat of the APPPC to all APPPC members, the Administrative Heads of Regional Plant Protection Organizations and the FAO International Plant Protection Convention (IPPC) Secretariat. This standard is available on the APPPC webpage found within the International Phytosanitary Portal: <http://www.ippc.int/En/rppo/jsp>

# INTRODUCTION

## Scope

This standard provides guidelines for members of the APPPC, particularly rubber growing countries, to assist them to improve or develop their phytosanitary measures, including prevention, eradication and control, against South American leaf blight of rubber (SALB). It covers all the areas of plant health dealing with the protection of member countries rubber industries from SALB.

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## **Definitions and abbreviations**

Except where noted, the definitions are from ISPM No. 5, *Glossary of phytosanitary terms*.

<b>Control (of a pest)</b>	Suppression, containment or eradication of a pest population [FAO, 1995]
<b>devitalization</b>	A procedure rendering plants or plant products incapable of germination, growth or further reproduction [ICPM, 2001]
<b>eradication</b>	Application of phytosanitary measures to eliminate a pest from an area [FAO, 1990; revised FAO, 1995; formerly eradicate]
<b>inspection</b>	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]
<b>intermediate quarantine</b>	Quarantine in a country other than the country of origin or destination [CEPM, 1996]
<b>pathway</b>	Any means that allows the entry or spread of a pest [FAO, 1990; revised FAO, 1995]
<b>Pest Free Area</b>	An area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained [FAO, 1995]

**Pest Risk Analysis**

The process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it [FAO, 1995; revised IPPC, 1997; ISPM No. 2, 2007]

**phytosanitary import requirements**

Specific phytosanitary measures established by an importing country concerning consignments moving into that country [ICPM, 2005]

**phytosanitary procedure**

Any official method for implementing phytosanitary measures including the performance of inspections, tests, surveillance or treatments in connection with regulated pests [FAO, 1990; revised FAO, 1995; CEPM, 1999; ICPM, 2001; ICPM, 2005]

**point of entry**

Airport, seaport or land border point officially designated for the importation of consignments, and/or entrance of passengers [FAO, 1995]

**surveillance**

An official process which collects and records data on pest occurrence or absence by survey, monitoring or other procedures [CEPM, 1996]

**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]

**Outline of requirements**

Guidelines for the protection of APPPC rubber growing countries against SALB, based on the 2007 *Pest risk analysis for South American leaf blight (SALB) of rubber (Hevea)*, are proposed by considering five major areas:

- the prevention of the introduction of SALB into Asia and Pacific region using import requirements, and systems for points of entry inspections, laboratory diagnostics, and surveillance;

- the establishment of eradication or control programmes in case of entry of SALB;
- the development of training programmes on inspection and diagnostic methods, surveillance, eradication and control programmes;
- the minimum resources, in terms of for personnel and facilities, for protection against SALB;
- the establishment of coordination and cooperation activities for SALB programmes.

Systems for preventing the introduction of SALB include the establishment of import requirements for host material (including budded stumps and bud wood, seeds, *in vitro* plants and foliage) and non-host material (inanimate goods or non-host organic material, travellers from SALB endemic countries and other items). Risk management can include measures such as inspection for freedom from infection; surface sterilization and post-entry quarantine for plants for planting; seed treatment for seeds; and removal, destruction or heat treatment for contamination of non-viable host material. The operational structures supporting the prevention of the introduction of SALB include points of entry inspection systems, a laboratory diagnostic system and SALB surveillance systems.

Rubber growing countries should also, within their SALB protection programme, develop and establish contingency plans for eradication or control programmes in case the disease is found in a country. Training programmes for inspection, diagnostic and disinfection procedures, surveillance, eradication and control measures, and staff management need to be developed. A minimum resource level of personnel and facilities should be established for SALB protection programmes.

In managing a national SALB protection programme, NPPOs should ensure that a centralized committee is set up to coordinate activities and that appropriate links are made with other related bodies to exchange information. For a regional coordination programme, the commission may consider establishing an SALB cooperation committee, the activities of which could be supported by all member countries.

## **BACKGROUND**

The South American leaf blight (SALB) is caused by the fungus *Microcyclus ulei* (P. Henn) v. Arx and is the most destructive disease of rubber. It has been a major constraint in the production of rubber in South America. The disease could also cause great economic damage to the rubber growing countries of Asia and the Pacific region if it were to be introduced into the area. This was recognized by countries of the region when establishing the Plant Protection Agreement for the Asia and Pacific region (the Agreement) in 1956, with the promulgation of Article IV and Appendix B to the Agreement which dealt specifically with SALB. This obliged members to prohibit the import of: plants or seed of the genus *Hevea* from outside the region; plant material of genus *Hevea* not capable of further growth or propagation (such as fresh or dried herbarium specimens); and any plants of other than genus *Hevea* from SALB endemic areas into their countries unless certain stringent phytosanitary import requirements were met.

When revising the Agreement in 1999 to bring it in line with the WTO Agreement on the Application of Sanitary and Phytosanitary Measures, the 1956 provisions relating to SALB were found to be inconsistent. APPPC decided that a pest risk analysis (PRA) on SALB should be developed by APPPC member countries.

The PRA for SALB was completed and accepted by the 25<sup>th</sup> session of the APPPC in August 2007 (subsequently referred to as the SALB PRA). The PRA is the guideline used to develop this standard on SALB.

### **Purpose**

This standard provides guidelines for APPPC member countries that grow rubber to prevent the entry, spread and establishment of SALB, taking into account the SALB PRA.

The guidelines cover five major areas:

- the prevention of the introduction of SALB into Asia and Pacific region using import requirements, and systems for points of entry inspections, laboratory diagnostics, and surveillance;
- the establishment of eradication or control programmes in case of entry of SALB;

- the development of training programmes on inspection and diagnostic methods, surveillance, eradication and control programmes;
- the minimum resources, in terms of for personnel and facilities, for protection against SALB;
- the establishment of coordination and cooperation activities for SALB programmes.

## **REQUIREMENTS**

### **1. The prevention of the introduction of SALB**

The prevention of the introduction of SALB into rubber growing countries can be achieved by an integrated programme including:

- strict phytosanitary import requirements (enabled through legislation) that reduce any potential risk from imports to a level acceptable to the importing country
- an inspection system at points of entry that ensures compliance with import requirements
- a laboratory diagnostic system for the identification of the pathogen
- an efficient surveillance system that ensures early detection of the pest.

#### **1.1 Import requirements**

Import requirements should be determined based on the SALB PRA which describes the pathways of entry and the relative risks of those pathways taking into account consequences and the appropriate level of protection of the individual country.

The SALB PRA noted the pathways for the entry of the pathogen as:

- Budded stumps and budwood
- foliage (stem and leaf material not for planting)
- flowers, fruit and seeds
- plants *in vitro*
- rubber wood
- travellers
- inanimate goods or non-host organic material (contaminated by spores)

- inanimate goods or non-host organic material contaminated by host plant material

Appendix 1 is a summary of the assessments of introduction, spread and consequences from the SALB PRA.

The NPPO of each rubber growing member country should consider import requirements as follows:

### ***1.1.1 Host material (Hevea spp.) as identified in the SALB PRA***

As identified in the SALB PRA, planting material of *Hevea* from SALB endemic countries (see Appendix 2) is categorised as high risk. Importing countries should apply measures as necessary. Rubber growing countries should consider limiting the entry of high risk material to designated entry points. Measures are described in the SALB PRA and include the following.

For budded stumps and bud wood\*:

- prohibition of imports if an importing country does not have the capacity to apply effective measures to mitigate risk to an appropriate level of protection
- restriction of quantity based on the capacity of the pose-entry quarantine (PEQ) station
- restricting the length of each budwood stick to less than 1 metre
- pre-export inspection and treatment
- measures applied on arrival (in an appropriately secure facility)
- restriction of imports to Government Research Institutes only and using PEQ stations
- other PEQ facilities or intermediate quarantine for at least one year and certified free from SALB by an SALB expert.

For seeds:

- restrictions on the quantities of seed imported
- only healthy seeds should be imported

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\* budded stumps includes seedling stumps

- washing with a surface sterilant and dressing with a fungicide immediately prior to export.

For *in vitro* plants:

- growing the plants aseptically on agar for more than three months followed by appropriate inspection or testing.

For foliage:

- the prohibition of foliage of *Hevea* from SALB countries unless treated to remove the risk.

### ***1.1.2 Non-host material (SALB endemic countries)***

Non-host material includes travellers, inanimate goods and non-host organic material.

#### *Travellers from SALB endemic countries*

Where a rubber growing country has determined a level of risk associated with travellers from SALB endemic countries, the travellers should be required to make a declaration to the NPPO point of entry inspector if they have visited a SALB endemic country in the last 21 days and have visited a rubber plantation. Special care should be taken with such items such as camping equipment and hiking boots, farm equipment and decorative plant material as these are more likely to contain non-viable host material greater than 1 cm<sup>2</sup> that may be contaminated with SALB. Measures to remove possible contamination may include cleaning, disinfection or destruction.

#### *Inanimate goods*

Cargo such as machinery that has been used in rubber plantations as well as household effects (gardening tools/equipment) in SALB endemic countries may be contaminated with non-viable host material carrying the pathogen. Measures include steam cleaning to remove all organic material which should be destroyed. Machinery should be dismantled if necessary. Where suspect material cannot be removed or the material cannot be destroyed (e.g. herbarium material) the material should be heat treated for a minimum of 30 minutes at 56 °C or a level that achieves the importing countries appropriate level of protection.

### *Non-host organic material*

Other items, such as planting material and foliage, of non-SALB hosts may need to be assessed for possible SALB contamination and phytosanitary procedures and appropriate treatment applied.

#### **1.1.3 Risk management methods**

A number of risk management methods from section 4 of the SALB PRA are given in Appendix 3.

## **1.2 Points of entry inspection systems**

When determined necessary, consignments from SALB endemic countries, including traveller's hand-carried items, should be subjected to inspection by NPPO inspectors at points of entry.

Points of entry inspection systems should be such to ensure that consignments and travellers comply with import/entry requirements.

NPPOs should follow the general guidelines provided in ISPM No. 23: *Guidelines for inspection*.

Specific points relating to inspection for risk items concerning SALB include:

- examination of documents associated with consignments to ensure that all permitted planting material has the correct certification
- examination of manifests to identify inanimate goods or non-host organic material that might be contaminated with the spores of the pathogen or host material that need to be inspected
- trained personnel to be stationed at entry points to recognize live and dead plant material of *Hevea*
- inspection of consignments, including used machinery, that have been in the rubber plantations of SALB infected countries
- equipment and chemicals for disinfection and disposing of any suspect material or non compliance consignment should be available to inspectors

- to ensure appropriate security, systems for packing and transporting suspect material to diagnostic laboratories should be available
- phytosanitary action, as noted in ISPM No. 20 *Guidelines for an import a phytosanitary import regulatory system*, section 5.1.6.1, should be taken where non-compliance occurs or SALB is detected.

### **1.3 Laboratory diagnostic system**

Countries should have, or have access to, appropriate laboratory diagnostic tools. This may include the following:

- diagnostic facilities for the identification of suspect fungal isolates. These laboratories should be established at or near the designated entry points where possible.
- the laboratories should follow standard procedures for the diagnosis of the pathogen(s) on specimens.

### **1.4 SALB surveillance systems**

NPPOs in rubber growing countries should establish and maintain national systems for surveillance for SALB – see ISPM No. 6. It is essential that any incursion of the pathogen is detected before it establishes and becomes widespread.

Additional surveillance programmes may be implemented in countries if the disease occurs in neighbouring country or is intercepted at an entry point. Such surveillance systems would be ongoing. Specific surveillance systems are described in Appendix 4.

## **2. Eradication or control programmes**

To be prepared in the event that the disease is found in a country, the NPPOs of APPPC rubber growing countries should develop and establish contingency plans for eradication or control programmes according to ISPM No. 9 *Guidelines for pest eradication programmes*. The plans should include a system for preventing the movement of potentially infected or contaminated materials within and out of infected areas. Such plans could include the following components:

## Operational procedures

- undertaking of a delimiting survey of the affected area
- undertaking other surveys as necessary
- documenting records of occurrences
- international notification
- carrying out an eradication feasibility study
- establishing and undertaking the eradication programme or control measures including surveillance, containment, treatment
- disinfection and destruction procedures
- systems for the prevention of the movement of possibly infected or contaminated material within and out of infected areas
- verification of eradication.

## Required resources

- a means of identifying the disease in the field plus a diagnostic laboratory support for confirmation of identifications
- identification of appropriate eradication methodologies
- administrative systems including a management structure and documentation procedures
- trained operational staff
- regulations providing authority for procedure implementation (movement prohibitions, setting up check-points, etc.)
- information management system
- communications programme, including media, public awareness
- financial support.

### **3. Training programmes**

NPPOs of rubber growing countries should establish training programmes for the staff on inspection, diagnostic and disinfection procedures; surveillance; eradication and control measures; and management of programmes for SALB. The components of such programmes are listed below:

### **3.1 Inspection, diagnostic and disinfection procedures**

- procedures of inspection
- procedures of clearance
- inspection of document and import requirements
- recognizing the symptoms of SALB and its pathogen
- recognizing host plants and host plant parts
- laboratory diagnostic protocols and procedures for identification of the pathogen
- disinfection or destruction of infected or non-compliant materials
- disinfection procedures for personnel handling diseased material.

### **3.2 Surveillance programmes**

- early detection systems
- identification of symptoms and the pathogen
- surveillance procedures and sampling techniques
- documentation and reporting.

### **3.3 Eradication and control measures**

- knowledge on eradication and control procedures
- safe handling of chemicals and equipment
- use of a geographical information system to map affected areas
- application of fungicides
- eradication programmes (including simulated outbreak exercises).

### **3.4 Management of programmes**

- eradication programme management
- programme documentation and recording
- communication with growers, industry representatives government departments, NGOs and public
- communications/media.

## **4. Minimum requirements for personnel and facilities**

The NPPO of each rubber growing country should have as a minimum resource for protection against SALB:

- designated expert(s) on SALB
- trained inspectors for consignment inspection and for surveillance programmes
- diagnostic capabilities to detect and identify the pathogen
- PEQ facilities
- access to aerial spraying organizations or companies
- disinfection facilities including:
  - dipping tank with sodium hypochlorite
  - hot water jet system for disinfection
  - incinerator
  - UV Chamber
  - access to chemicals and any necessary registration for use

## **5. National and regional coordination and cooperation**

### **5.1 National**

The NPPO of each rubber growing country should coordinate the activities of the SALB programme or, where appropriate, establish a centralized body or committee (if one does not already exist) to do this. The activities of such a body or committee could cover the following areas:

- resource management
- programme documentation, evaluation and improvement procedures
- centralized communication with growers, industry representatives, government departments and NGOs
- establishment and maintenance of a national focal point
- surveillance planning
- public awareness initiatives and programmes.

### **5.2 Regional**

The NPPO or the committee would also establish links with related bodies or committees in other rubber growing countries to exchange information and establish regional programmes where necessary to:

- seek technical assistance
- seek financial assistance

- ensure availability of technical expertise through regular regional and international workshops, training and seminars on SALB
- provide training on SALB
- cooperate with other regional and international organizations that deal with rubber and with the NPPOs of non-rubber growing countries (that could, for example, supply opportunities for intermediate quarantine, undertaking research)
- cooperate with SALB endemic countries in training, research, the safe transfer of rubber plant germplasm including the verification of phytosanitary systems and information exchange.

The Commission may consider the establishment of an SALB Cooperation Committee to oversee and coordinate various regional activities for the prevention of the introduction of SALB into the region. The NPPOs of all member countries should support regional activities for the prevention of the entry of the disease into the area where this is appropriate.

## APPENDICES

### Appendix 1

**Table 1. Summary of the assessments of introduction, spread and consequences to rubber growing countries** (source SALB PRA, 2007 Table 6)

Vector	Probability of entry	Probability of establishment	Probability of spread	Likely impact	Level of risk
<b>Host material (<i>Hevea</i> species)</b>					
<b>Budded stumps or budwood</b>	High	High	High	High	High
<b>Foliage (stem and leaf material not for planting)</b>	Low	High	High	High	Moderate
<b>Flowers, fruit and seeds</b>	Low	High	High	High	Low
<b>Plants <i>in-vitro</i></b>	Negligible	N/A	N/A	N/A	Negligible
<b>Non-host material</b>					
<b>Inanimate goods or non-host organic material</b>	Negligible	N/A	N/A	N/A	Negligible
<b>Inanimate goods or non-host organic material contaminated by host plant material</b>	Low (if <1 cm <sup>2</sup> )	High	High	High	Low (if <1 cm <sup>2</sup> )

## Appendix 2

### **SALB Endemic countries** (source SALB PRA, 2007 Plate 5)

1. Belize
2. Bolivia
3. Brazil
4. Colombia
5. Costa Rica
6. Dominican Republic
7. Ecuador
8. El Salvador
9. French Guiana
10. Guatemala
11. Guyana
12. Haiti
13. Honduras
14. Mexico
15. Nicaragua
16. Panama
17. Paraguay
18. Peru
19. Surinam
20. Trinidad and Tobago
21. Venezuela

## Appendix 3

### **Risk management methods for SALB**

(Source SALB PRA, 2007 Section 4)

This is a direct copy of the relevant section of the SALB PRA (with the reference to ISPM No. 5 being updated). The section numbers are changed to fit this appendix. References are to be found in the SALB PRA.

### **RISK MANAGEMENT**

The guiding principle for risk management should be to manage risk to achieve the required degree of safety that can be justified and is feasible within the limits of available options and resources. Pest risk management (in the analytical sense) is the process of identifying ways to react to a perceived risk, evaluating the efficacy of these actions, and identifying the most appropriate options (ISPM No. 11 2004). The effectiveness of any risk management measures depends on our perception and understanding of the disease and the risk pathways. Phytosanitary measures drawn up on the basis of science and practicality are essentially easy to implement, have minimal economic impact and are discriminating.

Based on the assessment of risks completed in the previous chapters of this risk analysis, risk commodities have been divided into the following groups.

1. Viable host material:
  - 1.1 Plants for planting: Whole plants and cuttings, and plants in-vitro;
  - 1.2 Seeds, flowers and fruit.
2. Non-viable (inanimate) host material:
  - 2.1 Cargo pathway (including sea freight, airfreight and mail);
  - 2.2 Passenger pathway (including accompanied luggage).

For goods or passengers originating from an area not known to be free of SALB, the following risk management measures may be applied.

## **1. Management options for viable host material**

Viable host material includes any plant parts that are being imported into the region for the purposes of propagation or could be propagated by conventional means.

### **1.1 Plants for planting**

The IPPC definition of plants for planting includes whole plants and cuttings, and plants *in-vitro* (ISPM No. 5 2009). For the purposes of this risk analysis only budded stumps and budwood have been considered for measures as they are the most likely form to be transported between countries for planting.

#### ***1.1.1 Budded stumps and budwood***

Management of the phytosanitary risks associated with the import of budded stumps and budwood for propagation should start in the country of origin. Efforts should be made to ensure that, as far as is reasonable and possible, budded stumps and budwood exported to the PRA area should be free of SALB. The United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ): *Postentry Quarantine Manual for State Inspectors* (2006) states that to achieve an appropriate level of assurance from plant inspections, plants should be inspected over two growing seasons. It is therefore considered appropriate that mother plants of budded stumps and budwood should undergo periods pre-export and post entry inspection for signs of SALB before being distributed in the PRA area.

In the case of SALB and the susceptible *Hevea* species, the period of greatest disease expression is at the time of new foliage growth. Therefore budded stumps and budwood should only be harvested from mother plants that have been recently inspected during a period of optimal disease expression and no signs of SALB were detected. To further lessen the likelihood of contamination, harvesting of budded stumps and budwood should only occur when the bark has been hardened (brown in colour) and during the low-disease season (e.g. dry weather). Budded stumps and budwood should be no longer than 1 metre when exported, and the material dipped into a suitable surface sterilant followed by a suitable systemic fungicide. All packaging material should be destroyed on arrival in the PRA area.

During the post-entry quarantine inspection period plants should be maintained in an environment that both stimulates SALB expression and limits the ability of SALB to escape the facility and infect surrounding host plants. No fungicides effective against SALB should therefore be applied to plants during the inspection period (new leaf growth) as fungicides may mask disease expression. SALB containment can be achieved either through the use of high security quarantine facilities or by ensuring no host plants are within 3 km of the boundaries of the facility. To ensure any infected plants are removed from the post-entry quarantine facility as possible, plants should be inspected daily by staff trained to detect signs of SALB infection. Suitably qualified plant pathologists should also inspect the plants every two weeks to verify the daily inspections by facility staff.

If SALB is positively identified in the quarantine facility, all host plants in the facility should be treated with an appropriate fungicide, and another inspection period instigated.

Based on the aforementioned recommendations, the following measures should be applied to budded stumps and budwood before export from the SALB affected country or region, during transport to the PRA area, and on arrival in the PRA area.

### ***Pre-export inspection and treatment***

- Mother plants should be inspected by suitably qualified plant pathologist for signs of SALB infection and deemed to be free of SALB infection. Inspections should take place immediately before the harvesting of budded stumps or budwood and during a period considered optimal for disease expression;
- Harvesting of budded stumps and budwood should only occur when the bark has been hardened (brown in colour) and during the low-disease season (e.g. dry weather). Budded stumps and budwood should be no longer than 1 metre when exported;
- Budded stumps and budwood should be packaged for export in a manner that limits the likelihood of infestation during transport;
- Budded stumps and budwood should be dipped in an appropriate surface sterilant and a systemic fungicide effective against *M. ulmi*;
- Budded stumps should be free from soil.

### ***Measures on arrival (in an appropriately secure facility)***

- Budded stumps and budwood should be dipped in an appropriate surface sterilant and a systemic fungicide effective against *M. ulei*;
- All packaging material should be destroyed or appropriately sterilized, and the budded stumps and budwood repackaged after treatment.

### ***Post-entry quarantine***

- Imported budded stumps and budwood should be grown in a suitable post entry quarantine facility for at least one year or after new foliage has been produced at least six times;
- Plants should be inspected for signs of SALB daily by suitable trained facility staff and fortnightly by suitably qualified plant pathologists;
- Should any signs of SALB be detected, plants showing signs should be destroyed and all other *Hevea* plants within the facility should be treated with suitable fungicide (treatment may require six or more applications);
- Prior to release from the facility all plants in the facility should be inspected by a suitable qualified plant pathologist for signs of SALB infection;
- Plants may be released from the post entry quarantine facility only after having all plants in the facility have been free from any signs of SALB for at least one year or after new foliage has been produced at least six times.

### ***Intermediate quarantine***

Intermediate quarantine offers a further option to mitigate risk. This system can have some logistical, maintenance and financial problems when used for rubber, but it may operate successfully in some specific circumstances.

#### ***1.1.2 Plants in vitro***

Plants *in vitro* should not be considered a risk pathway for the entry of *M. ulei* if the cultures are axenic. However, at the moment the practice is not commercially used.

## **1.2     *Seeds and fruit***

As the risk from seeds and fruit material relates to surface contamination only, all such products exported from SALB regions should be surface sterilized immediately prior to export.

Flowers and fruits should be washed with a surface sterilant such as 200 ppm of sodium hypochlorite (Chee 2006). Only healthy seeds should be selected for export, washed with water and soaked in formalin (5 percent) for 15 minutes, and then air dried and dressed with thiophanate methyl, benomyl or mancozeb (Chee 1978; Santos and Pereira 1986).

## **2.       Management options for non-viable host material**

Non-viable host material is essentially leaves or other parts of a host plant (susceptible *Hevea* species) that are imported either deliberately or as contaminants into the PRA area from countries or areas not known to be free of SALB. These types of host material are not able to be propagated by normal means.

### **2.1     Cargo pathway**

Cargo from SALB infested countries or areas should be screened for goods or shipments that are likely to contain or be contaminated by non-viable host material. A profile list should be established that identifies cargo most at risk of containing non-viable host material.

Cargo such as used machinery (cars, logging equipment, chainsaws, cutters etc.) that may have been used in rubber plantations should be thoroughly steam cleaned of all organic material larger than 1 cm<sup>2</sup>, and dismantled if there are parts that can not be easily cleaned. Household effects should be inspected for gardening equipment that may be contaminated by organic material.

Any organic material that is thought to be from a susceptible *Hevea* species, is larger than 1 cm<sup>2</sup>, and can not be removed from the goods or can not be destroyed (e.g. herbarium material), should be heat treated for a minimum of 30 continuous minutes at 56 °C or greater.

## **2.2 Passenger pathway**

Passengers and accompanied luggage arriving within 21 days from areas not known to be free of SALB should be inspected for both viable and non-viable host material. Special care should be taken with such items as camping equipment and hiking boots, farm equipment, and decorative plant material as these are more likely to contain or be contaminated by non-viable host material greater than 1 cm<sup>2</sup>. Measures may include cleaning, disinfection or destruction.

## **3. Residual risk after measures**

While the measures above, if strictly and effectively enforced, should be expected to manage the phytosanitary risks posed by SALB to the PRA area, it should still be considered possible that slippage (undetected risk items) may result in the establishment of SALB in the region. Efforts should be made to manage this residual risk by establishing an effective monitoring system that would be expected to detect an establishment event early enough to allow for an effective eradication programme to be completed.

### SALB surveillance system

#### 1. Responsibilities of the Survey and Monitoring Officer

- Plan surveys and monitoring programmes
- Produce format and guidelines on survey procedures
- Identify agencies and persons to implement surveys
- Monitor, coordinate and direct all survey
- Report immediately the event of detection of SALB or any other disease and pests not hitherto found
- Assist in the identification of SALB

#### 2. Types of surveillance

There are several types of survey each conducted for different purposes as outlined in ISPM No. 6 *Guidelines for surveillance*. These may include: initial detection surveys, delimiting surveys, monitoring or evaluation surveys and nationwide surveys.

##### 2.1 Detection Survey

The initial detection surveys will be conducted to determine if the disease is present for the first time. A detection survey should include the following components:

###### 2.1.1.1 Areas

Areas that should be inspected include rubber estates, smallholdings and nurseries which are likely to be exposed to:

- travellers and air carriers
- planting materials from SALB endemic areas.

###### 2.1.1.2 Coverage

- points of entry, parcel/post office, tourist routes,  
Any rubber trees found within 2 km from the site
- Rubber estate, small holding and rubber research institute  
All rubber tree growing areas within a plantation/institute.

### *2.1.1.3 Sampling procedure*

All trees in the nurseries should be inspected. For mature plantings, the numbers of sampling point is one (1) every 5 hectares.

### *2.1.1.4 Survey frequency*

The survey in the nurseries should be carried out monthly during the wet season and fortnightly on mature stands during refoliation irrespective of weather.

## **2.2 Farmer-based detection survey**

For rubber growing areas (small holders or plantation-owned), a detection survey must be farmer-based. The owners of these estates or smallholdings shall be provided with leaflets biennially informing them to be vigilant for SALB and to report immediately to the Survey and Monitoring Officer in the event of any suspected presence of SALB in their respective holdings. This instructional and informative leaflet should be prepared and distributed by the Survey and Monitoring Coordinator for dissemination to all estates and smallholders.

## **2.3 Delimiting surveys**

When an infection of SALB is suspected or detected in an area, a delimiting survey should be conducted immediately to determine the extent of the infection. This involves inspection (as outlined under the Sampling Procedure, section 2.1.1.3) of all the surrounding fields starting from the centre of the infected area and extending to a radius of 5 km (beyond the affected areas).

## **2.4 Monitoring/Evaluation surveys**

The purpose of the monitoring and evaluation surveys is to monitor the effectiveness of the eradication measures that have been carried out and to establish whether the disease has been contained, eradicated or has spread to other areas surrounding the infested zone. Hence monitoring and evaluation surveys will have to be conducted once the eradication procedures have been initiated. They need to continue until eradication is declared or until it is determined that eradication is not possible. If the incursion is contained, ongoing monitoring surveys will be necessary.

## **2.5 Nationwide survey**

In the case of a detection of SALB, a nationwide survey should be conducted to determine if the disease has spread or an incursion has occurred in other part of the country. The results may initiate emergency measures to be implemented by other countries.

## **3. Reporting of surveys**

After completion of each survey round, the report from each survey should be submitted immediately to the coordinator. Any suspected detection of SALB should be notified to the NPPO immediately.

### Importation requirements for *Hevea* plant material (This Appendix is for information only)

#### 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 Organization
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 1 m, 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 1 m.
- 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 or 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 sterilized.
- 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 p. 5-8.

## **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 1 m.
- 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 200 ppm 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. Programme 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.

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