Section 1.3. Booklet

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 percent 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 and Pacific Plant Protection Commission (APPPC) was established in 1956 based on APPPC 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 (Thurston, 1973). In addition, efforts are being taken to increase knowledge of relevant pathologists and quarantine officers.

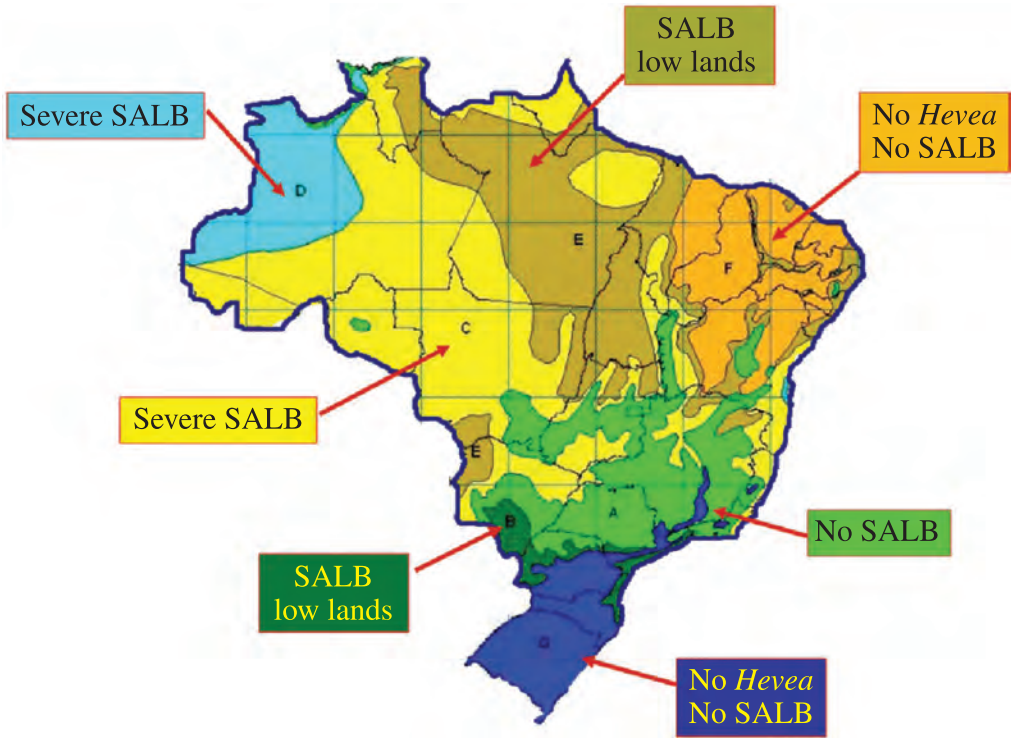
This publication briefly reviews the biology of SALB and its causal agent *M. ulei* (P. Henn.) v. Arx. The publication will be a useful guide to plant pathologists and plant quarantine officers who are dealing with SALB.

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 2 500 mm) with long period of high humidity (>80 percent 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 3). 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.

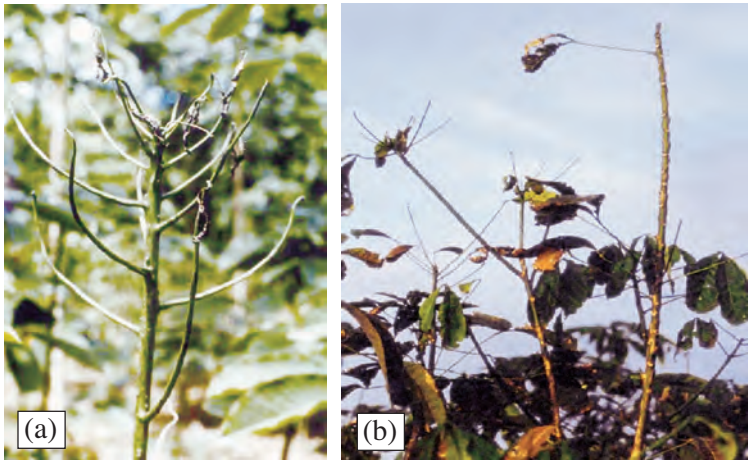
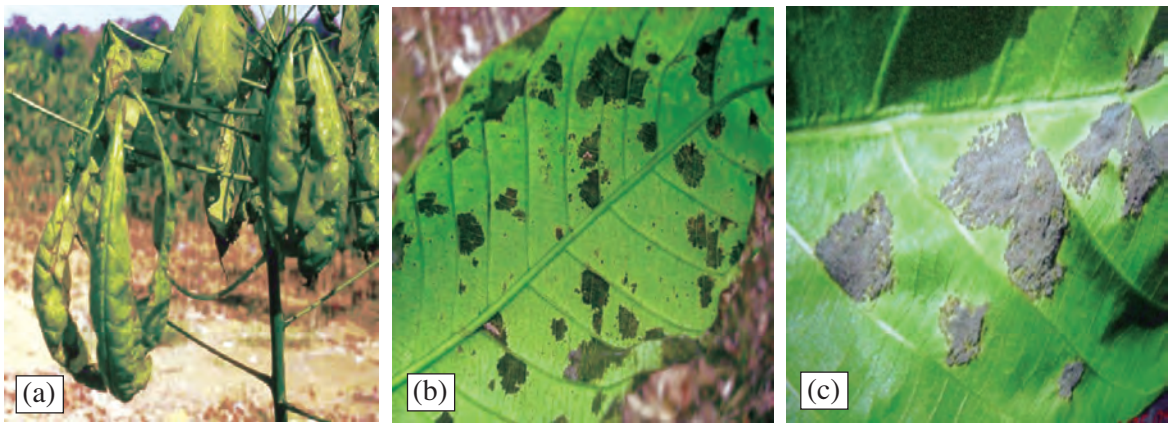


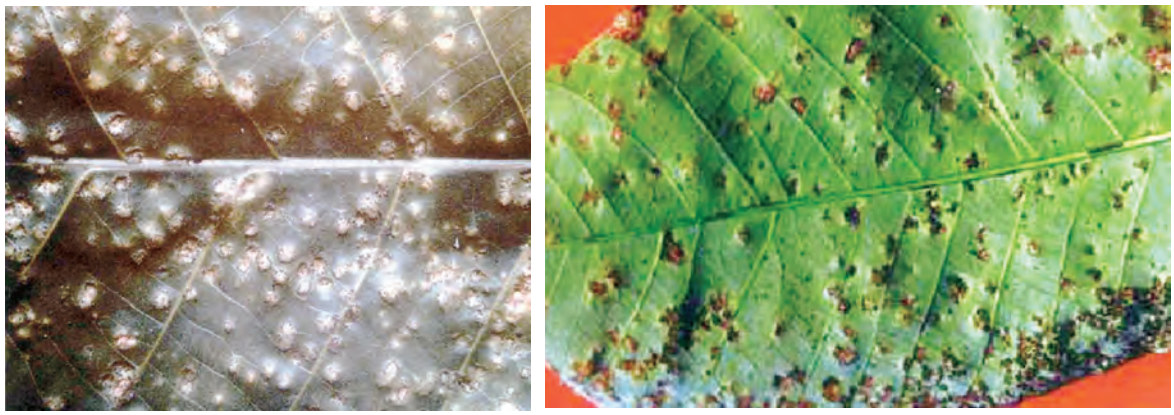
Figure 3. Symptoms on intact young leaves: (a) Distorted infected leaves; (b) and (c) Conidial lesions on the lower surface of the leaves.



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 μm in diameter and these fruiting bodies produce the pycnospores.

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

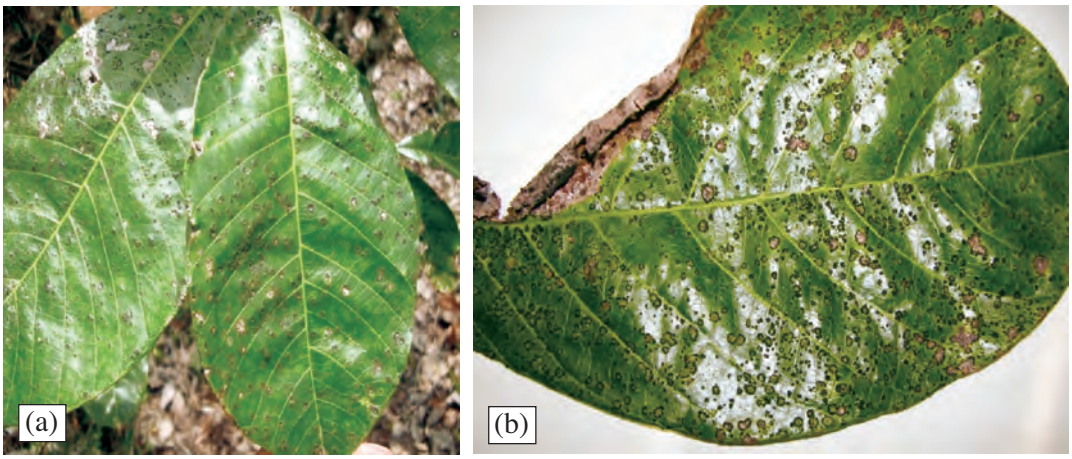


Figure 6. Symptoms on old leaves showing the shot holes formed following necrosis of tissues at the centre of the perithecia

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).

Figure 7. Infection on other parts of rubber plants

(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

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*)

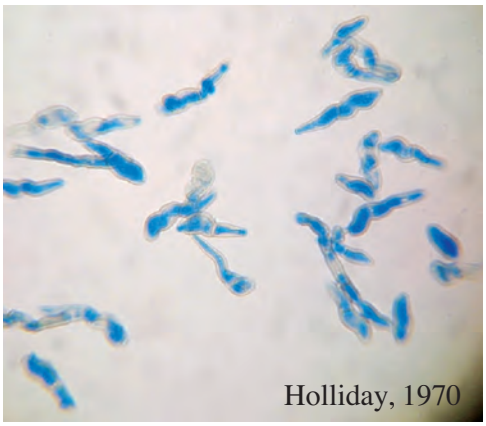
The pathogen of South American leaf blight (SALB) is the obligate fungus *Microcyclus ulei* (P. Henn.) v. Arx. The fungus was previously known by other names such as *Dothidella ulei* P. Henn., *Melanopsammopsis ulei* (P. Henn.) Stahel, *Fusicladium macrosporum* Kuyper (refers to the conidial state of 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.

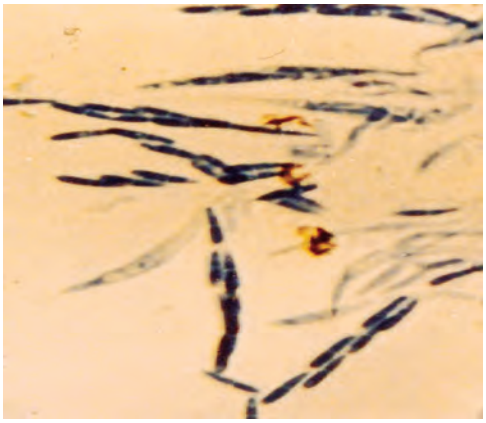
The pycnospores are dumbbell shape and small (6-10 μm long and 2-5 μm in width). The ascospore is oblong shaped and is made up of two cells of unequal size. The size of the ascospores also varies (Table 1).

Figure 9. Spores of *M. ulei*



(a) Conidia with two cells

(b) Pycnospores



(c) Ascospores

Table 1. Spores of *M. ulei*

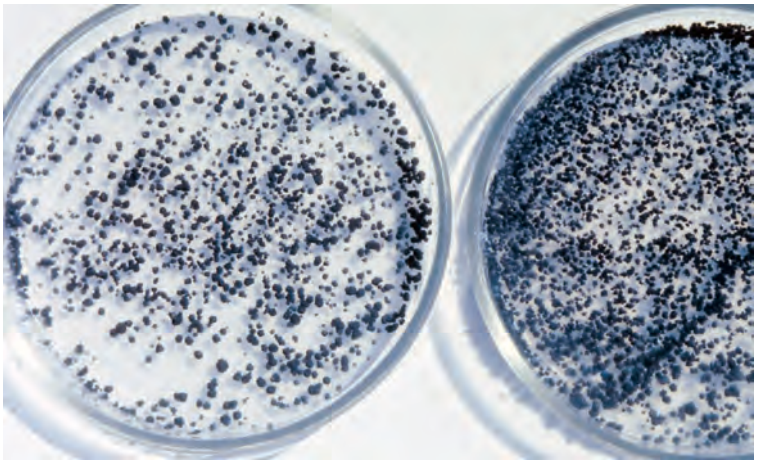
Spore	Description	Size (Reference)
Conidia	Mostly septate with two cells with broader proximal cell, dark grey to olive green in colour, twisted; sometimes unicellular.	• 12-30 × 5-8 μm (Langford, 1945) • 23-65 × 5-10 μm (Holliday, 1970b) • 23-65 × 5-10 μm for septate and 15-34 × 5-9 μm for aseptate conidia (Chee and Holliday, 1986)
Pycnospores	Dumbbell-shaped with the ends twice the width of the centre.	• 6-10 μm (Holliday, 1970b) • 6-10 μm (Chee and Holliday, 1986)
Ascospores	Septate with two cells of unequal size, ellipsoidal, hyaline	• 3-5 × 10-15 μm (Langford, 1945) • 2-5 × 12-20 μm (Holliday, 1970b) • 2-5 × 12-20 μm (Chee and Holliday, 1986)

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 ºC, 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 ºC and at 65 to 95 percent 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 percent of the conidia germinated after one week and the percentage germination declines to three to six percent after three weeks of storage. Detached conidia remained viable for about nine days at 65 percent RH and for six days at 80 to 90 percent RH (Chee, 1976d).

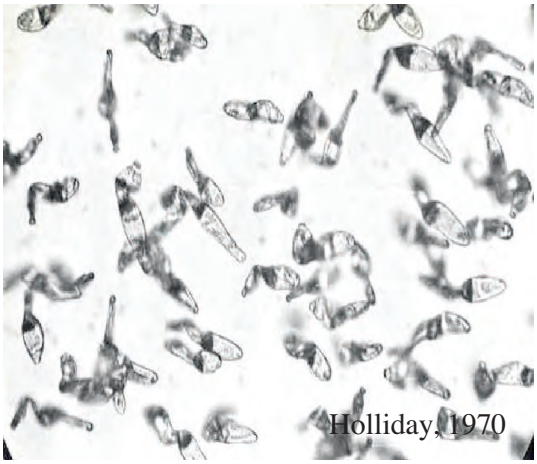
Table 2. Viability of spores and perithecia of *M. ulei*

Spores	Storage condition	Viability period
Conidia (detached)	24 ºC and 65-95 percent R.H.	3-4 weeks
Conidia (intact)	24 ºC and 85 to 100 percent R.H.	2 weeks
	24 ºC and under dessication	16 weeks
Ascospores	24 ºC and 85 to 100 percent R.H.	9 days
Perithecia	24 ºC and 65 percent R.H.	3 weeks
	24 ºC 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 ºC) 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 percent 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 material for seven dyas

Materials	Germination (percent)
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

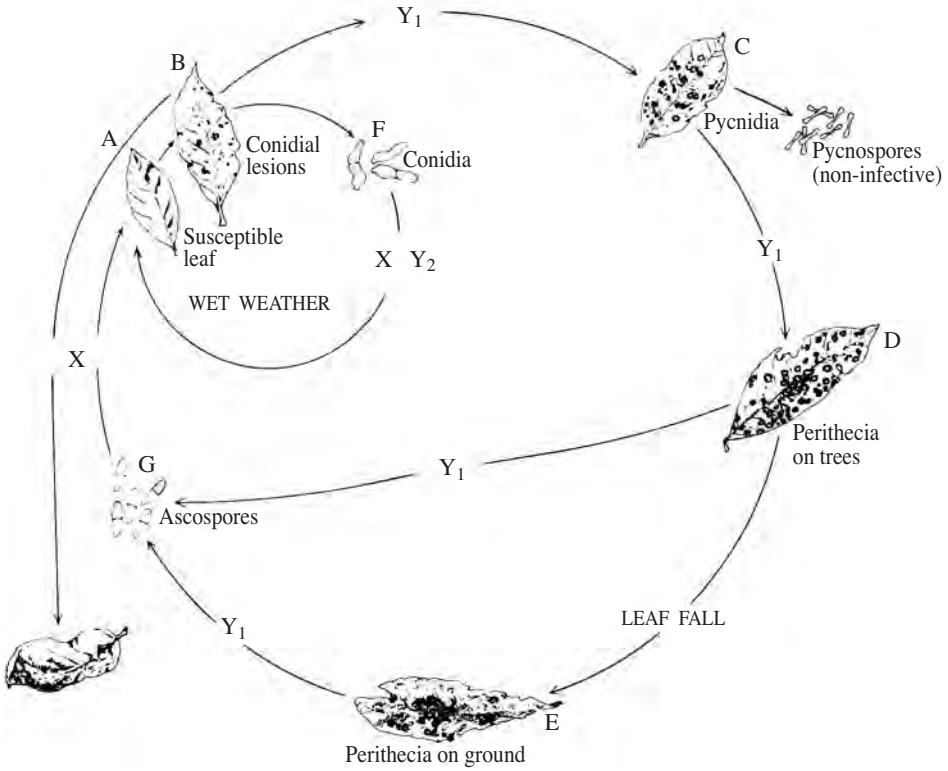
5.1 Disease dispersal

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 (Blazquez 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 ºC (Chee, 1976). However, conidia and ascospores germinated between 14-36 ºC and 14-29 ºC 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 ºC and high humidity favours sporulation. Maximum infection occurred at 24 ºC and less at 18 ºC. Infection was high at 100 percent RH as compared to 65 percent 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 hours (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 hours. 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 pycnospores had not caused infection following artificial inoculation though the pycnospores 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; Giesemannn, *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

Table 4. Historical destruction of rubber cultivation by SALB

Country	Fate of earlier plantations
Brazil	• Ford Motor Co. established a plantation of 3 200 ha at Fordlandia in 1928 and the plantation was abandoned in 1933. • Another plantation of 6 478 ha was established by Ford Motor Co. at Belterra in 1936 and was abandoned in 1943. • 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 186 as by then 100 000 ha out of 150 000 ha established were seriously affected by SALB.
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

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.

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

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

REFERENCES

1. Albuquerque, P.E.P., Pereira, J.C.R. and Santos, A.F. dos. (1987). Efficiency of impactation of fungicides by thermal fogging in leaves of *Hevea* spp. *Revista Theobroma*, 17: 189-199.
2. Alencar, H., Peixoto, E. and Ferreira, H.I.S. (1975). Controle do mal das-folhas (*Microcyclus ulei*) da seringueira na Bahia. II. Relacao custo/beneficio da aplicacao aerea de fungicida, regiao de Itubera, 1972-73. *Revista Theobroma,* 5: 12-20.
3. Almeida, H.A., Santana, S.O. and Sa, D.F. (1987). Edaphic-climatic zoning for rubber in southeast Bahia, with emphasis for incidence of the South American leaf blight. *Revista Theobroma*, 17: 111-123.
4. Altson, R.A. (1955). South American leaf blight. *Journal Rubber Research Institute Malaysia,* 14: 338-354.
5. Aziz S.A.K. (1976). South American leaf blight: a proposed national and regional plan for emergency eradication. ANR Technical Committee Meeting on SALB, Bogor, 1976, 7 pp.
6. Bergamin Filho, A. (1984). Disease progress of South American leaf blight of rubber in different Brazilian region. *European Journal Forest Pathology*, 14, 386-391.
7. Berger, P. (1992). Foliar phenolic compounds of the rubber tree and their implication in the resistance to *Colletotrichum gloeosporioides* and *Microcyclus ulei*. Thesis, Universite de Montpellier 2 (France), 235 pp.
8. Bezerra, J.L., Castro, A.M.G., Vale, F.X.R., Rao, B.S., Souza, A.R., Araujo, A.C. and Neves, M.I. (1980). Controle quimico de *Microcyclus ulei* no Brasil atraves do PROMASE. Seminario Nacional da Seringueira, 3, 1980, Manaus. *Anais Brasilia, SUDHEVEA, 1980,* Vol. I, pp. 130-161.
9. Blazquez, C.H. and Owen, J.H. (1957). Physiological studies of *Dothidella ulei*. *Phytopathology,* 47: 727-732.
10. Blazquez, C.H. and Owen, J.H. (1963). Histological studies of *Dothidella ulei* on susceptible and resistant *Hevea* clones. *Phytopathology*, 53: 58-65.
11. Bos, H. and McIndoe, K.G. (1965). Breeding of *Hevea* for resistance against *Dothidella ulei*. *Journa Rubber Research Institute Malaysia,* 19: 98-107.
12. Brookson, C.W. (1956). Importation and development of new strains of *Hevea brasiliensis*. *Journal Rubber Research Institute Malaysia,* 14: 423-447.
13. Camargo, A.P. de, Cardoso, Rosa M.G. and Schmidt, N.C. (1967). Comportamento e ecologia do 'mal-das-folhas' da seringuiera nas condicoes climaticas do Planalto Paulista. *Bragantia*, 26: 1-17.
14. Camargo, A.P. de, Schmidt, N.C. and Cardoso, Rosa M.G. (1975). South American leaf blight epidemics and rubber phenology in Sao Paulo. *Proceedings International Rubber Conference*, 1975, Kuala Lumpur, 3: 251-265.
15. Cano, H.H., (1997). Physiological races of *Microcyclus ulei* in Mexico. *Proceedings Scientific Technological Meeting on Forestry, Agriculture and Husbandry Research in Vera Cruz State, Mexico,* 27-28 November 1997, p. 143-145.
16. Chee, K.H. (1976c). South American leaf blight of *Hevea brasiliensis*: spore dispersal of *Microcyclus ulei*. *Annals Applied Biology*, 84: 147-152.
17. Chee, K.H. (1976d). Factors affecting discharge, germination and viability of spores of *Microcyclus ulei*. *Transaction British Mycological Society*, 66: 499-504.
18. Chee, K.H. (1977). Combating South American leaf blight of *Hevea* by plant breeding and other measures. *Planter, Kuala Lumpur*, 53: 287-296.
19. Chee, K.H. (1978a). Evaluation of fungicides for control of South American leaf blight of *Hevea brasiliensis*. *Annals Applied Biology,* 90: 51-58.
20. Chee, K.H. (1978b). South American leaf blight of *Hevea brasiliensis*: culture of *Microcyclus ulei*. *Transaction British Mycological Society*, 70: 341-344.
21. Chee, K.H. (1980a). The suitability of environment conditions in Asia for the spread of South American leaf blight of *Hevea* rubber. *Planter, Kuala Lumpur*, 56: 445-454.

22. Chee, K.H. (1980b). Management of South American leaf blight. *Planter, Kuala Lumpur*, 56: 314-325.

23. Chee, K.H. (1985). An analysis of possible preventative measures against the introduction of South American leaf blight to Malaysia. *Regional Conference in Plant Quarantine Support for Agricultural Development, 1985, Kuala Lumpur, Malaysia,* p. 261-263.

24. Chee, K.H. and Holliday, P. (1986). South American leaf blight of *Hevea* rubber. Monograph No. 13. Malaysian Rubber Board, 50 p.

25. Chee, K.H., Zhang, K.M. and Darmono, T.W. (1986). The occurrence of eight races of *Microcyclus ulei* on *Hevea* rubber in Bahia, Brazil. *Transaction British Mycological Society,* 87, 15-21.

26. Commonwealth Mycological Institute (1975). Distribution maps of plant diseases, No. 27.

27. Compagnon, M.P. (1976). Review on progress and spread of South American leaf blight. ANRPC Technical Committee Meeting on SALB, Bogor, 1976, 16 pp.

28. Davis, W. (1997). The rubber industry's biological nightmare. *Fortune*, August 4, p. 36-46.

29. Delamadi, L.C., Neto, D.C. and Rocha, V.F. (2009). Avaliacao do potencial do uso de *Dicyma pulvinata* no controle biologico mal-das-folhas (*Microcyclus ulei*) de seringueira (*Hevea barasiliensis*). *Ciencia Florestal*, 19: 183-193.

30. Edathil, T.T. (1986). South American leaf blight: a potential threat to the natural rubber industry in Asia and Africa. *Tropical Pest Management*, 32: 296-303.

31. Feldman, F., Junqueira N.T.V. and Lieberei, R. (1989). Utilization of VA-mycorrhiza as a factor in integrated plant protection. *Agriculture, Ecosystem Environment*, 29: 131-38.

32. Feldman, F., Idczak, E., Martins, G., Nunes, J., Gasparotto, L., Preisenger, H., Moraes, V.H.F. and Lieberei, R. (1995). Recultivation of fallow low lying areas in Central Amazonica with equilibrated polycultures: Response of useful plants to monoculture with VA-mycorrhizal fungi. *Angewandte Botanik*, 69: 111-118.

33. Fernando, D.M and Liyanage, A. de S. (1975). *Hevea* breeding for leaf and panel disease resistant in Sri Lanka. *Proceedings international Rubber Conference, 1975, Kuala Lumpur*, Vol. III, pp. 236-246.

34. Figari, A. (1965). Sustancias fenolicas toxicas al hongo *Dothidella ulei* en jojas de clones de *Hevea brasiliensis*. *Turrialba*, 15: 103-110.

35. Furtado, E.L., Sambugaro, R. and Mattos, C.R.R. (2004). SALB management. *IRRDB/Michelin/CIRAD International Workshop on SALB*, 2004, Bahia, Brazil, Mimeograph, pp. 7.

36. Garcia, D. (2004). Breeding CMB (CIRAD-Michelin-Brazil) clones. *IRRDB/Michelin/CIRAD International Workshop on SALB*, 2004, Bahia, Brazil, Mimeograph, pp. 3.

37. Garcia, D., Cazaux, E., Rivano F. and D'Auzac, J. (1995a). Chemical and structural barriers to *Microcyclus ulei*, the agent of South American leaf blight in *Heve*a spp. *European Journal of Forest Pathology*, 25: 282-292.

38. Garcia, D., Mattos, C.R.R., Clement-Demange, A. and LeGuen, V. (2004). Genetic parameter estimations of three traits used to evaluate South American leaf blight (SALB) in rubber tree. *Crop Breeding and Applied Biotechnology* 2: 453-462.

39. Garcia, D., Mattos, C.R.R., Goncalves, P. S. and LeGuen, V. (2004). Selection of rubber clones for resistance to South American leaf blight and latex yield in the germplasm of Michelin Plantation of Bahia, Brazil. *Journal of Rubber Research*, 7: 188-198.

40. Garcia, D., Sanier, C. Macheix, J.J. and D'Auzac, J. (1995b). Accumulation of scopoletin *in Hevea brasiliensis* infected by *Microcyclus ulei* and evaluation of its fungitoxicity to three leaf pathogens of rubber tree. *Physiological and Molecular Plant Pathology,* 47: 213-223.

41. Garcia, D., Troispoux, V., Grange, N., Rivano, F. and D'Auzac, J. (1999). Evaluation of the resistance of 36 *Hevea* clones to *Microcyclus ulei* and relation to their capacity to accumulate scopoletin and lignin. *European Journal of Forest Pathology*, 29: 323-338.

42. Gasparotto, L. and Junqueira, N.T.V. (1994). Ecophysiological variability of *Microcyclus ulei*, causal agent of rubber tree leaf blight. *Fitopatologia Brasileira,* 18: 22-28.

43. Gasparotto, L., Zambolim, L. Junqueira N.T.V., Maffia, L.A. and Vale F.X.R. (1991). Epidemiology of South American Leaf blight of rubber tree: Manaus Region. *Fitopatologia Brasileira,* 16: 18-21.

44. Gasparotto, L., Zambolim, L. Ribeiro do Vale, F.X. and Junqueira N.T.V. (1989). Effect of temperature and humidity on the infection of the rubber tree (*Hevea* spp.) by *Microcyclus ulei*. *Fitopatologia Brasileira,* 14: 38-41.

45. Goncalves, J.R.C. (1968). The resistance of FX and IAN rubber clones to leaf disease in Brazil. *Tropical Agriculture Trinidad*, 45: 331-336.

46. Goncalves, P. de S., Paiva, J.R. de and Souza, R.A. de (1983). Retrospectiva e atualidade do melhoramento genetico da seringueira (*Hevea* spp.) no Brasil e em paises Asiaticos. *Serie Documentos No. 2, EMBRAPA*, Brazil, 69 pp.

47. Giesemann, A., Biehl, B. and Lieberei, R. (1986). Identification of scopoletin as a phytoalexin of the rubber tree *Hevea brasiliensis*. *Journal Phytopathology*, 117: 373-76.

48. Hagen, J., Gasparotto, L., Moraes, V.H.F., and Lieberei, R. (2003). Reactions of cassava leaves to *Microcyclus ulei*, causal agent of South American leaf blight of rubber trees. *Fitopatologia Brasileira* 28: 477-480.

49. Hilton, R.N. (1955). South American leaf blight. A review of the literature relating to its depredations in South America, its threat to the Far East and the methods available for its control. *Journal Rubber Research Institute Malaysia,* 14: 287-337.

50. Holliday, P. (1969). Dispersal of conidia of *Dothidella ulei* from *Hevea brasiliensis*. *Annal Applied Biology*, 63: 435-447.

51. Holliday, P. (1970a). *Microcyclus ulei* In 'IMI Description of Fungi and Bacteria', No. 23, Sheet 225, Cab International, U.K.

52. Holliday, P. (1970b). South American leaf blight *(Microcyclus ulei)* of *Hevea brasiliensis*. *Phytopathological Papers no. 12*, Commonwealth Mycological Institute, England, 31 pp.

53. Ismail Hashim (1978). Histological and biochemical studies on South American leaf blight of *Hevea* species. Ph. D. Thesis, University of the West Indies, Trinidad and Tobago.

54. Ismail Hashim (1979). Possible mechanism of *Hevea* resistance to South American leaf Blight. *ANRPC SALB Technical Committee Meeting*, 1979, Chiang Mai, Thailand.

55. Ismail Hashim (1988). Detection and characterisation of benomyl resistant strains of *Microcyclus ulei*. *Journal Natural Rubber Research,* 3: 155-162.

56. Ismail Hashim and Almeida, L.C.C. de (1987). Identification of races *and in vitro* sporulation of *Microcyclus ulei, Journal Natural Rubber Research,* 2: 111-117.

57. Ismail Hashim, Chee, K.H. and Duncan, E.J. (1978). Reaction of *Hevea* leaves to infection with *Microcyclus ulei*. *Journal Rubber Research Institute Malaysia,* 26: 67-75.

58. Ismail Hashim, Chee, K.H. and Wilson, L.A. (1980). The relationship of phenols and oxidative enzymes with resistance of *Hevea* to South American leaf blight. *Phytopathologische Zeitschrift,* 97: 332-345.

59. Ismail Hashim and Pereira, J.C.R. (1986). Influence of resistance of *Hevea* on development of *Microcyclus ulei*. *Journal Natural Rubber Research*, 4: 212-218.

60. Ismail Hashim and Pereira, J.C.R. (1989). Lesion size, latent period and sporulation on leaf discs as indicators of resistance of *Hevea* to *Microcyclus ulei*. *Journal Natural Rubber Research*, 4: 56-65.

61. Ismail Hashim, Wilson, L.A. and Chee, K.H. (1978). Regulation of indole acetic acid (IAA) oxidase activities in *Hevea* leaves by naturally occurring phenolics. *Journal Rubber Research Institute Malaysia,* 26: 105-111.

62. Jayasinghe, C.K. (1992). South American leaf blight: Likelihood behaviour in Sri Lanka and strategies in management. *Bulletin of the Rubber Research Institute Of Sri Lanka*, 29: 21-26.

63. Jayasekera, N.E.M. and Fernando, D.M. (1977). *Hevea* introduction (non-Wickham) into Sri Lanka. *Workshop on International Collaboration on Hevea Breeding, Kuala Lumpur*, Mimeograph, 4 pp.

64. Junqueira, N.T.V., Chaves, G.M., Zambolin, L., Gasparotto, L. and Alfenas, A.C. (1986). Variabilidade fisiologica de *Microcyclus ulei* (Physiological variability of *Microcyclus ulei*). *Fitopatologia Brasileira*, 11: 823-833.

65. Junqueira N.T.V., Chaves G.M., Zambolim L., Alfenas, A.C. and Gasparotto, L. (1988). Reaction of rubber tree clones to various isolates of *Microcyclus ulei*. *Pesquisa Agropecuaria Brasileira*, 23: 877-893.

66. Junqueira, N.T.V, Gasparotto, L.; Kalil Filho, A.N.; Lieberei, R. and Lima, M.I.P.M. (1989). Identificacao de fontes da resistencia ao *Microcyclus ulei*, agente causal do mal das folhas da seringueira. *Fitopatologia Brasileira*, 14: 147.

67. Junqueira, N.T.V., Chaves, G.M., Zambolin, L., Alfenas, A.C. and Gasparotto, L. (1984). Efeito do numero do repicagems, idade de cultura de armezenada e luminosidade sobre a esporulacao e patonicidade de *Microcyclus ulei* agente etilogico do mal das folhas de seringueira. *Fitopatologia Brasileira*, 9: 328.

68. Junqueira, N.T.V., Chaves, G.M., Zambolim, L., Romeiro, R.S. and Gasparotto, L. (1987). Isolation, culture and sporulation of *Microcyclus ulei*, causative agent of South American leaf blight of *Hevea* rubber trees. *Revista Ceres*, 31: 322-331.

69. Junqueira, N.T.V. and Gasparotto, L. (1991). Controle biologico de fungos estromaticos causados de doencas foliares em seringueira. In 'Bettiol, W., ed., *Controle Biologicos de Doencas de Plantas'*, EMBRAPA-CNPDA, Brazil, Document 15, pp. 307-331.

70. Junqueira, N.T.V, Lieberei, R., Kalil Filho, A.N. and Lima, M.I.P.M. (1990). Components of partial resistance in *Hevea* clones to rubber tree leaf blight, caused by *Microcyclus ulei*. *Fitopatologia Brasileira*, 15: 211-214.

71. Junqueira, N.T.V., Lima, M.I.P.M., Gasparotto, L. and Luiz, A.J.B. (1992). Integrated control of rubber tree leaf blight, association between genetic resistance and chemical control. *Pesquisa Agropecuaria Brasileira*, 27: 1027-1034.

72. Junqueira, N.T.V., Moraes, V.H.F., Lieberei, R. and Gasparotto, L. (1993). Induced polyploidy potential for improving resistance in *Hevea* clones to Rubber Tree Leaf Blight. *Fitopatologia Brasileira*, 18: 12-18.

73. Kajornchaiyakul, P., Chee, K.H., Darmono, T.W., and Almeida, L.C. de (1984). Effect of humidity and temperature on the development of South American leaf blight (*Microcyclus ulei*) of *Hevea brasiliensis*. *Journal Rubber Research Institute Malaysia*, 32: 217-223.

74. Langford, M.H. (1945). South American leaf blight of *Hevea* rubber trees. *Technical Bulletin U.S. Department of Agriculture*, 882, 31 pp.

75. Lebai Juri, M., Bahari, I., Lieberei, R. and Omar, M. (1997). The effects of X-rays, UV, temperature and sterilants on the survival of fungal conidia, *Microcyclus ulei*, a blight of *Hevea* rubber. *Tropical Science,* 37: 92-98.

76. LeGuen, V., Garcia, D., Doare, F. And Mattos, C.R.R. (2011). A rubber tree's durable resistance to *Microcyclus ulei* is conferred by a qualitative gene and a major quantitative resistance factor. *Tree Genetics and Genomes,* 7: 877-889.

77. LeGuen, V., Garcia, D., Mattos, C.R.R., Doare, F. Lespinasse, O.G. and Seguin, M. (2000). Bypassing of a polygenic *Microcyclus ulei* resistance in rubber tree analysed by QTL detection. *New Phytologist*, 173: 335-345.

78. LeGuen, V., Garcia, D., Mattos, C.R.R. and Clement Demange (1995). Evaluation of field resistance to *Microcyclus ulei* of a collection of Amazonian rubber tree (*Hevea brasiliensis*) germplasm. *Crop Breeding and Applied Biotechnology*, 2: 141-146.

79. LeGuen, V., Guyot, J., Mattos, C.R.R., Seguin, M. and Garcia, D. (2008). Long lasting rubber tree resistance to *Microcyclus ulei* characterized by reduced conidial emission and absence of teleomorph. *Crop Protection*, 27: 1498-1503.

80. LeGuen, V., Lespinasse, O.G. and Rodier-Goud, M. (2003). Molecular mapping of genes conferring field resistance to South American leaf blight (*Microcyclus ulei*). *Theoretical Applied Genetics*, 108: 160-167.

81. LeGuen, V., Rodier-Goud, M., Troispoux, V., Xiong, T.C., Brottier, P., Billot, C. and Seguin, M. (2004). Characterisation of polymorphic microsatellite markers for *Microcyclus ulei*, causal agent of South American leaf blight. *Molecular Ecology Notes*, 4: 122-124.

82. Lespinasse, D., Grivet, L., Troispoux, V., Rodier-Goud, M., Pinard, F. and Seguin, M. (2000a). Identification of QTLs involved in the resistance to South American leaf blight (*Microcyclus ulei*) in the rubber tree. *Theoretical and Applied Genetics*, 100: 975-984.

83. Lespinasse, D., Rodier-Goud, M., Grivet, L. and Leconte, A. (2000b). A saturated linkage map of rubber tree (*Hevea* spp.) based on RFLP, AFLP, microsatellites and isozymes markers. *Theoretical and Applied Genetics*, 100: 127-138.

84. Lieberei, R. (2007). South American leaf blight of the rubber (*Hevea* spp.) tree: New steps in plant domestication using physiological techniques and molecular markers. Annals of Botany 100: 1125-1142.

85. Lieberei, R., Junqueira, N.T.V. and Feldman, F. (1989). Integrated disease control in rubber plantations in South America. *Proceedings Integrated Pest Management in Tropical and Subtropical Cropping System,* 1989, Germany, p. 445-456.

86. Lim, T.M. (1982). Fogging as a technique for controlling rubber leaf diseases in Malaysia and Brazil. *Planter, Kuala Lumpur*, 58: 197-212.

87. Lima, M.P.I.M., Gasparotto, L., Araujo, A.L. and Dos Santos, A.R. (1992). Surto do mal das folhas (*Microcyclus ulei*) em seringal enxertado com copa do clone IAN 6158 em Manaus. *Fitopatologia Brasileira,* 17: 192.

88. Liyanage, A. de S. (1981). Long distance transport and deposition of spores of *Microcyclus ulei* in Tropical America – a possibility. *Bulletin Rubber Research Institute Sri Lanka*, 16, 3-8.

89. Liyanage A. de S. and Chee, K.H. (1981). The occurrence of a virulent strain of *Microcyclus ulei* on *Hevea* Rubber in Trinidad. *Journal Rubber Research Institute Sri Lanka,* 58, 73-78.

90. Mainstone, B.J., McManaman, G. and Begeer, J.J. (1977). Aerial spraying against South American leaf blight of rubber. *Planter's Bulletin Rubber Research Institute Malaysia,* 148: 15-26.

91. Martains, E.M.F., Moraes, W.B.C., Cardosa, P.M.G. and Kuc, J. (1970). Purification and identification a substance connected with resistance in rubber (*Hevea brasiliensis*). Biologico, 36: 112-114.

92. Mattos, C.R.R. (1999). Culture media containing green coconut water for sporulation of *Microcyclus ulei*. *Fitopatologia Brasilieira*, 24: 470.

93. Mattos, C.R.R. (2004). Crown grafting – a way of fighting the *Microcyclus ulei* – The experience at the Michelin Bahia Plantation. *IRRDB/Michelin/CIRAD International Workshop on SALB*, 2004, Bahia, Brazil, 3 pp.

94. Mattos, C.R.R., Garcia, D., Pinard, F., LeGuen, V. (2007). Variability of *Microcyclus ulei* from South East Bahia. *Fitopatologia Brasileira*, 28:502-507.

95. Medeiros, A.G. (1973). Tecnica simples para isolar *Microcyclus ulei* (P. Henn.) v. Arx, fungo responsavel pela 'Queima sul-Americana' das folhas da seringueira. *Revista Theobroma*, 3: 57-76.

96. Mello, S.C.M. de (2004). Dicyma pulvinata, a biological control agent for South American leaf blight (*Microcyclus ulei*). *IRRDB/Michelin/CIRAD International Workshop on SALB*, 2004, Bahia, Brazil, Mimeograph, pp. 5.

97. Mello, S.C.M., Estevenato, C.E., Brauna, L.M., Capdeville, G., Queroz, P.R. and Lima, L.C. (2007). Antagonistic process of *Dycima pulvinata* against *Fusicladium macrosporum* on rubber tree. *Boletim de Pesquisa e Desenvovimento*, 169, 17 p.

98. Mello, S.C.M, Santos, F., Silva, M. da J.B.T. (2006). *Dicyma pulvinata* isolates colonizing *Microcyclus ulei* stroma in rubber. *Pesquisa Agropecuaria Brasileira*, 41:359-364.

99. Miller, J.W. (1966). Differential clones of *Hevea* for identifying races of *Dothidella ulei*. *PIant Disease Reporter*, 50: 187-190.

100. Moraes, V.F. and Moraes L.A.C. (2008). Effects of SALB resistant budded crowns on the yield and physiological parameters of *Hevea* latex.

101. Neto, B.F., Furtado, E.L., Cardoso, R.M.G., Oliveira, D.A. and Roloim, R.R. (1991). Systemic fungicide effects on lifecycle of *Microcyclus ulei*, agent of South American leaf blight. Summa Phytopatologica 17: 238-245.

102. Ong, S.H. (1980). Breeding for disease resistance. *Hevea breeding Course, Lecture notes*. Pp. 1-13.

103. Ong, S.H., and Tan H. (1987). Utilization of *Hevea* genetic resources in the RRIM. *Malaysian Applied Biology,* 16: 145-155.

104. Pereira, J.L., Rao, B.S. and Ribeiro, J.L. (1980). Role of oil in fungicide formulations in the control of *Microcyclus ulei*. *Seminario Nasional de seringueira, 1980, Anais,* Vol. 1, p. 223-252.

105. Pinheiro, E. (1995). Reducing SALB risks – Cultivating rubber in 'escaped areas'. Paper presented at a colloquium at the Rubber Research Institute of Malaysia, 1995, 5 pp.

106. Pinheiro, E. and Libonati, V.F. (1971). O emprego do *Hevea pauciflora* M.A. como fonte genetica de resistencia ao mal das folhas. *Polimeros*, 1: 31-39.

107. Pinheiro, E. and Lion, A. (1976). Perspectivas do emprego da *Hevea pauciflora* na enxertia da copa de seringueira. *Seminario Nacional da Seringueira, Rio Branco, Acre, Mimeograph*, 11 pp.

108. Pita, F.A.D.O., Junqueira, N.T.V., Alfenas, A.C. and Cano, M.A.O. (1992). Phenolic regulation of resistance to *Microcyclus ulei* infection in *Hevea* progenies. *Pesquisa Agropecuaria Brasileira*, 25: 1193-1200.

109. Rao, B.S. (1973a). Potential threat of South American leaf blight to the plantation rubber industry in the Southeast Asia and the Pacific region. *FAO Plant Protection Bulletin*, 21: 107-113.

110. Rao, B.S. (1973b). South American leaf blight: chances of introduction and likely behaviour in Asia. *Quarterly Journal Rubber Research Institute Sri Lanka,* 50: 216-222.

111. Rivano, F. (1997). La maladie sud-americaine des feuilles de l'heveae I. Variabilite du pouvoir pathogene de *Microcyclus ulei*. *Plantations, recherche, developpement,* Mai-Juin, pp. 104-110.

112. Rivano, F. (2004). Rubber growing in Latin America: a new challenge. *IRRDB/Michelin/CIRAD International Workshop on SALB*, 2004, Bahia, Brazil, Mimeograph, 6 pp.

113. Rocha, H.M., Aitken, W.M. and Vasconcelos, A.P. (1975). Control of South American leaf bllight (M*icrocyclus ulei*) of the rubber tree in Bahia: Aerial spraying of fungicides in the region of Itubera. *Revista Theobroma,* 5: 3-11.

114. Rocha, H.M., Medeiros, A.G. and Vasconcelos, A.P. (1973). Selecao de fungicidas para o controle de 'mal-das-folhas' *(Microcyclus ulei*) en condicoes de viveiro. *CEPEC Informe Tecnico 1972 and 1973*, p. 54.

115. Rocha, H.M., Medeiros, A.G. and Vasconcelos, A.P. (1978a). Comparacao de fungicidas para controle do 'mal-das'folhas' de seringueira. *Fitopalogia Brasiliera*, 31, 163-167.

116. Rocha, H.M. and Vasconcelos Filho (1978). Epidemiology of the South American leaf blight of rubber in the region of Itubera, Bahia, Brazil. *Turrialba*, 28: 325-327.

117. Rodrigues-Machado, R.F., Sena-Gomes, A.R., Rocha, H.M. and Vasconcelos, A.P. (1974). Programa especial de pulverizacao aerea de seringais na Bahia. *CEPLAC/SUDHEVEA*, Mimeograph, 18 pp.

118. Rogers, T.H. and Peterson, A.L. (1976). Control of South American leaf blight on a plantation scale in Brazil. *Proceedings International Rubber Conference,* 1975, Kuala Lumpur, Malaysia, 3: 266-277.

119. Sambugaro, R. (2003). Caracterizacao anatomica foliar de clones de seringueira visando resistancia ao *Microcyclus ulei*. Thesis, Universidade Estadual Paulista, 61 pp.

120. Sanier, C., Berger, P., Coupe, M., Macheix, J.J., Petat, J.M., Rivano, F., Sainst-Blanquat, A. de and D'Auzac, J. (1992). Relationship between resistance to *Microcyclus ulei* and clonal foliar phenolics of rubber trees. *Journal of Natural Rubber Research*, 7: 38-59.

121. Santos, A.F. dos and Pereira, J.C.R. (1985). Efficiency of fungicides in the control of *Microcyclus ulei in vitro* and *in vivo*. *Revista Theobroma*, 15: 185-190.

122. Santos, A.F. dos and Pereira, J.C.R. (1986a). Evaluation of systemic fungicides in the control of *Microcyclus ulei*. *Fitopatologia Brasileira*, 11: 171-176.

123. Santos, A.F. dos and Pereira, J.C.R. (1986b). Evaluation of protective and systemic fungicides and their mixtures in the control of *Microcyclus ulei*. *Revista Theobroma*, 16: 141-147.

124. Silva, L.G.C. (2007). Mapping risk of occurrence of South American leaf blight of rubber using Geographical Information Systems (GIS). *Thesis, Universidade Federal do Vicosa, Brazil*, 38 pp.

125. Simmonds, N.W. (1990). Breeding horizontal resistance to South American leaf blight of rubber. *Journal Natural Rubber Research*, 5: 102-113.

126. Soepadmo, E. (1975). *Microcyclus ulei* – Bahaya laten bagi industri karet alam di Indonesia. *Menara Perkebunan*, 43: 303-304.

127. Subramanian, S. (1969). Performance of recent introductions of *Hevea* in Malaysia. *Journal Rubber Research Institute Malaysia*, 21: 11-18.

128. Subramaniam, S. (1970). Performance of *Dothidella*-resistant *Hevea* clones in Malaysia. *Journal Rubber Research Institute Malaysia*, 23: 39-46.

129. Tavares, E.T., Tigano, M.S., Sueli, C.M.M., Martins, I. and Cordeiro, C.M.T. (2003). Molecular characterization of Brazilian *Dicyma pulvinata* isolates. *Fitopatologia Brasileira,* 29: 148-154.

130. Thurston, D.H. (1973). Threatening plant diseases. *Annual Revue Phytopathology*, 11: 27-52.

131. Wijewantha, R.T. (1965). Some breeding problems in *Hevea brasiliensis*. *Journal Rubber Research Institute Ceylon,* 41: 12-22.

132. Wirjomidjojo, R. (1962). First report on performance of *Dothidella ulei* resistant clones in Experimental Garden, Tjiomas. *Menara Perkebunan*, 31: 181-185.

133. Zhang, K.M., Chee, K.H. and Darmono, T.W. (1986). Survival of South American leaf blight on different substances and recommendations on phytosanitary measures. *Planter, Kuala Lumpur*, 62: 128-133.