SECTION 3

OPERATIONAL GUIDELINES FOR IMPORTATION OF *HEVEA* PLANTS FOR PLANTING TO PROTECT AGAINST SOUTH AMERICAN LEAF BLIGHT

CONTENTS

Contents *(continued)*

Page

OPERATIONAL GUIDELINES FOR IMPORTATION OF *HEVEA* PLANTS FOR PLANTING TO PROTECT AGAINST SOUTH AMERICAN LEAF BLIGHT

1. PURPOSE

The purpose of the Operational Guidelines for Importation of *Hevea* Plants for Planting to Protect against South American Leaf Blight (SALB) is to establish an effective uniform quarantine procedures relating to the protection from SALB. The main objective of the operational guidelines is to reduce the risk of entry and spread of SALB in the APPPC region. The operational guidelines is for Competent Authority (CA) (PQ officers, PQ inspectors, plant pathologists) and other related personnel in the Asia and Pacific Plant Protection Commission (APPPC) rubber growing countries to deal with importation of rubber planting materials from SALB endemic countries.

2. SCOPE

The SOP documents the various certification and measures to be followed by Competent Authority during importation of planting materials from SALB endemic areas. The SOP is divided into several major sections:

- References, glossary, definitions, references;
- Resources;
- Quarantine procedures:
  - Registration and Processing application for importation;
  - Quarantine procedure for pre import requirements;
  - Set/establish requirements and approve PEQ facilities;
  - Inspection of document and consignment at point of entry;
  - Transportation;
  - Handling and Quarantine of planting materials;
  - Surveillance;
- Appendices.

3. REFERENCES

Protection against South American Leaf Blight (2011), RAP Publication 2011/7, FAO of the United Nations, APPPC, Bangkok.

Glossary of Phytosanitary Terms, ISPM No. 5 (2010), FAO, Rome.

Guidelines for Inspection, ISPM No. 23 (2005), FAO, Rome.

Guidelines for a phytosanitary import regulatory system, ISPM No. 20 (2004), FAO, Rome.

Guidelines for surveillance, ISPM No. 6 (1998), FAO, Rome.

Training requirement for plant quarantine inspectors, 2004, APPPC RSPM No. 2, RAP Publication 2004/24, FAO, Bangkok.

Design and operation of post-entry quarantine stations for plants, ISPM No. 34 (2010), FAO, Rome.

4. GLOSSARY OF ACRONYMS AND DEFINITIONS

4.1 Acronyms

APPPC	Asia and Pacific Plant Protection Commission
CA	Competent Authority
IPPC	International Plant Protection Commission
NPPO	National Plant Protection Organization
PQ	Plant quarantine
PEQ	Post entry quarantine
PRA	Pest Risk Analysis
SALB	South American leaf blight

4.2 Definitions

Budded stumps	Planting material produced by grafting bud from a selected scion onto rootstocks.
Bud-grafting/budding	The process of inserting a slice of bark with bud obtained from a scion onto an insertion made on a rootstock to a budded plant.
Buffer zone	An area in which a specific pest does not occur or occurs at low level and is officially controlled, that either encloses or is adjacent to an infested area.
Certificate	An official document which attests to the phytosanitary status of a consignment affected by phytosanitary regulations
Clearance of a consignment	Verification of compliance with phytosanitary regulations
Competent Authority	The national authority with the officially approved responsibility and competency and is responsible to ensure and supervise the implementation importation of planting materials and quarantine measures
Consignment	A quantity of plants, plant products and/or other articles being moved from one country to another and covered when required by a single phytosanitary certificate.
Country of origin	Country where the plants were grown
Detection survey	Survey conducted in an area to determine if pests are present.
Enclosed quarantine facility	Facilities of the station may include glasshouse constructed of breakage-resistant glass or twin-walled polycarbonate, or a laboratory.
Import Permit	An official document authorizing importation of a commodity in accordance with specified phytosanitary import requirements
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.
Intermediate quarantine	Quarantine in a country other than country of origin or destination.
Monitoring survey	Ongoing survey to verify the characteristics of a pest population.
National Plant Protection Organization (NPPO)	Official service established by a government to discharge the functions of the IPPC

Open field quarantine facility	A suitable site, field or nursery where imported consignment released from enclosed PEQ facility are grown and subjected to post entry quarantine measures.
Pest Risk Analysis (PRA)	The process of evaluating biological or other specific or economic evidence to determine whether a pest should be regulated and the strength of any regulatory measures to be taken against it.
Phytosanitary Certification	Use of phytosanitary procedures leading to the issue of a phytosanitary certificate
Phytosanitary Import Requirements	Specific phytosanitary measures established by an importing country concerning consignment moving into that country.
Plants	Living plants and parts thereof, including **seeds** and **germplasm** [FAO, 1990; revised IPPC, 1997]
Plants for Planting	**Plants** intended to remain **planted**, to be **planted** or **replanted** [FAO, 1990]
Point of entry	Airport, seaport or land border point officially designated for the importation of consignment.
Post entry quarantine (PEQ)	Quarantine applied to a consignment after entry
Quarantine pest	A pest of potential economic importance to the area endangered and not yet present there or present but not yet widely distributed and being officially controlled.
Regulated pest	A quarantine pest or a regulated non-quarantine pest.
Survey	An official procedure conducted over a defined period of time to detect the characteristics of a pest population or to determine which pest species occur in an area.
Visual examination	The physical examination of plants, plant products or other regulated articles using the unaided eye, lens, or microscopes to detect pests and contaminants without testing or processing.

5. BACKGROUND

South American leaf blight (SALB) is the most serious disease of rubber. The pathogen of SALB is the fungus *Microcyclus ulei* (P. Henn) v. Arx. The disease had destroyed many early plantations and it is still the main hindrance to the expansion of a viable natural rubber growing industry in Central and South America. SALB prolongs the immaturity period of the rubber plant and reduces latex yield resulting from repeated and prolong defoliation. Several scientists predict that the natural rubber industry in Asia and Pacific region would be decimated within five years on the entry of SALB (references?). The early rubber planters had the vision to introduce the Plant Protection Agreement for the Asia and Pacific region (the Agreement) in 1956. The Article IV and Appendix B of the Agreement that dealt specifically with SALB prohibited 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 except for research purposes.

The Agreement was revised in 1999 to bring it in line with the WTO Agreement on the Application of Sanitary and Phytosanitary Measures. Consequently, a pest risk analysis (PRA) on SALB was done and adopted by the APPPC in 2007. The PRA identified the pathways and vectors, and categorized their risks. Budded stumps and budwood were given high probability of entry high level of risks. The other vectors i.e. the foliage, seeds, fruits and flowers were given low probability of entry and low to moderate level of risk. The SALB PRA recommends general measures for importation of rubber planting materials

from SALB endemic countries into Asia and the Pacific region. The measures are detailed in the APPPC RSPM No. 7 – Guidelines for Protection against South American leaf blight that includes:

- Strict phytosanitary import requirements;
- An inspection system at point of entry;
- A laboratory diagnostic system;
- An efficient surveillance system.

The procedures to implement the above measures are detailed in the current publication.

6. FLOW CHART

This flow chart should be read in conjunction with the work plan of the importation of the budded stump or budwood of *Hevea* sp.

Description	Responsibilities
1. Prepare and submits application a. Propose PEQ facilities	Importers
2. CA Quarantine Facility a. Receive and acknowledge application; b. Register application; c. Process application; d. Investigate pre-export condition; e. Assess proposed PEQ facilities; f. Decide on status of application; g. Inform importer on status of application or for clarification; h. Issue Import Permit and any other relevant documents.	CA
3. Inspect, harvest and treat planting materials in exporting country and transport to importing country. a. NPPO of the exporting countries certifies the planting materials which meet the import requirements	Importer, exporter & NPPO of the exporting and importing countries (need based)
4. Intermediate Quarantine Station (optional) a. Intermediate country must be a non-rubber growing country b. NPPO of the importing country approved the intermediate country c. Same procedures as in point of 5 and 6. d. NPPO of the intermediate country issue a PC	NPPO of the importing country and NPPO of intermediate country
5. Point of Entry a. Inspect outside of the consignment and verify documents at the point of entry b. For non-compliance consignments, the options include destruction, deportation, seeking further clarification or other remedial action c. Transfer the consignment to approved post entry quarantine facility	NPPO

6. For importation from intermediate quarantine country a. Only budwood to be imported b. Quarantine period in enclose post entry quarantine facility for minimum of 3 months or until the first flushing of leaves.	NPPO
7. Post-entry Quarantine Facility a. Inspect consignment b. Destroy packaging materials and unwanted seeds c. Treat seed and budwood d. Germinate seed in seedbeds and transplant seedlings into polybags or carry out bud grafting for budwood e. Inspect seedlings or budded plants for disease symptoms f. Quarantine plants for at least 12 months g. Destroy plants infected with SALB and other quarantine pests h. Plant shall undergo an initial quarantine period in an enclosed quarantine facility until after the second flush of growth then maybe transfer to open quarantine facility for the remainder of quarantine period.	NPPO
8. Surveillance around the PEQ facility for the sign of SALB.	NPPO of importing country

7. LEGAL AUTHORITY

The laws and regulations pertaining to the importation of rubber planting materials from SALB endemic countries shall be established. The regulations must comply and satisfy the international and national requirements. The personnel involved shall have legal mandate and administrative authority to perform the required activities. The Competent Authority (CA) and its personnel will have legal power for their activities and actions taken.

8. MANAGEMENT RESPONSIBILITY

The NPPO will identify a specific Plant quarantine unit and personnel at national and regional levels that will deal with importation of rubber planting materials from SALB endemic countries. The line of command and the duties and functions of an officer at various national and regional levels will be established. The NPPO shall plan and execute measures on importations of rubber planting materials from SALB endemic countries. The NPPO to assess other potential pest of *Hevea* that may be of quarantine concern.

9. RESOURCES

9.1 Trained and qualified personnel

The NPPO shall ensure that adequate, trained and competent personnel to undertake various related activities are available. In addition, a list of local and foreign pathologists well versed on SALB and related subjects shall be kept for consultation and assistance on diagnostic and advisory purposes (if necessary).

9.2 General facilities

The Plant Quarantine Department will be provided with ample office space and relevant facility and equipment for secretarial, data storage, communication and training (Appendix 1).

9.3 Specific Facilities and Equipments

9.3.1 Diagnostic Laboratory

A laboratory to carry out pathological and diagnostic activities will be made available. The laboratory will be equipped with adequate equipment, chemicals and consumables needed to effectively carry out these functions (Appendix 1). Apart for general pathological functions, the laboratory will also have rooms and equipment for serological (ELISA) and molecular diagnostic (PCR) methodologies. The laboratory will also be allocated with a suitable insect proof glasshouse. Laboratory, glasshouse and other facilities should follow the guideline as specified in plant containment facilities requirement (Appendix 9).

(Note: Appendix 9: in draft – Safety in the laboratory code of practice part 3: Bio containment and biosafety in microbiological facility)

9.3.2 Post entry quarantine facilities (Enclosed and open field facilities)

Post entry quarantine (PEQ) facilities will be established in accordance with **Appendix 1 of ISPM No. 34 – Requirement for PEQ Station for Pests** that are highly mobile or easily dispersed (e.g. rust fungi, airborne bacteria).

The enclosed PEQ facility shall be designed and constructed to ensure it is insect-proof and is large enough for carrying out quarantine treatments and maintaining the rubber plants for the quarantine period. The open field PEQ facility (rubber nursery) shall be located in an area at least 3 km away from any rubber plant. The open field PEQ facility shall be secured by a fence.

9.3.3 Intermediate Quarantine

Importation of planting materials that are considered as high risk may undergo intermediate quarantine in non-rubber growing country where SALB is not present.

For importation from intermediate quarantine country:

a. Only budwood to be imported
b. Quarantine period in enclose post entry quarantine facility for minimum of 3 months or until the first flushing of leaves.

The quarantine in the intermediate country must fulfill the following conditions:

Point of Entry

- Inspect outside of the consignment and verify documents at the point of entry
- For non-compliance consignments, the options include destruction, deportation, seeking further clarification or other remedial action
- Transfer the consignment to an approved post entry quarantine facility

Post-entry Quarantine Facility

- Inspect consignment
- Destroy packaging materials and unwanted seeds
- Treat seed and budwood

- Germinate seed in seedbeds and transplant seedlings into polybags or carry out bud grafting for budwood
- Inspect seedlings or budded plants for disease symptoms
- Quarantine plants for at least 12 months in an enclosed quarantine facility
- Destroy plants infected with SALB and other quarantine pests

9.4 SALB Database

The Plant Quarantine Division shall be equipped with equipments and software for data storage and online communication and reporting. The Plant Quarantine Authority will establish a SALB database that contains information on South American leaf blight and its causal pathogen *Microcyclus ulei*, available expertise on SALB, diagnostic laboratories, surveillance system and data, documentation on quarantine procedures and linkages. The database will also contain information on pest control companies preferably those with airplanes or helicopters, suppliers of spraying equipment and chemicals effective against *M. ulei*.

10. QUARANTINE PROCEDURES

10.1 Registration and processing of application

Any importation of *Hevea* plants for planting will be subject to the conditions and the laws of the importing countries. Private application to import *Hevea* plants for planting will also be considered in collaboration with the importing country NPPO.

10.1.1 Register application for importation of planting materials from importers

On receipt of the application for importation of rubber planting materials from an importer, the receiving officer is to verify and record the particulars of the applicant including the name of the applicants in a prescribed form containing the information in Annex 1. If necessary, the applicant will provide other relevant particulars and information required for approval of the importation.

10.1.2 Process application and issue relevant certificates

The application shall be processed by relevant personnel and the personnel shall decide whether to approve or reject the application guided by the rules and regulations for importation of rubber planting materials from SALB endemic countries (Appendix 2 and the work plan). Once the decision to permit importation is made, the relevant PQ officer will inform the importer on the status of the application and issue the Import permits.

10.1.3 Record the application

The application shall be acknowledged, recorded and stored in proper places.

10.2 Certify PEQ facilities

The enclosed quarantine facility need to be registered with the NPPO and certified in accordance with ISPM No. 34: Appendix 1. For the facilities that not registered the following procedure will apply

10.2.1 Preparation and Inspection

The imported planting materials (budwood or seeds) shall be processed and treated on arrival in an enclosed PEQ facility. After two growth flushes, the plants will be transferred to an open field PEQ facility to complete the quarantine period. If the PEQ facilities do not exist, they shall be constructed in accordance to ISPM No. 34. Existing facilities shall be inspected, modifications and renovations made if necessary. The suitability of the facilities for the quarantine purposes of the planting materials will be inspected and certified.

Annex 1. Application for Permit to Import Rubber Planting Materials

1. Particulars of applicant	1.1 Name of applicant: 1.2 Name of Organization: 1.3 Contact address: Telephone: Fax: E-mail: 1.4 Contact person:
2. Particulars of planting materials to be imported	2.1 Type of planting materials and quantity: • Seeds (quantity); • Budwood (quantity); 2.2 Detail information on planting materials: • Species: • Clones: • Wild progeny
3. Purpose of importation	3.1 Objectives
4. Source of planting materials	4.1 Country and states: 4.2 Name of plantation or nursery:
5. Exporter	5.1 Name of Organization: 5.2 Contact address: Telephone: Fax: E-mail: 5.3 Contact person:
6. Shipment itinerary	6.1 Point of entry: 6.2 Dates to import/arrival: 6.3 Direct or indirect importation: 6.4 Carrier:
7. Proposed PEQ facility	7.1 Location of facility 7.2 Specification (include e.g. layout, photo of the facility)

10.2.2 Certification

The suitability of the PEQ facilities shall only be given when the following conditions are met:

- *Location:* The enclosed PEQ facility if possible should be near to the entry point. The PEQ facility should be 3 km away from any rubber plant. The open field PEQ should be easily accessible for inspection;
- *Construction:* The enclosed PEQ facility will meet ISPM No. 34 – Appendix 1. The PEQ facility shall be insect proof and equipped with double doors. The height of the roof should be sufficiently high to accommodate the height of the growing rubber plants and made of transparent materials for light transmission. Details as Appendix 9 (to be refer later);
- *Adequate space:* The PEQ facility shall have sufficient space to accommodate the quantity of the imported planting materials for the required period of quarantine and allow sufficient space for maintenance (watering, fertilization, inspection) and permits good growth of plants without suffering from aetiolation;
- *Security:* The PEQ facility is sited in a safe location. The open field PEQ facility should be adequately fenced;

Items	Comments	Approval
Location		
Construction		
Adequate space		
Security		
Soil fertility		
Drainage, irrigation and watering system		
	Name of officer:	Signature:

10.3 Inspection and Examination of documents and consignment

10.3.1 Inspection of consignment

PQ Inspectors shall examine the necessary documents to ensure that consignments comply with export/ import requirements (Appendix 3 and the work plan). The documents to be examined are:

- Permit to import consignment issued by the NPPO of importing country;
- Phytosanitary Certificate issued by NPPO of exporting country;
- Report on preparation and treatments of planting materials in exporting country;
- Shipment reports;
- Quarantine Inspectors should conduct visual inspection of the consignment i.e. packaging to ensure that they are in good conditions.

10.3.2 Certify and release of consignment

Consignment with documentation that complies with rules and regulations should be released and immediately moved to the PEQ facility.

10.4 Inspection, treatment, processing and quarantine of planting and packaging materials at PEQ facility

10.4.1 Disposal of waste materials

The packaging and packing materials and debris and wastes derived from the budwood (i.e. the remainder of the budwood after the bud eyes had been removed for budding operation), damaged budwood, poor quality seeds and ungerminated seeds shall be disposed accordingly. They shall be burned, autoclaved or soaked for at least 30 min in 10 percent formaldehyde/sodium hypochlorite.

10.4.2 Inspection of planting materials

The planting materials (budwood or seeds) shall be examined and good quality planting materials shall be selected. Damaged and poor quality budwood and seeds shall be disposed. The budwood is considered damaged when the bud eyes are not viable anymore often due to long storage. Selection for good seeds is based on the appearance and weight as good seeds are shiny and heavy and the micropiles are intact. In addition, good seeds will bounce when thrown onto hard surfaces while poor seeds will not bounce.

10.4.3 Treatment of planting materials

The budwood and the seeds shall be treated as follows:

- Dipped in surface sterilant e.g. formaldehyde or sodium hypoclorite solution and treated with a fungicide in Appendix 7

10.4.4 Germination of seeds

The seed germination shall be carried out as follows:

- The seeds shall be germinated in seedbeds or appropriate containers filled with aged sawdust and fine river sand.
- The germinated seeds with sufficient length of radicles shall then be transplanted to designated polybags filled with soil. The seeds that failed to germinate after a month in the seedbeds shall be collected and disposed according to 14.4.2.

10.4.5 Budding

The bud-eyes shall be harvested from budwood and grafted onto rootstock seedlings maintained in the PQ facility for sometimes for acclimatization. The leftover of the budwood shall be disposed as specified in 14.4.2. The budding tapes shall be removed and disposed by autoclaving or sterilized by dipping in formaldehyde/sodium hypochlorite solution.

10.5 Quarantine and inspection

10.5.1 Quarantine period

The plants are to be kept under quarantine for one year or until the plants produce six whorls of leaves.

10.5.2 Inspection and diagnosis of pests and diseases

The plants shall be inspected for disease symptoms as follows:

- The plants shall be examined daily by a trained technician and weekly by a qualified plant pathologist for symptoms of SALB or any quarantine pests and diseases.
- Inspection shall concentrate on the lower surface of the young leaves (reddish in colour) of the plants.
- Record each inspection (dates, pests detected).
- The identification of pests and diseases shall be based on:
  - The characteristic symptoms of SALB on young leaves;
  - The morphology of the spores under a microscope;
  - The diagnostic aids, Appendix 5;
  - In the event of inability to diagnose the disease, a competent authority should be consulted;
- Fungal isolation and culturing may be conducted according to Appendix 6.

10.5.3 Certification and release

At the end of quarantine period the plants shall be released after being certified free of SALB and other quarantine pests and diseases.

- The plant pathologist shall certify that the plants are free of SALB and other quarantine pests;
- If the diseases are not detected during the quarantine period, the plant pathologist shall recommend that the plants are to be released to the importer;
- In the event SALB or any quarantine pests and diseases are detected and confirmed, the infected plants in the PEQ facility shall be destroyed immediately by uprooting and dipping the whole plant in formaldehyde/sodium hypochlorite solution and burned or autoclaved. The other plants that have not shown any disease symptoms should be treated with effective fungicides and continue under quarantine for another 12 months. The importer should be immediately informed;
- The seedlings and budded plants once certified free of SALB and other quarantine pests and diseases will be released.

10.6 Planting and maintenance of plants in open field PEQ

10.6.1 Planting

Plants shall be planted in rows at reasonable distance (3 m × 3 m) to facilitate good plant growth and permits inspection and treatment. The plants will be regularly watered and fertilized.

10.6.2 Inspection

The plants are inspected daily by a trained technician and weekly by a qualified plant pathologist to detect for symptoms of SALB and other quarantine pests and diseases. If the need arises for further confirmation, the leaves or other plant organs with suspected symptoms are sampled, placed in special containers and brought to the laboratory for further diagnosis.

10.7 Disease surveillance

10.7.1 Responsibilities

The NPPO shall appoint a Survey and Monitoring Officer who will be responsible for planning, conducting, monitoring and reporting result of the surveys. The NPPO shall determine that the survey procedures (Appendix 8) are carried out and properly recorded.

10.7.2 Types of surveys

Two types of surveys shall be conducted:

- *Detection survey*. A detection survey shall be initiated following importation of rubber planting materials from SALB endemic countries to determine if SALB occurs for the first time. The survey shall be conducted within 3 km around the point of entry and 3 km around the PEQ facilities. The NPPO shall identify all the rubber plantings (nurseries, estates and smallholdings) within the boundary and the NPPO shall undertake the survey after informing and briefing the stakeholders. The survey should commence one month after arrival of imported plants and shall be continued for one year. The survey will target young leaves. All plants in the nursery shall be inspected fortnightly. For mature rubber, monitoring should be done fortnightly during the leaf change period (wintering) and monthly during normal growth period. The number of sampling points is one point per hectare. In addition, all rubber holding owners and/or operators within the zone shall be supplied with relevant information on SALB. They will inform the NPPO when SALB is suspected.
- *Delimiting survey*. In the event of detection of SALB, the NPPO shall initiate and implement a delimiting survey immediately so as to determine the extent of disease spread.
- *Disease Eradication and quarantine*. The NPPO shall decide and determine the eradication and quarantine measures. The eradication and quarantine measure shall be implemented as soon as possible upon detection of SALB. The procedures for eradication and quarantine of a SALB infected and surrounding area (Appendix 8) shall be adhered.

11. DOCUMENT MANAGEMENT AND RECORD CONTROL

11.1 Document management

The NPPO dealing with importation of rubber planting materials shall adopt these guidelines. The NPPO will document and store relevant information in the database. Any revision and changes made to the guidelines or any other related documents shall be recorded and communicated to relevant personnel and authorities possessing the guidelines. The obsolete documents will be replaced by the revised version. The NPPO will ensure that the documents are easily available to related personnel.

11.2 Record control

The NPPO will maintain the records of relevant activities related to importation of rubber planting materials in special files and folders that includes:

- Particulars on various applications;
- Procedures and treatments undertaken for each importation;
- Records of surveillance activities and technical visits
- Records of pests and diseases intercepted from imported consignments of planting materials or planting materials grown in PEQ facilities that includes diagnostic protocols, and pest and disease identifications (photographs, microscope slides).

The records should be properly compiled and indexed for easy retrieval. The records should be maintained for at least five years.

12. TRAINING

The NPPO will identify personnel entrusted to carry out quarantine activities related to importation of planting materials. The NPPO will review the competency of the personnel, identify the training needs and initiate implementation of training programme that includes funding, target audience, lecturers and training materials, training venues and dates.

Appendix 1

RESOURCES

1. Equipment for secretarial, data storage, communication and training

- Computers and printers;
- Software for data management;
- Reference books;
- Diagnostic and laboratory manuals.

2. General Pathology Equipments

- Major equipments – Autoclaves, incubators, ovens, water distillers, bio-safety cabinets and fume chambers, laminar flow cabinets, refrigerators, weighing balances, microscopes, cameras, pH meters, water baths, shakers, microwave ovens, thermometers, isolation needles, grinders, glassware, hot plates and stirrers, distilled water apparatus, centrifuges.
- PCR and related accessories – Thermocycler, electrophoresis apparatus, gel documentation unit with printer, PCR work station, pipettes, nucleic acid chemicals.
- ELISA and related accessories – Elisa readers
- Disease treatment and disposing equipments – Sprayers, moist heat chambers, dipping tanks, incinerators, pesticides and hot water jet apparatus.
- Chemicals and consumables – Medias (PDA, PSA, MEA etc.), Chemicals for making medias (agar, sucrose, glucose, malt extract, potato extract etc.); Chemicals for sterilization (formalin, mercuric chloride, sodium hypochlorite, alcohols etc.); Glassware (Petri plates, test tubes, flasks, beakers, glass slides and cover slips); Stains (trypan blue or cotton blue, hematoxylin etc.); PCR primers, ELISA antiserum.
- Requirements for sampling and transportation – Cooler boxes, containment boxes.

Appendix 2

IMPORT REQUIREMENTS FOR *HEVEA* PLANTING MATERIALS

(Sourced from "RSPM No. 7: Guidelines for protection against South American leaf blight of rubber")

The NPPO of rubber growing country shall impose or undertake the following import requirements for *M. ulei* host materials:

- Restriction of the quantity of importation (NPPO of importing country to decide)
- Pre-export inspection and treatments in the exporting country by a pathologist;
- Measures applied on arrival at entry point;
- For budwood
  - Restriction of quantity
  - Restricting the length
  - Treating with a surface sterilant and dressing with a systemic fungicide (effective to control *M. ulei*)
- For seeds
  - Restrict the quantities of seeds imported
  - Only healthy seeds to be imported
  - Treating with a surface sterilant and dressing with a systemic fungicide (effective to control *M. ulei*)

Appendix 3

MANAGEMENT OPTIONS FOR VIABLE HOST PLANTS

(Sourced from "Guidelines for protection against South American leaf blight of rubber")

1. Pre-export inspection and treatments:

- Mother plants should be inspected by a suitably qualified pathologist for symptoms of SALB infection. The inspection will be carried out immediately before the harvesting of budwood.
- Only brown budwood should be harvested and the length should not exceed one metre. The budwood should be harvested during low disease season. Budwood should be dipped in a surface sterilant and a fungicide effective against *M. ulei*.
- Budwood should be properly packaged to ensure minimum infestation during export.

2. Intermediate Quarantine

Importation of planting materials that are considered as high risk may undergo intermediate quarantine in non-rubber growing country where SALB is not present.

For importation from intermediate quarantine country:

a. Only budwood to be imported

b. Quarantine period in enclose post entry quarantine facility for minimum of 3 months or until the first flushing of leaves.

3. Measures on arrival:

- The outside of the consignment must be inspected for any unwanted pests.
- *bud*wood and seeds should be dipped in a surface sterilant and a fungicide effective against *M. ulei*.
- The packaging materials and should be destroyed by incinerating them or soaked in a surface sterilant.

4. Post entry quarantine

- The budwood operation using the budwood on preplanted rootstocks shall be carried out in certified PEQ facility.
- The seedlings and budded plants should kept in the PEQ facility for at least a year or until the plants possess six leaf whorls
- Plants should be inspected daily by a trained technician and weekly by a qualified plant pathologist.
- Any plant suspected of being infected by SALB should be immediately destroyed and all the remaining plants should be treated with a fungicide effective against *M. ulei*.

Appendix 4

RESPONSIBILITIES OF NPPO FROM EXPORTING AND IMPORTING COUNTRIES AND THE IMPORTER

(Sourced from "Work plan for the importation of budded stumps or budwood of *Hevea* spp. from (exporting country) into (importing country)")

1. Responsibilities of NPPO of exporting country

- Follow all requirements of the work plan and the import permit issued by the importing country;
- Propagating materials should be harvested from mother plants with no or minimum symptoms of SALB;
- Propagating materials with symptoms of SALB or other diseases will not be exported;
- Supervise harvesting of budwood (brown budwood of less than 1 m long) and budded stumps (without sprouting) during low disease season;
- The nursery where the budded stumps are prepared or where budwood is harvested should be registered;
- Ensure that budded stumps, budwood or seeds are surface sterilized and treated with fungicides effective against *M. ulei*;
- Inspect the requirement and issue a PC certifying that the consignment conforms to the import requirements of the importing country.

2. Responsibilities of NPPO of importing country:

- Issue an import permit specifying the import requirements;
- May elect to send a plant pathologist to the exporting country to inspect the conditions of the planting materials for export and supervise treatments and packing of the planting materials;
- Inspect the consignment on arrivals to ensure they are free of pests and other regulated items;
- Transport the consignment to PEQ facilities in enclosed vehicles;
- Treat planting materials with surface sterilant and fungicides;
- Ensure that the packaging materials and remainder of budwood and non viable seeds are destroyed or sterilized;
- Examine plants for signs of exotic pests and diseases.

3. Responsibilities of Importer:

- The grower will contact the NPPO of exporting country and request them to provide inspection and certification services according to the IP and provide them with the necessary information;
- Abide by all rules and regulations and recommendations of the accredited plant pathologist pertaining to importation;
- Provide details on the source of planting materials and other information required by the NPPO;
- Maintain accurate records of activities and identity and source of planting materials.

Appendix 5

INSPECTION AND DIAGNOSTICS

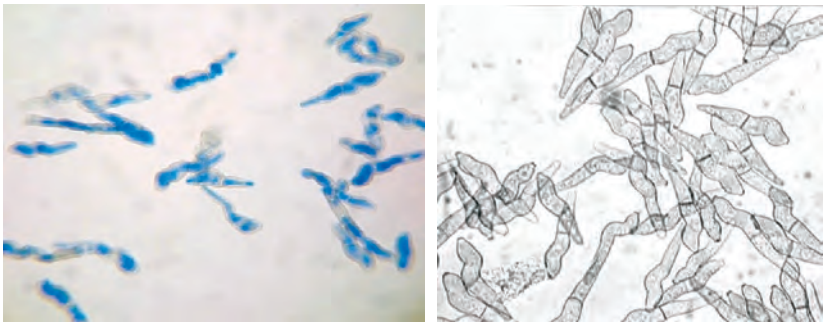
1. Inspection

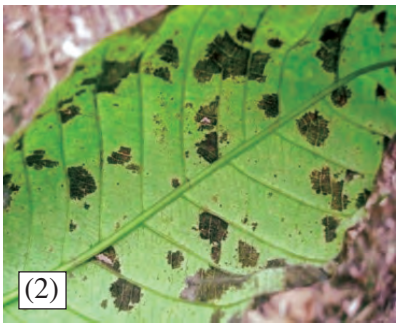
Levels of inspection

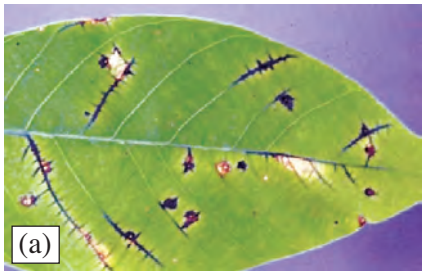
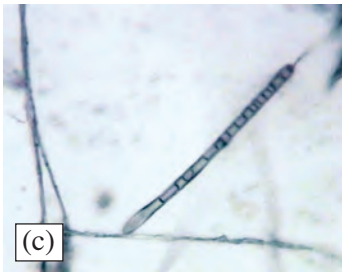
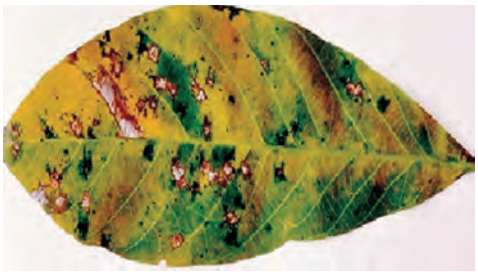
Level	Site	Activity
I	Point of entry	• Inspection of documents; • Visual inspection of imported consignment.
II	Enclosed quarantine facility	• Inspection of seedlings and budded plants for disease and pest symptoms visually or aided by hand lenses, binocular or compound microscopes
III	Open quarantine facility	• Inspection of seedlings for symptoms of diseases and pests visually or aided by hand lenses, binocular or compound microscopes
IV	Laboratory	• Identification of spores • Isolating and culturing of pathogens

Good rubber seeds are shining and reasonably heavy. Good budwood and budded stumps possess viable bud eyes i.e. they are not dead.

2. Diagnostic aids

Disease (Pathogen)	Symptoms
South American leaf blight (*Microcyclus ulei*)	 The conidia of *M. ulei*. They are septate with two cells; the distal cell is narrower than the proximal cell. Observe for the characteristic twist of the conidia.

Disease (Pathogen)	Symptoms
	 (1)  (2) The initial signs of SALB infection are the crinkling and distortion of the young leaflets (1). Later, dark irregular shaped lesions form on the lower surface of leaflets. The lesions are covered with dark colored spores (2).
	  The perithecia appearing as dark raised bodies occur on the upper leaf surface of mature leaflets.
Colletotrichum leaf disease (*Colletotrichum gloeosporioides*)	  The necrotic leaftips of leaflets infected by *C. gloeosporioides* and brown lesions are surrounded by yellow region. The lesions are raised and can be felt when touched.
Oidium leaf disease (*Oidium heveae*)	  White powdery mycelia on upper surface of leaflet

Disease (Pathogen)	Symptoms
Corynespora leaf fall (*Corynespora cassiicola*)	 (a)  (b)  (c)  The leaf spots (a) and herring bone (b) symptoms and the stick-like spore (c). The herring bone is the characteristic symptoms of the disease. On some leaves the symptoms may appear as spots.
Phytophthora abnormal leaf fall (*Phytophthora palmivora*)	 Drops of latex on the darkened lesions on leaf petiole
Fusicoccum leaf blight (*Fusicoccum* sp.)	 Large lesions with concentric rings
Bird's eye spot (*Drechslera heveae*)	 Spots with white centres surrounded by brown region

Appendix 6

ISOLATING AND CULTURING *MICROCYCLUS ULEI*

1. Isolation method

Cultures of *M. ulei* can be obtained by transferring fresh conidia from disease lesions onto growth medium (Chee, 1978; Junqueira *et al*., 1984). The spores should be obtained from young leaves with fresh disease lesions. Contamination is more common if the conidia are obtained from old lesions or from leaves which are harvested on a rainy day. The conidia can be transferred onto the isolation medium in several ways:

(i) Carefully touching the top surface of the lesion with moist tip of an isolating needle and gently placing the conidia onto the surface of the isolation medium agar slants contained in test tubes (Junqueira *et al*., 1984).

(ii) A small piece (2 × 2 mm) of the isolation agar medium is cut and placed on the tip of the isolating needle. The agar block is then gently touched to the top surface of the lesion and transferred onto the surface of the isolation medium (Medeiros, 1973).

(iii) The leaf sections bearing fresh lesions are gently tapped to dislodge the conidia onto the surface of the isolation medium or water agar in Petri plates. With the aid of a stereo microscope, the spores are located and a small piece of agar with the conidia is cut with the aid of a biscuit cutter isolation needle, and transferred onto the surface of the isolation medium. This method is most suitable when fungal culture from a single spore is required. Chee (1978) stated that a good method to isolate *M. ulei* is to deposit fresh conidia on plain water agar and then transfer the spores to potato sucrose medium.

(iv) The culture can also be established from isolation of the ascospores. Leaf section bearing fresh perithecia were stuck to the inner surface of the Petri dish cover. The perithecia are moistened by spraying cold water and the cover is replaced on top of Petri plate containing the isolation medium. The ascospores released onto the medium are identified under a compound microscope and transferred onto the isolation medium using a biscuit cutter isolation needle. Single ascospore culture is obtained using this technique.

(v) The fungus can also be isolated by plating fresh infected leaf tissues on growth medium. Sections of young leaves with fresh lesions are cut into small pieces and surface sterilised in either sodium hypochlorite or mercuric chloride solutions. Holliday (1970) was more successful in isolating the fungus by plating leaf tissues which are cut across the disease lesions than plating leaf sections cut around a disease lesion.

2. Growth Medium

Various media had been used to isolate *M. ulei*. The most commonly used media are water agar, potato dextrose agar and potato sucrose agar. If water agar is used, the culture has to be transferred to richer medium once its growth is visible, usually after three weeks. The main problem usually encountered during the isolation process is the presence of microbial contamination. Bacterial contamination of the culture can be reduced by adding antibiotics e.g. chloroamphenicol at 50 mg/l.

The better medium for culturing *M. ulei* was potato sucrose medium containing 2.5 or 5 percent sucrose. A modified potato sucrose medium amended with multivitamins and minerals was indicated to improve growth of the fungus. The growth of *M. ulei* on artificial medium is very slow. Normally the fungal stroma is visible only after two weeks attaining a diameter of about 2 mm and reaching a diameter of two centimetres in one month.

Sporulation in culture is induced by incubation under alternating light and dark period. Sporulation is also increased by adding vitamins and green coconut water in the medium. For long storage, sporulating colonies of the fungus grown on agar slants in test tubes are submerged in sterile mineral oil, however rejuvenation of these cultures are often very slow. For short term, *M. ulei* can be stored in a medium containing egg yolk. The conidia can also be preserved on infected leaves for several months in dry condition. Some of the conidia remained viable for a few months on leaves stored in a dessicator either at room temperature or in a refrigerator.

Appendix 7

FUNGICIDES EFFECTIVE AGAINST *M. ULEI*

Common name
Triadimefon
Benomyl
Thiophanate Methyl
Mancozeb
Chlorothalonil
Fenarimol
Propiconazole
Triforine
Triadimenol

Appendix 8

DISEASE SURVEYS AND ERADICATION PROCEDURES

1. Detection Survey

- The survey will be conducted to determine if the disease is present for the first time;
- Areas – The areas to be surveyed include rubber estates, smallholdings and nurseries especially those exposed to planting materials imported from SALB endemic countries;
- Coverage – Any rubber holdings within 3 km of entry points and PEQ stations;
- Sampling procedure – All trees in the nursery to be inspected. For mature rubber, one sampling point for every 5 ha;
- Frequency – Monthly for nurseries and fortnightly for mature rubber during the annual leaf change refoliation period.
- Farmer based detection survey – Owners or operators of rubber holdings and nurseries shall be supplied with information on SALB. They will be requested to inform the Survey and Monitoring Officer in the event SALB is suspected.

2. Delimiting survey

A delimiting survey shall be implemented immediately after detection of SALB to determine the extent of infection. The survey should cover 3 km radius from the boundaries of the infested area.

3. Eradication and Quarantine Treatment

The responsibility of the NPPO shall include:

- Inform the stakeholders and higher authorities on the outbreak;
- Carry out a feasibility study on disease eradication;
- Establish and undertake an eradication programme;
- Eradicating infected trees and fallen leaves by felling and burning the trees and spraying of fungicides effective against *M. ulei*.
- If a decision is made that eradication is not feasible, then the disease shall be contained
- Quarantine the affected area by restricting human and vehicle movement within and out of the affected area.
- Verify effectiveness of eradication or disease containment.

4. Monitoring surveys

The purpose of the monitoring survey is to verify the effectiveness of the eradication procedures and to determine whether the disease has spread to new areas. The survey shall commence when the eradication procedure is conducted and shall be continued until the disease is eradicated or until a decision is made that eradication is a futile exercise.

SALB diagnostic laboratories

Development of Diagnostic Laboratories for SALB of Rubber(potential)

Country	Laboratory	Containment Level	Test that can be perform	Contact Person
China				
India	Rubber Research Institute of India, Kottayam, Kerala.	Quarantine Level 2	Cultural and Morphological Test Serological Test Molecular Test – PCR Sequencing for DNA	Dr James Jacob Director Rubber Research Institute of India Rubber Board, Kottayam, 686009 Kerala, India Tel: +914812353311 E-mail: james@rubberboard.org.in
Indonesia	Centre for Plant Quarantine Laboratory, Jakarta	Quarantine Level 3	Cultural and Morphological Test Serological Test Molecular Test – PCR Sequencing for DNA	Dr Eliza S. Rusli Deputy Director Plant Quarantine Seed Division Indonesia Agriculture Quarantine Agency Tel/Fax: 6221 781 6482 E-mail: eliza_rusli@yahoo.com
	Crop Protection Laboratory, Medan (North Sumatera)	Quarantine Level 1	Cultural and Morphological TestMolecular Test – PCR	Mr Nurnowo Paridjo Director Estate Plant Protection Ragunan, Jakarta, Indonesia Tel: (Mobile): 08128010778 Fax: 62217815684 E-mail: nurnowa_paridjo@yahoo.com
	Rubber Research Laboratory, Medan (North Sumatera)	Quarantine Level 1	Cultural and Morphological Test Serological Test Molecular Test – PCR	Dr Karyudi Centre for Rubber Research Sei Putih Galang, Deli Serdang, North Sumatera Tel: (Mobile): 08126559566 Fax: 62617980046 E-mail: karyudi@indoset.net.id

Country	Laboratory	Containment Level	Test that can be perform	Contact Person
Malaysia	PEQ Laboratory, Serdang, Selangor	Quarantine Level 2	Cultural and Morphological Test Serological Test Molecular Test – PCR	Mr Yusof Othman Deputy Director Crop Protection and Plant Quarantine Division, Tel: +603-26977180 E-mail: yusofothman@doa.gov.my
	Crop Protection and Plant Quarantine Laboratory Division, Kuala	Quarantine Level 1	Cultural and Morphological Test Serological Test Molecular Test – PCR Sequencing for DNA	
	LumpurMalaysian Rubber Board (LGM), Sungai Buloh, Selangor	Quarantine Level 1	Cultural and Morphological Test Serological Test Molecular Test – PCR Sequencing for DNA	
Philippines	Post Entry Quarantine Station, Los Banos, Laguna	Quarantine Level 3	Cultural and Morphological Test Serological Test Molecular Test – PCR	Mr Clarito M. Barron, Ph.D BPI Director E-mail: cmbarron@ymail.com
	University of the Philippines, Los Banos, Laguna	Quarantine Level 3	Cultural and Morphological Test Serological Test Molecular Test – PCR	
Sri Lanka				
Thailand	Plant Quarantine Research Group Laboratory, Plant Protection Research And Development Office, Bangkok, Thailand	Quarantine Level 2	Cultural and Morphological Test Serological Test Molecular Test – PCR	Mr Surapol Yinassawapan Director Plant Quarantine Research Group Plant Protection Research and Development Office Bangkok 10900 Thailand Tel: +662 940 6670 ext. 109 Fax: +662 579 8516

Country	Laboratory	Containment Level	Test that can be perform	Contact Person
Vietnam	Central Laboratory for Plant Quarantine, 149 Ho Dac Di, Dong Da, Hanoi, Vietnam	Quarantine Level 3	Cultural and Morphological Test Serological Test Molecular Test – PCR Sequencing for DNA	Dr Duong Minh Tu Director Tel: (84) 4 904101090 E-mail: duongminhtu60@gmail.com
	PEQ1 Laboratory, Dong Ngac, Tu Liem, Hanoi, Vietnam	Quarantine Level 2	Cultural and Morphological Test Serological Test Molecular Test – PCR	Dr Nguyen Quy Duong Deputy Director Tel: (84) 4 989589477 E-mail: kdtvsnk1@vnn.vn
	PEQ2 Laboratory, 28 Mac Dinh Chi, District 1, Ho Chi Minh City	Quarantine Level 2	Cultural and Morphological Test Serological Test Molecular Test – PCR	Dr Nguyen Huu Dat Director Tel: (84) 8 903775574 E-mail: kdtvsnk2@vnn.vn
	Rubber Research Institute Laboratory, Ho Chi Minh City	Quarantine Level 2	Cultural and Morphological Test Serological Test Molecular Test – PCR	Mr Phan Thanh Dung Deputy Director Tel: (84) 8 918320888 E-mail: ptdrriv@gmail.com

Country	Laboratory	Containment Level	Test that can be perform	Contact Person
Other Asian and Pacific Countries				
Bangladesh				
Brunei Darussalam				
Cambodia				
Lao PDR				
Myanmar				
Papua New Guinea				

Notes:

- Infectious materials cannot moved between APPPC rubber growing country
- For diagnostic purposes, DNA of *M. ulei* should be extracted in the country of detection and this can be moved to other country which have the facility
- For identification and confirmation of pathogen, the extracted DNA may be sent to laboratories in non rubber growing countries or country endemic to SALB such as CAB International Laboratory or laboratory in a country such as Brazil.
- APPPC should write to NPPO of Brazil seeking their agreement to provide SALB identification services
- Rubber growing countries of APPPC should have close collaboration in the event of an incursion as a formal agreement (to be initiated by APPPC)
- It was noted that minor producing countries of APPPC may need assistance in developing the capacity to manage incursion of SALB especially in the area of diagnostic, surveillance and incursion response
- General awareness workshop should be conducted by APPPC inviting all rubber growing countries with their statistic
- All laboratory handling infectious SALB materials should be at least at quarantine level 2 and preferably at quarantine level 3

FAO Regional Office for Asia and the Pacific
39 Phra Atit Road, Bangkok 10200, Thailand
Tel: (66 2) 697 4000 Fax: (66 2) 697 4445
E-mail: FAO-RAP@fao.org
Website: http://www.fao.org/asiapacific

ISBN 978-92-5-107228-8

9 789251 072288

I2730E/1/05.12