

# **SOUTH AMERICAN LEAF BLIGHT (*Microcyclus ulei*) OF HEVEA RUBBER**

**Ismail Hashim**

## **1. INTRODUCTION**

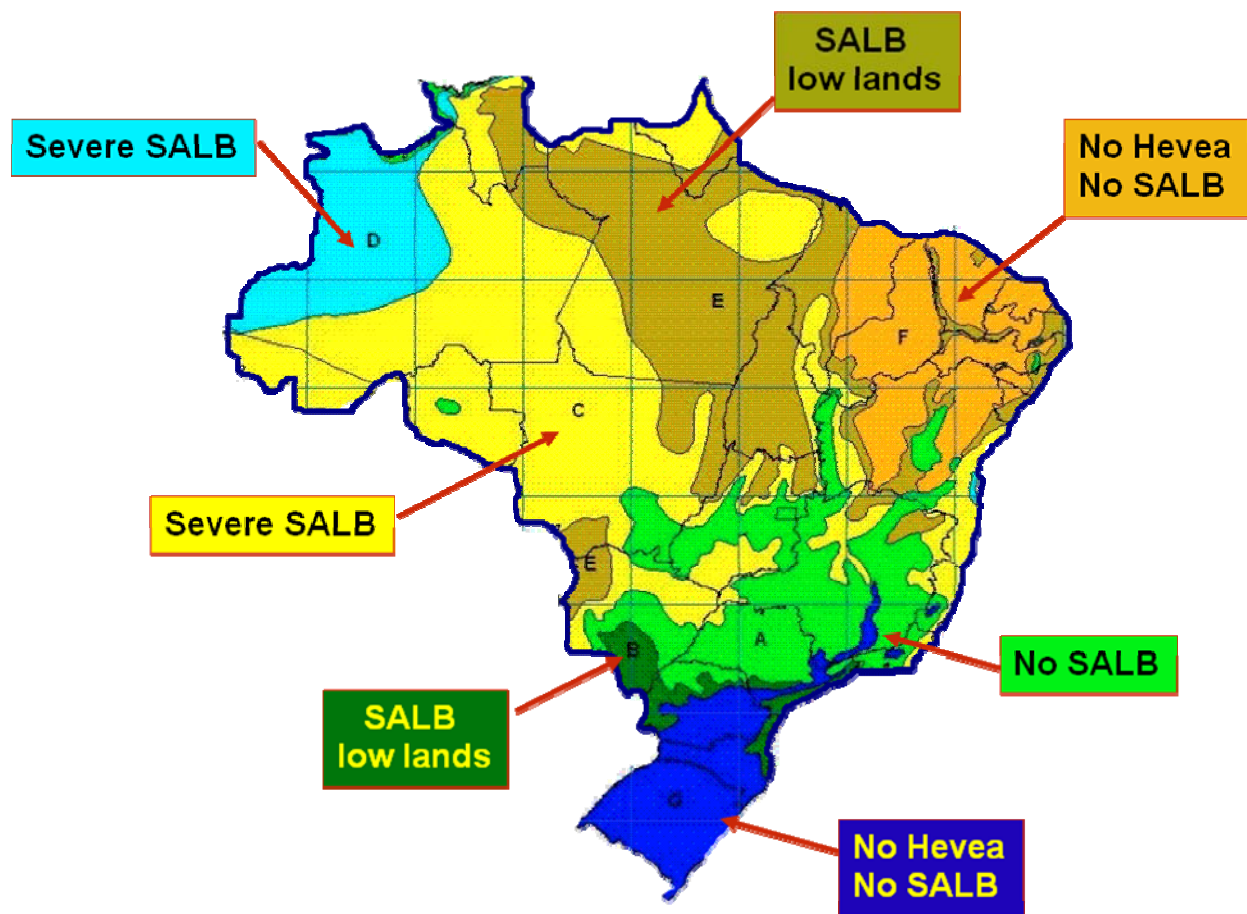
South American leaf blight (SALB) is the most serious disease of the rubber tree due to its devastating effects. Historically, SALB had destroyed several rubber plantations established in the 1930s in Central and South America. Until today, it is the most important factor limiting a vibrant rubber planting industry in tropical Central and South America where the disease is endemic (Lieberei, 2007; Sambugaro, 2003). The Asian rubber growing countries that produce more than 90% of the world's natural rubber are very concerned of the threat of SALB (Rao, 1973a; Edathil, 1986; Jayasinghe, 1992; Soepadmo, 1975). This is because the climatic conditions in these major rubber producing countries are conducive to serious SALB infection (Chee, 1980b; Rao, 1973b; Silva, 2007). These countries are taking serious quarantine actions to reduce the chance of introduction of the disease (Aziz, 1976; Chee, 1985; Rao, 1973a). The Asia Pacific Plant Protection Commission (APPPC) was established in 1956 based on APPP agreement which includes measures to protect the region from SALB. These actions had been useful in preventing the entry of SALB into the Asian rubber growing countries (Thurstun, 1973). In addition, efforts are being taken to increase knowledge of relevant pathologists and quarantine officers.

This publication briefly reviews the biology of SALB and its causal agent *M. ulei* (P. Henn.) v. Arx. The publication will be a useful guide to plant pathologists and plant quarantine officers who are dealing with SALB.

## **2. DISTRIBUTION OF SALB**

The disease was first detected in the early 1900s on rubber plants from the Amazon jungle. The disease later spread from the Amazon forest to cultivated rubber in other areas within the Americas in tandem with the expansion of monoculture rubber cultivation in large holdings or plantations. It was detected in Guyana in 1910, Trinidad in 1916, Venezuela in 1944, Costa Rica in 1935 and Mexico in 1946. Hilton (1955) presented a detailed account on the early cultivation of rubber in Central and South America and the occurrence and destruction of SALB in these countries.

The SALB region is now confined to the American tropics from Mexico to the north and Brazil to the south. The disease is now present in Mexico, Guatemala, Panama, Honduras, Belize, Costa Rica, Nicaragua, Trinidad and Tobago, Haiti, Dominican Republic, Guyana, French Guiana, Surinam, Venezuela, Colombia, Peru, St Lucia, Ecuador, Bolivia, El Salvador, Paraguay and Brazil. (Commonwealth Mycological Institute, 1975; Compagnon, 1976; Hilton, 1955; Holliday, 1970b). In Mexico, SALB occurs at Vera Cruz, Oaxaca and Chiappas regions about 400 km from Mexico City (Rivano, 2004). In Brazil, SALB is particularly serious in the hot and humid Amazon region and also in the states of Bahia and Espirito Santo (Bergamin Filho, 1984). SALB is less serious in the states of Sao Paulo and Mato Grosso as the climatic condition in these states is less favorable for SALB due to the longer dry period and lower rainfall (Campanharo *et al.*, 2011, Holliday, 1970b). SALB is serious in areas with high annual rainfall (about 2500 mm) with long period of high humidity (>80% R.H.) with no distinct dry period for several months (Holliday, 1970b).



Map showing the distribution of SALB

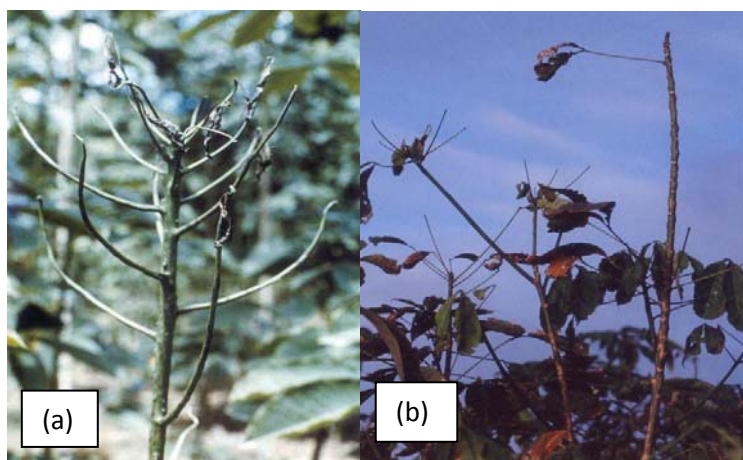
### 3. SYMPTOMS OF SOUTH AMERICAN LEAF BLIGHT

#### 3.1 Young leaves

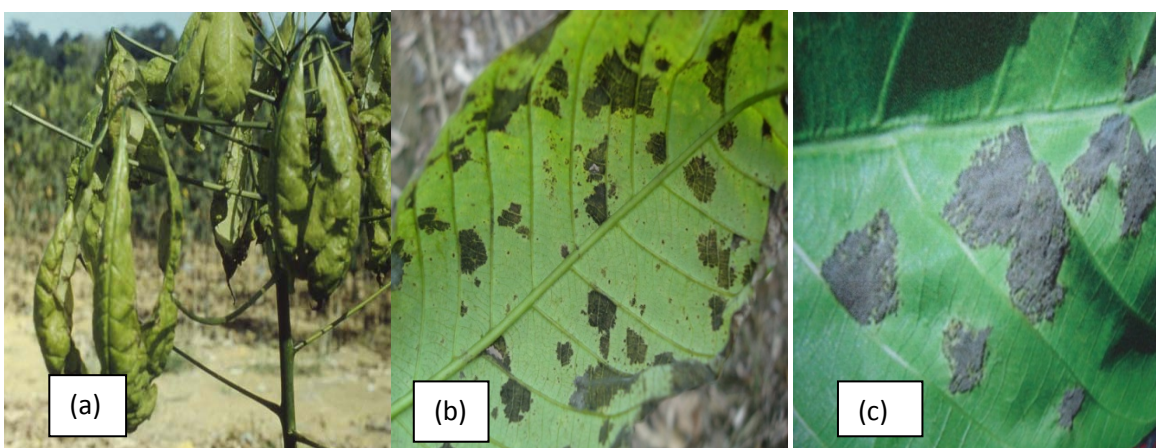
Shortly after infection of young rubber leaflets, the first visible symptom is the distortion in shape of the leaflets (Figure 1). Two to 12-day-old leaves showed symptoms of SALB about 2-3 days after inoculation (Blazquez and Owen, 1957). Heavily infected susceptible leaflets shrivel, turn black and drop off (Figure 1b). The petioles remain on the stem for several more days before they also drop off (Figure 2). A few days after infection, irregular-shaped disease lesions develop on the undersurface of the young brown-colored leaflets. Then, the lesions produce abundant conidia and appear dark to olive green in colour (Figure 2). The size of lesions and the amount of conidia produced are influenced by the age of leaflets, the susceptibility of the clones and the prevailing weather conditions.



**Figure 1. Symptoms on young leaves: (a) Deformation of young leaflets; (b) Severely infected leaflets that had shriveled and turned black.**



**Figure 2. Defoliation of leaves: (a) Petioles remain intact on the branches for sometimes; (b) Shoots without leaves.**



**Figure 3. Symptoms on intact young leaves: (a) Distorted infected leaves; (b) and (c) Conidial lesions on the lower surface of the leaves.**

### 3.2 Immature green leaves

About two to three weeks after infection started, the leaf tissues on the upper surface of leaf immediately above the disease lesions on the lower leaf surface turn yellowish and later small round black raised structures called the pycnidia are formed (Figure 4). The pycnidia are 120-160  $\mu\text{m}$  in diameter and these fruiting bodies produce the pycnosporos.

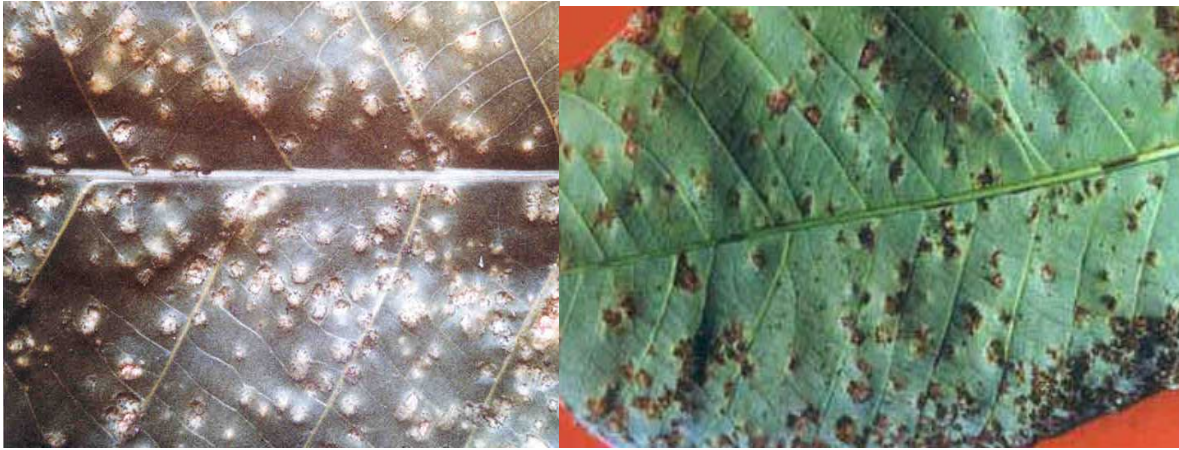
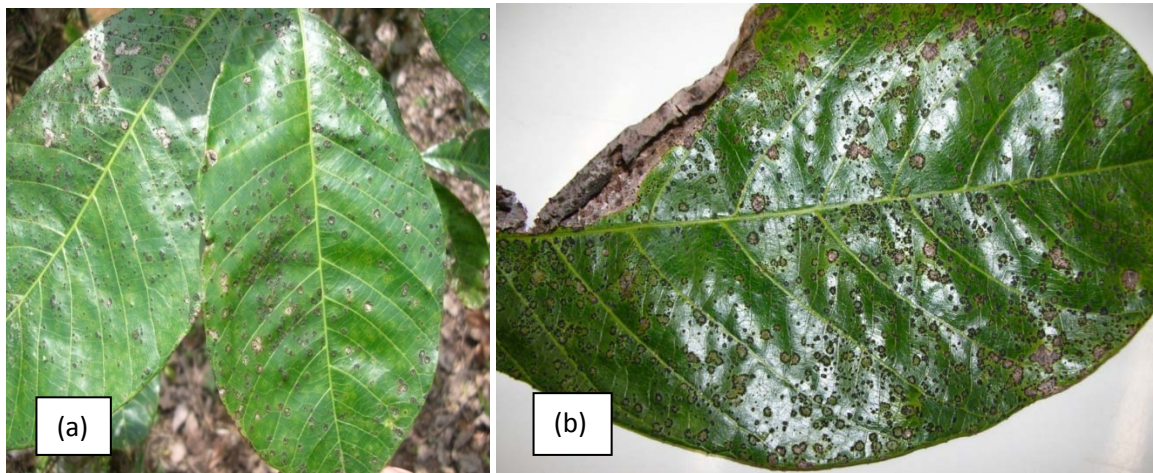


Figure 4. The pycnidia on the upper and lower leaf surfaces

### 3.3 Mature green leaves

Several weeks later, the round dark raised structures enlarge and form another dark colored raised bodies called the perithecia especially around the edges of the disease lesions (Figure 5). The perithecia produce ascus that bears the ascospores. The number of perithecia varies with severity of infection and susceptibility of leaves. In certain cases, the whole upper surface of the lamina is covered with numerous perithecia (Figure 5b). As the leaf ages, the leaf tissues at the centre of the lesions die, turn papery white and later tear off leaving shot-holes in the leaf (Figure 6).



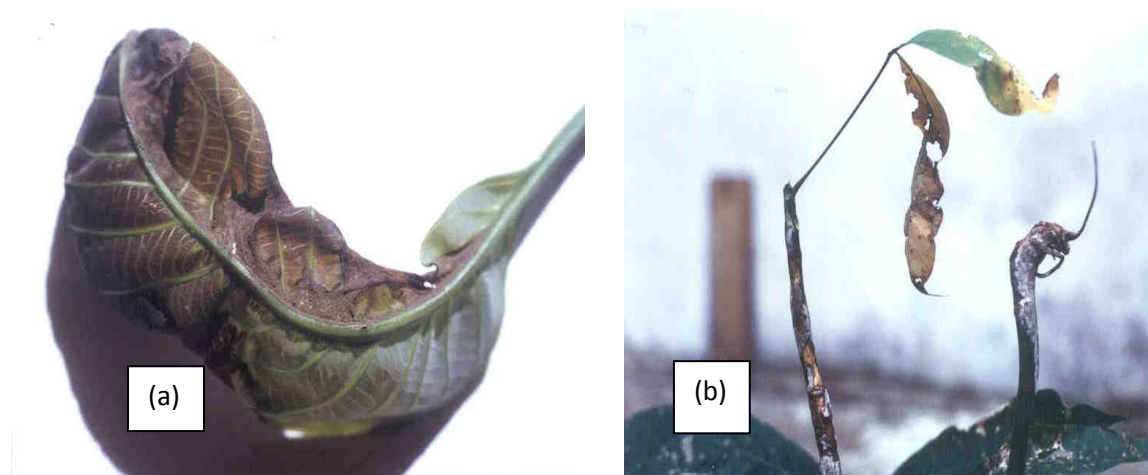
**Figure 5. The perithecia on the upper surface of mature leaves**



**Figure 6. Symptoms on old leaves showing the shot holes formed following necrosis of tissues at the centre of the perithecia**

### **3.4 Other plant parts**

*M. ulei* also infects other parts of the plant and the symptoms on the inflorescence, petiole, stem and fruits are as shown in Figure 7. Infection of the stem may cause tip dieback (Figure 7b).



**(a) Infection on lamina and midrib (b) Infection on young shoot causing shoot dieback**



(c) Infection on leaf petiole



(d) Infection on inflorescence



(e) Infection of young pods

**Figure 7. Infection on other parts of rubber plants**

The canopy density of trees severely infected by SALB is poor and the tree has dead branches (Figure 8). Severely infected plants through time may also die.



**Figure 8. Severely infected trees: (a) Trees with poor canopy density (b) dead trees**

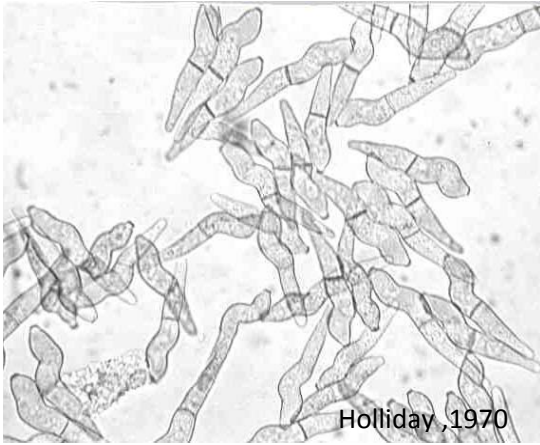
#### **4. THE PATHOGEN (*Microcyclus ulei*)**

The pathogen of South American leaf blight (SALB) is the obligate fungus *Microcyclus ulei* (P. Henn.) v. Arx. The fungus was previously known by other names such as *Dothidella ulei* P. Henn., *Melanopsammopsis ulei* (P. Henn.) Stahel, *Fusicladium macrosporum* Kuyper (refers to the conidial state of the fungus) and *Aposphaeria ulei* P. Henn. (refers to the pycnidial state of the fungus). It was shown that the various names actually refer to the same fungus. The identification and the related historical development on naming the fungus were extensively presented by Hilton (1955) and Holliday (1970a; 1970b).

##### **4.1 Spores**

*M. ulei* is in the group Ascomycetes and the fungus produces three types of spores in sequence viz. the conidia (Figure 9a), the pycnospores (Figure 9b) and the ascospores (Figure 9c). The conidia are produced abundantly during the asexual stage while the pycnospores and the ascospores are produced during the sexual stages of the fungus. The conidia are mainly two-celled with a broad proximal cell and a tapered distal cell. The unique character of the conidia is that they are twisted. Various sizes of the conidia had been reported (Table 1). The size of the conidia varied with location and season. The conidia, sometimes, have only one cell and the one-celled conidia are more common during dry weather conditions and in laboratory cultures.

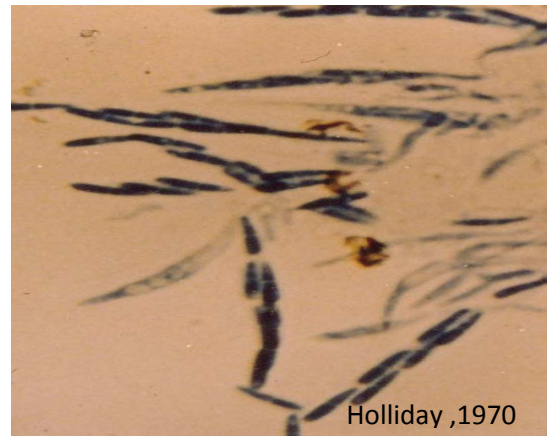
The pycnospores are dumbbell shape and small (6-10  $\mu\text{m}$  long and 2-5  $\mu\text{m}$  in width). The ascospore is oblong shaped and is made up of two cells of unequal size. The size of the ascospores also varies (Table 1).



**(a) Conidia with two cells**



**(b) Pycnospores**



**(c) Ascospores**

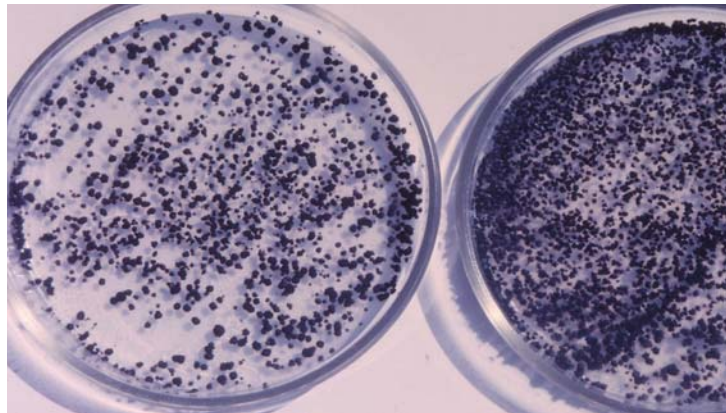
**Figure 9. Spores of *M. ulei***

**TABLE 1. SPORES OF *M. ULEI***

Spore	Description	Size (Reference)
Conidia	Mostly septate with two cells with broader proximal cell, dark grey to olive green in colour, twisted; sometimes unicellular.	<ul style="list-style-type: none"> <li>• 12-30 x 5-8 µm (Langford, 1945)</li> <li>• 23-65 x 5-10 µm (Holliday, 1970b)</li> <li>• 23-65 x 5-10 µm for septate and 15-34 x 5-9 µm for aseptate conidia (Chee and Holliday, 1986)</li> </ul>
Pycnospores	Dumbbell-shaped with the ends twice the width of the centre.	<ul style="list-style-type: none"> <li>• 6-10 µm (Holliday, 1970b)</li> <li>• 6-10 µm (Chee and Holliday, 1986)</li> </ul>
Ascospores	Septate with two cells of unequal size, ellipsoidal, hyaline	<ul style="list-style-type: none"> <li>• 3-5 x 10-15 µm (Langford, 1945)</li> <li>• 2-5 x 12-20 µm (Holliday, 1970b)</li> <li>• 2-5 x 12-20 µm (Chee and Holliday, 1986)</li> </ul>

#### 4.2 Culture of *M. ulei*

Eventhough *M. ulei* was earlier termed as an obligate parasite, the fungus has been successfully isolated and cultured on artificial media. Various media had been developed that contained special additives such as leaf extracts, vitamins, coconut water, etc. (Blazquez and Owen, 1957; Chee, 1978b; Langdon, 1966; Langford, 1945; Mattos, 1999; Medeiros, 1977). The potato sucrose medium supports good growth of *M. ulei* (Chee, 1978b). The growth of the fungus is very slow and forms stroma either raised above the surface of the medium or flattened along the media surface (Figure 10). Conidia are produced on artificial medium especially on special medium for spore production (Chee, 1978b, Junqueira *et al.*, 1984; 1987; Mattos, 1999). Exposure to intermittent light and dark periods effectively enhanced conidial production (Chee, 1978b).



**Figure 10. Stroma of *M. ulei* in culture**

### 4.3 Physiological races of *M. ulei*

Several physiological races of *M. ulei* exist and the occurrence of new races of *M. ulei* had caused breakdown of resistance of certain clones. Variability in the fungus was observed by several earlier scientists (Langdon, 1965; Langford, 1945). The existence of four races of *M. ulei* was established by Miller (1966). Miller also established a set of clones to differentiate Race 1, Race 2, Race 3 and Race 4 of the fungus. More races and other physiological strain of *M. ulei* were described. Chee *et al.* (1986) differentiated nine races of *M. ulei* from Bahia, Brazil using a set of differential clones. Later, Ismail and Almeida (1987) confirmed the existence of four races (Race 2, Race 3, Race 4, and Race 6) of *M. ulei* in Bahia. A more virulent strain of *M. ulei* was reported in Trinidad and Tobago (Chee, 1978; Liyanage and Chee, 1981). More variability in the population of *M. ulei* was indicated in Brazil (Furtado *et al.*, 1995; Junqueira *et al.*, 1986; Mattos *et al.*, 2007), French Guiana (Rivano, 1997) and Mexico (Cano, 1997). Three physiological groups of *M. ulei* were separated among the 16 isolates of *M. ulei* studied (Junqueira *et al.*, 1986). Rivano (1997) identified seven 'virulence factors' and differentiated 11 physiological races of *M. ulei* among the 16 isolates used. Gasparotto and Junqueira (1994) indicated the existence of ecophysiological variability among isolates of *M. ulei*. Until today, the number of physiological races of *M. ulei* is not certain. Mattos *et al.* (2007) identified 36 variations in infection types from isolates obtained in Bahia state using a set of differential clones. The number of strains of *M. ulei* may be as large as 50 (Pinheiro, 1995). However, there is no doubt that several races, more than four, of *M. ulei* exist nowadays.

### 4.4 Viability of spores

The viability period of the spores is influenced by weather conditions especially moisture and temperature. The spores in their fruiting bodies remain viable for longer period than detached spores. Several specific studies were conducted to determine the viability period of spores at different temperature and humidity. These studies indicate that the spores could survive for a reasonably long period (Table 2). Under moist conditions at 24 °C, the perithecia on green leaves were viable for 12 days and for nine days for those on fallen brown leaves (Chee, 1976d). Conidia placed on glass slides and maintained at 24 °C and at 65 to 95% relative humidity (RH) for three weeks still germinated (Chee, 1976d). The percentage germination of the conidia varied with the period of storage whereby 12 to 27 % of the conidia germinated after one week and the percentage germination declines to 3 to 6% after three weeks of storage. Detached conidia remained viable for about nine days at 65% RH and for six days at 80 to 90% RH (Chee, 1976d).

**TABLE 2. VIABILITY OF SPORES AND PERITHECIA OF *M. ULEI***

Spores	Storage condition	Viability period
Conidia (detached)	24 °C and 65-95% RH	3-4 weeks
Conidia (intact)	24 °C and 85 to 100% R.H.	2 weeks
	24 °C and under dessication.	16 weeks
Ascospores	24 °C and 85 to 100 % R.H.	9 days
Perithecia	24 °C and 65% R.H.	3 weeks
	24 °C and 100 R.H.	12 days

Chee, 1976d

It is interesting to note that the spores stored under cold and dry condition survived for a longer period. The conidia and ascospores obtained from leaves stored in a refrigerator for a reasonably long period

still germinated. Spores on glass slides stored under desiccation for 16 weeks still germinated (Chee, 1976d). The ascospores kept under desiccation survived for 15 days (Chee, 1976d). In fact, spores stored under freezing temperature (-74 °C) still survived (Lebai-Juri, 1995). Under dry condition, the conidia shrivel (Figure 11) and become turgid under high moisture.



**Figure 11. Shrunken desiccated conidia**

In another study, the spores remained viable for a certain period when they are deposited on common materials (Zhang *et al.*, 1986). The conidia deposited on paper, glass, leather and cloth for a week still germinated with 5.8 -31.5% germination (Table 3). Some of the conidia deposited in soil for 10 days still germinated.

**TABLE 3. VIABILITY OF CONIDIA OF MICROCYCLUS ULEI  
STORED ON SELECTED MATERIALS FOR SEVEN DAYS**

<b>Materials</b>	<b>Germination (%)</b>
Cloth	31.6
Plastic	29.3
Leather	26.5
Glass	26.0
Metal	6.3
Paper	5.8
Rubber leaf	21.0

Zhang *et al.* (1986)

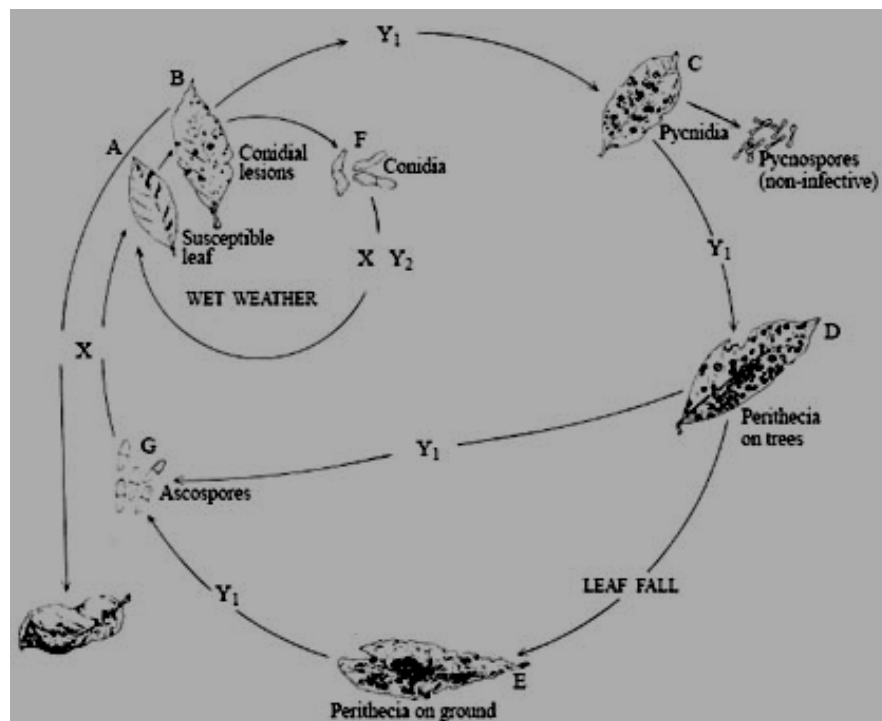
## 5. DISEASE SPREAD AND INFECTION

### 5.1 Disease dispersal

The spread of SALB is attributed to wind and rain splash (Holliday, 1970; Liyanage, 1981). Wind-borne spores had also been accredited to the spread of the disease from the natural habitat to the early cultivated rubber of Brazil (Hilton, 1955). Insects and other animals may also spread the disease locally (Chee, 1980a).

### 5.2 Spore germination

The conidia obtained from disease lesions germinate readily under moist condition. In distilled water, conidia germinated within one hour and most of the conidia germinated within three hours (Holliday, 1970). The distal cell germinated first and followed later by the proximal cell. Similarly, the ascospores germinated within two to six hours (Chee, 1976). On detached leaves, the conidia germinated three hours after inoculation (Blasquez and Owen 1963; Ismail *et al.*, 1978; Kajornchaiyakul *et al.*, 1984). Spore germination is influenced by weather conditions. The optimum temperature for germination of conidia and ascospores is 24 °C (Chee, 1976). However, conidia and ascospores germinated between 14-36 °C and 14-29 °C respectively.



Disease cycle of SALB (from Chee & Holliday 1986)

### 5.3 Epidemiology

Epidemiology studies indicate that spore production, spore liberation and infection vary with weather conditions (Chee, 1976c; Gasparotto *et al.*, 1989; 1991; Holliday, 1969; Rocha and Filho, 1978). The optimum temperature for spore production is 24 °C and high humidity favours sporulation. Maximum

infection occurred at 24 °C and less at 18 °C. Infection was high at 100% RH as compared to 65% RH (Chee, 1976c).

There is diurnal periodicity in liberation of conidia. The number of conidia liberated was low at night and the number started to rise in the early morning and reached a peak at midday and declined thereafter. In Trinidad and Tobago, the peak was at about 10 00 h (Chee, 1976c; Holliday, 1969) while in Brazil, the peak was at noon (Rocha and Filho, 1978). Conidia liberation is influenced by rainfall as the amount of conidia released increased after a rain (Chee, 1976c; Holliday, 1968). In the case of the ascospore, on a dry day, ascospore release is higher at night reaching a peak at 06 00 h. On a wet day, ascospores are also liberated especially after rain.

#### **5.4 Disease infection**

The conidia and the ascospores are responsible for infection. The pycnosporangia had not caused infection following artificial inoculation though the pycnosporangia germinated *in vitro* (Holliday, 1970). The histology of disease infection has been studied (Blazquez and Owen, 1963; Ismail *et al.*, 1978) and summarized by Lieberei (2007). Following germination, the hyphae may penetrate directly through the cuticle into the epidermal layer or the hyphae may first form appressoria from which the hyphae penetrate into the epidermal layer. The reaction of the host to infection is influenced by the degree of resistance of the leaves. In susceptible leaves, the fungus spreads intercellularly in the leaf. However, in certain highly resistant or immune clones, disease spread was inhibited by hypersensitive host cell collapse accompanied by discoloration due to accumulation of phenolic compounds (Berger, 1992; Figari, 1965; Giesemann, *et al.*, 1986; Martains, *et al.*, 1970 and Pita *et al.*, 1992).

### **6. ECONOMIC IMPORTANCE AND DISEASE CONTROL**

#### **6.1 Economic importance**

SALB is the most serious disease of the rubber plant. Its economic destruction has been shown during the early attempts to establish rubber plantations in Brazil in 1930s and 1940s (Table 4). Ford Motor Co. started to establish a large plantation at Fordlandia in 1928 in Brazil. The planting materials were seeds obtained from the regions around Tapajos, Solimoes and Machado Rivers (Goncalves *et al.*, 1983). Soon after the establishment of the plantation, the rubber plants were seriously infected by SALB. Then, the company abandoned this plantation in 1933 and subsequently established another plantation at Belterra in 1934 and by 1942, about 6,570 hectares had been planted using local as well as oriental materials. This plantation was also seriously infected by SALB. The severe infection by SALB forced Ford Motor Co. to abandon these two plantations. It is interesting to note that these plantations were destroyed and abandoned about seven years after their establishment. Several other international companies attempted to establish rubber plantations. Goodyear Company established a plantation in Panama and also in Brazil at Belem, Para and Una, Bahia. Firestone Company established its rubber plantations in Bahia and eventually this plantation was sold to Michelin Company and nowadays Michelin is the only international company operating a large rubber plantation in Brazil specifically in Bahia and Mato Grosso states. Thus, SALB is still the limiting factor to natural rubber cultivation in Central and South America. Rubber cultivation in Brazil expanded especially under a special programme called PROBOR. From 1967 to 1986, about 150,000 ha of rubber were cultivated. Unfortunately, it was reported that in 1986, about 100,000 ha was infected by SALB and the project was prematurely terminated.

**TABLE 4. HISTORICAL DESTRUCTION OF RUBBER CULTIVATION BY SALB**

Country	Fate of earlier plantations
Brazil	<ul style="list-style-type: none"> <li>• Ford Motor Co. established a plantation of 3,200 ha at Fordlandia in 1928 and the plantation was abandoned in 1933.</li> <li>• Another plantation of 6,478 ha was established by Ford Motor Co. at Belterra in 1936 and was abandoned in 1943.</li> <li>• In 1972 a special rubber planting programme called PROBOR was established and the programme was supposed to continue until 1994. However, the programme was prematurely terminated in 1986 as by then 100,000 ha out of 150,000 ha established were seriously affected by SALB.</li> </ul>
Surinam	A plantation was established in 1911 and was abandoned in 1918.
Panama	Goodyear Plantation established an estate in 1935 and the plantation was abandoned in 1941.

Lieberei, 2007

Several renowned plant pathologists predicted that SALB would be devastating in South East Asia. The weather condition in South East Asia is similar to those found in the SALB endemic areas in Brazil (Chee, 1980; Silva, 2007). Moreover, the rubber clones planted in Asia are susceptible to SALB. An outbreak of SALB would destroy the rubber growing industry in South East Asia within a short period. Richard Evans Shultes, a well known rubber botanist, predicted that within five years, the rubber industry in South East Asia would be compromised (Davis, 1997).

SALB is most damaging when it infects the young leaves and shoots developing during the annual leaf change season. Severely infected leaves fall-off and the repeated cycle of infection and defoliation resulted with trees with poor canopy throughout the year. The growth of young rubber plant is reduced and the immature period of the plants is increased. In Asia, it is common that the newly plants will mature within six years or earlier. In the SALB endemic countries, the immaturity period may be extended even to 13 years. Prolonged infection of SALB may kill younger rubber plants. The latex yield of SALB infected trees is also reduced. The yield loss couples with the extra management costs and extra agronomic inputs required especially on pest and disease control reduce the economic viability of rubber cultivation in SALB endemic countries until today.

## 6.2 Quarantine measures

Several diseases for example potato late blight, coffee rust and Dutch elm's disease, had crossed oceans and established themselves in new areas. The spread was attributed either to wind-borne spores or importation of infected plant materials. Hence, SALB is always a threat to the rubber cultivation in Asia or Africa in view of the expansion of rubber cultivation in many South American countries and the increase in communication between SALB endemic countries with the Asian and African rubber growing countries. The threat of SALB to the Asian rubber growing countries was realized since 1950s (Altson, 1955; Hilton, 1955; Rao, 1973) and prompted the introduction of special quarantine measures. The establishment of the Asia and Pacific Plant Protection Agreement had been launched in 1955 as an effective means to reduce the risks of introduction of SALB into Asia (Lieberei, 2007; Thurston, 1973). Apart from other general actions, the agreement clearly stipulated measures to deal with SALB especially to regulate the importation of rubber planting materials.

The Association of Natural Rubber Producing Countries (ANRPC) once established the ANRPC Technical Committee on SALB and an ANRPC Agreement on SALB was established with the main objective to secure common and effective actions to deal with SALB. Unfortunately, the committee and the agreement were abolished. The major contribution of the Committee then was introducing measures to increase the preparedness of the member countries to deal with SALB. Most member countries established SALB Country Committees and also the SALB Contingency Plan that includes measures to eradicate the disease in the event of an outbreak. ANRPC also introduced training programme to increase the knowledge of plant quarantine and research personnel on SALB. With the co-operation of the International Rubber Research and Development Board (IRRDB), special training programme and SALB workshops were held in Brazil and also in some member countries. The IRRDB SALB Fellowship programme enables plant quarantine officers or plant pathologist to work on SALB for a period in Brazil. This strategy ensures that each member country has a personnel well verse with SALB. In addition, certain measures had been implemented then to tackle the possible entry of spores of *M. ulei* that lodge on bodies and clothing of persons visiting a rubber area infected with SALB. Thus it was recommended that these travelers break their return journey in temperate North America or Europe. This is to enable them to rid their bodies and used clothing from viable spores. Detergents, UV irradiation and moist heat killed spores of *M. ulei* (Chee, 1985; Lebai Juri *et al.*, 1997; Zhang *et al.*, 1986). Gamma irradiation was shown to kill the spores (Lebai Juri *et al.*, 1997). Previously, there were direct flights between SALB endemic countries to Thailand and Malaysia. These two countries implemented special measures to deal with these travelers and their personal belongings.

**TABLE 5. EFFECTS OF UV LIGHT AND OTHER QUARANTINE TREATMENTS ON SURVIVAL OF *M. ULEI* CONIDIA**

<b>Treatment</b>	<b>Spore survival</b>
UV irradiation	Some conidia (5-10%) germinated after 15 min exposure (Zhang and Chee, 1986), however 45 min and 60 min exposure (Lebai Juri <i>et al.</i> , 1997) caused total kill
X-ray irradiation	Killed the conidia (Lebai Juri <i>et al.</i> , 1997).
Commercial disinfectant, detergent, formalin liquid and gas or moist heat	Killed the conidia (Lebai Juri <i>et al.</i> , 1997; Zhang and Chee, 1986).

## 7. DISEASE MANAGEMENT

### 7.1 Chemical control

Earlier, application of fungicides is the most popular strategy to manage SALB. Therefore, it is not surprising that a great deal of research attention was given to chemical control. Many fungicides are effective against *M. ulei*. The older fungicides such as chlorothalonil, propineb, mancozeb and benomyl and the newer systemic fungicides (triadimefon, thiophanate methyl, prochloraz, propiconazole, and triadimenol, triforine, **azoxystrobin?**) were effective against *M. ulei* (Chee, 1978a; 1980; 1985; Chee and Holliday, 1986; Rocha *et al.*, 1975; Santos and Pereira, 1985; 1986a; 1986b). Reports of wide-scale applications of fungicides applied by fogging (Lim, 1982; Rocha *et al.*, 1973) or aerial spraying (Alencar *et al.*, 1975; Bezeera *et al.*, 1980; Mainstone *et al.*, 1977; Rocha *et al.*, 1975; Rogers and Peterson, 1976) had produced variable results. There is no shortage of effective fungicides, however the cost effectiveness of chemical treatment is not encouraging especially during low rubber price.

Being a deciduous plant, *H. brasiliensis* changes its leaves once a year. The annual leaf change or wintering process from shedding of mature leaves and sprouting of new leaves may take several weeks depending on weather conditions. Normally, the process is longer during wet seasons as the leaf shedding is not uniform among trees. The young leaves emerging after the annual leaf change (wintering) season should be sprayed weekly until most of the leaves are green and thus resistant to *M. ulei* infection. Many spray rounds are required thus affecting the economics of disease control.

In the nurseries, the fungicides are normally applied using portable mistblowers. The height of mature rubber is often above 20 meters and is a limiting factor to chemical treatment. Thus, larger or tractor mounted mistblowing machines are required. Fogging machines had also been used to control SALB (Lim, 1982; Rocha *et al.*, 1973), however the effectiveness of fogging had been questioned (Albuquerque *et al.*, 1987). Airplanes and helicopters had been used to treat large areas of mature rubber (Alencar *et al.*, 1975; Mainstone *et al.*, 1977; Rocha and Vasconcelos, 1975; Rogers and Peterson, 1976). Weekly aerial spraying of mancozeb for six rounds was effective to control SALB (Rogers and Peterson, 1976). The effectiveness of fungicide treatment was also improved by using suitable spray oils (Pereira *et al.*, 1980; Rao *et al.*, 1980). Controlling SALB with fungicides had improved latex yield (Alencar *et al.*, 1975; Chee, 1980).

Earlier, benomyl, a systemic fungicide was widely used to manage SALB. Unfortunately, benomyl resistant strains of *M. ulei* had developed in Bahia, Brazil where the chemical had been used (Ismail, 1988). Thus, measures should be taken to ensure that *M. ulei* does not develop resistance to a particular fungicide. The response of *M. ulei* to fungicides also varied with races of *M. ulei* (Zhang and Chee, 1986). They indicated that races 6 and Race 8 were less sensitive to benomyl and thiophanate methyl as compared to the sensitivity of Race 4 and Race 7.



**Figure 12. Spraying of fungicides using a tractor-mounted mistblower**

## **7.2 Biological control**

The potential of biological control of SALB had been investigated. The fungus *Hansfordia pulvinata* (later known as *Dicyma pulvinata*) on SALB has the most potential. *D. pulvinata* forms white mycelial colonies on *M. ulei* lesions and parasitize the pathogen (Mello, 2004). Several trials reported that *D. pulvinata* was effective in controlling *M. ulei* (Delmadi et al., 2009; Junqueira and Gasparotto, 1991; Junqueira, et al., 1991; Mello, 2004; Mello et al., 226; 2007). However, Junqueira and Gasparotto (1991) observed that *D. pulvinata* controlled SALB in a rubber area planted with many rubber clones but the fungus was not effective in an area planted with only one rubber clone as a single rubber clone could not maintain an effective population of *D. pulvinata*. Genetic studies indicated that *D. pulvinata* isolated from *M. ulei* was similar from all rubber regions (Tavares et al., 2003). So far, biological control with *D. pulvinata* has not been adopted to control SALB. The effectiveness of mycorrhiza was also investigated but it did not significantly affect disease severity though VA-mycorrhiza infected rubber plants were more resistant to SALB (Feldman et al., 1989; 1995).

## **7.3 Resistant clones**

When the earlier plantations at Fordlandia and Belterra were ravaged by SALB, there were some plants that were not infected by the disease. This indicates the existence of resistance to *M. ulei* in *Hevea* species. Thereafter, planting of resistant clone was adopted as a strategy to manage SALB and resistant clones were selected or bred. Earlier, the Ford Motor Co. started the breeding programme by crossing some of the resistant progenies found at Fordlandia and Belterra plantations with high yielding oriental clones (e.g. PB 86 and Tjir 1). The progenies of Ford breeding and selection programme are the F, FA, FB and Fx clones. Then since 1945, *Hevea* breeding was carried out by the Instituto Agronomico do Norte which produced the IAN clones. Some 'resistant clones' in the IAN, F and Fx series were planted commercially. Unfortunately, the resistance of these clones broke down with time when Race 2 and other new races of *M. ulei* emerged. A notable example is the breakdown of supposedly resistant clone IAN 717 and other clones that derived their resistance from F 4542, a *H. benthamiana* clone when Race 2 of *M. ulei* emerged (Langdon, 1965). Another clone Fx 3864 was considered tolerant to SALB and this clone was widely planted in Bahia, Brazil. However, Fx 3864 is now severely infected by SALB there. Therefore, in view of the perennial nature of rubber plant and the fast speed of disease spread,

breeding for SALB resistant clones should take into account the existence of numerous physiological races of *M. ulei*.

Numerous attempts were made to breed clones resistant to SALB in Central and South America (Goncalves, 1968; Goncalves *et al.*, 1983; Pinheiro and Libonati, 1971). Breeding for SALB resistance was also carried out in other continents. Bos and McIndoe, (1965) documented the early Firestone Plantation Co. breeding programme conducted in Africa. Similar attempts were made in Malaysia (Brookson, 1956; Subramaniam, 1970; Ong, 1980), Sri Lanka (Fernando and Liyanage, 1975; Jayasekara and Fernando, 1977; Wijewantha *et al.*, 1965), Indonesia (Wirjomidjojo, 1962) and France (Garcia, 2004). In Malaysia, several introduced clones (such as Fx 25), and selections from Madre de Dios and Rio Negro were used as the source of SALB resistance (Ong, 1980; Ong and Tan, 1987; Subramaniam, 1970).

The histological development of the fungus in resistant and susceptible leaves was studied in detail (Blazquez and Owen, 1963; Ismail Hashim, 1978; Ismail Hashim *et al.*, 1978; Lieberrei, 2007). These studies indicate that the fungus could penetrate susceptible and resistant leaves. However, the subsequent spread of mycelia was inhibited in resistant leaves. The inhibition could be attributed to occurrence of hypersensitive host cell collapse which occurred soon after inoculation of very resistant or immune clones (Blazquez and Owen, 1963; Ismail Hashim, 1978; Ismail Hashim *et al.*, 1978). Hypersensitive host cell collapse is associated with vertical resistance.

Since the fungus could penetrate into leaves of all clones, it was suggested that biochemical reactions is more important in the mechanism of resistance of *Hevea* to SALB after studying changes in phenol content and activities of selected enzymes (Ismail Hashim (1978; 1979); Ismail Hashim *et al.*, 1978a; 1978b; 1980). A yellow fluorescent substance was observed following infection of resistant leaves (Blazquez and Owen, 1957; Figari, 1965; Ismail Hashim, *et al.*, 1978a). This substance was identified as a glucoside of kaempferol (Martins, *et al.*, 1970). Another phenolic compound scopoletin was also observed following infection of resistant leaves (Garcia *et al.*, 1995a; 1995b; 1999; Giesemann, 1980). Scopoletin is a phytoalexin and the rapidity and amount of its occurrence was associated with resistance (Giesemann *et al.*, 1980).

Many *Hevea* clones exhibit vertical resistance to SALB characterized by hypersensitive reaction following infection and breaking down of resistance to new races of *M. ulei*. Since rubber is a perennial crop, vertical resistance is not of benefit in the long run. Therefore, breeding for clones with horizontal resistance has been suggested (Simmonds, 1990). Lesion size, latent period and spore production are useful parameters to identify clones with horizontal resistance (Garcia *et al.*, 1999; 2004; Ismail and Pereira, 1986; 1989; Junqueira *et al.*, 1990; LeGuen *et al.*, 1995; 2008). Clones with horizontal resistance exhibited smaller or less number of lesions, produce less number of spores and the spore generation period is short. Recently, Michelin jointly with CIRAD had produced or identified 13 clones that exhibited some degree of horizontal resistance to SALB (Garcia, 2004). Breeding clones with horizontal resistance to SALB has been adopted as an important strategy to manage SALB.

The art of *Hevea* breeding is now being assisted with new developments in molecular techniques. Recent research throws more light on the genetics of resistance of *Hevea* to *M. ulei* (LeGuen *et al.*, 2000, 2003; 2004; 2011; Lespinasse *et al.*, 2000a; 2000b). Lespinasse *et al.* (2000a) had identified the QTLs involved with resistance to SALB. They had also created a linkage map of *Hevea*. Le Guen *et al.*, (2011) indicated that horizontal resistance is conferred by a qualitative gene and a major quantitative resistance factor.

## 7.4 Polyploidy

The chromosome number of the diploid ( $2n$ ) *Hevea* spp. is 36. Polyploid rubber plants had been produced by treatments with chemical mutagens or X-ray irradiation. The susceptibility of these polyploid rubber plants to *M. ulei* had been evaluated and some polyploid plants were more resistant than the diploid plants (Junqueira, *et al.*, 1993).

## 7.5 Crown budding

Crown budding is the technique of bud grafting a specific rubber clone or progeny (crown clone) onto a trunk of another clone (trunk clone) which had itself been budded onto a seedling (root stock) to produce a 'three-part tree'. This technique is being utilized for managing SALB whereby clones resistant to SALB is used as crown clones to be budded onto high yielding trunk clone. Selected "resistant clones" such as Fx 3899, Fx 3810 and Fx 3925 had been budded onto high yielding oriental clones (Chee and Wastie, 1980; Moraes and Moraes, 2008). Later, other clones (IAN 6158) or *Hevea* species such as *H. pauciflora* and *H. rigidifolia* had been used as crown (Furtado *et al.*, 2004; Lima *et al.*, 1992; Mattos, 2004; Moraes and Moraes, 2008). Though the technology of crown budding is technically feasible to overcome SALB, the current limiting factor is the unavailability of suitable resistant crown. Another disadvantage is that certain crown-scion combinations are incompatible and result in uneven growth of the trunk parts.



Picture of crown budding

## 7.6 Disease escaped areas

The severity of SALB is influenced by the amount and duration of rainfall. Holliday (1970) observed that the incidence of SALB was low in areas with annual rainfall of 130-150 cm (7-8 cm/month) with long dry season of at least four consecutive months. These relatively dry areas are termed as SALB 'escaped areas'. The 'escaped areas' in Brazil had been identified and mapped out (Almeida *et al.*, 1987; Camargo, 1963; Camargo *et al.*, 1967; 1975; Silva, 2007). The major 'escaped areas' are in Sao Paulo and Mato Grosso states. Cultivation of rubber in the "escaped areas" has been a successful strategy to overcome

the ravages of SALB (Pinheiro, 1995; Rivano, 2004). In the 'escaped areas' of Sao Paulo and Mato Grosso, SALB susceptible oriental clones such as RRIM 600, GT 1 and PB 260 had been successfully planted and produce good yield (Furtado, *et al.*, 2004; Pinheiro, 1995).

## 8. CONCLUSIONS

SALB is a very serious disease of *Hevea* rubber that is the main hindrance to a viable commercial cultivation of rubber in Central and South America. Despite the existence of many fungicides, chemical control does not offer a cost effective solution of the disease. The only practical method is to plant resistant clones. Unfortunately, high yielding clones that are resistant to SALB are very limited. SALB is always a threat to the rubber industry in the major rubber producing countries in Asia and Africa. Effective quarantine measures should be taken to prevent the introduction and spread of SALB into Asia and the Pacific region.

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