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International Workshop on
Circular Leaf Spot Disease (Pestalotiopsis)
&
Other Economically Important Diseases of Rubber Plantations

"New Directions & Strategies for Sustainable Management"
September, 2023

Jointly organized by

Rubber Research Institute of Sri Lanka

International Rubber Research & Development Board

Editors

T.H.P.S. Fernando BSc, Mphil, PhD (SL)
M.K.R. Silva BSc Agri, MSc, PhD (SL)
K.V.V.S. Kudaligama BSc, Mphil, PhD (SL)

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Message from the Secretary General, International Rubber Research & Development Board

Pestalotiopsis (Circular Leaf Spot) Disease of Rubber: A Challenge to Global Natural Rubber Supply

The rubber tree *Hevea brasiliensis* has its origin in the Amazon rain forest. The product from the tree – Natural Rubber (NR) has now become an indispensable raw material for the humanity, largely due to its wide industrial applications particularly in tyres, which are essential for human mobility. The global consumption of NR is nearly twelve million tonnes. About 90% of the supply of NR is from South East Asia. The rubber tree was introduced into South East Asia from South America, due to the favourable climatic conditions and its high productivity. The trees were not affected by South American Leaf Blight (SALB) which depressed the production in South America.

The rubber production in South East Asia is under great threat since 2018 due to the wide spread occurrence of *Pestalotiopsis* leaf disease. This disease was first reported in Medan and Palembang in Indonesia during 2016. The area affected increased to 387,000 ha in 2018. The disease spread to Malaysia and by 2021 about 20,000 ha was affected. The current statistics show that 75,000 ha is affected in Malaysia 125,000 ha in Thailand and 40,000 ha in Sri Lanka. Other rubber growing areas are also affected to varying extents. The crop loss in the affected areas is reported to be more than 40 per cent.

The IRRDB has taken note of the threat caused by this disease and initiated actions to identify the causal pathogen, study the epidemiology and conducted research on effective control measures. The member Institutes were notified of the seriousness of the disease and took immediate research activities.

Following the discussions on global disease scenario, during the IRRDB Annual Conference 2018, held in Abidjan, Cote d' Ivoire, the IRRDB approached the Association of Natural Rubber Producing Countries (ANRPC) to revive the activity of the ANRPC Technical Committee on Crop Protection. Subsequently four meetings of the ANRPC Technical Committee were held, the latest being in Davao, Philippines in August 2023. The discussions focused mainly on ways and means to address the *Pestalotiopsis* leaf disease threat in Asia.

With the opening up of international travel after the Covid 19 epidemic, the IRRDB conducted workshops in India, Indonesia & Malaysia. Experts visited disease affected rubber plantations to assess the gravity of the situation and to evaluate the results of small-scale trials on disease management. Subsequently, a meeting was held in December 2022 at Port Dickson, Malaysia to work out strategies for future research. A draft research programme was drawn out. Financial support for this programme is being explored. Although interim measures on disease management by chemical means are recommended in the affected regions, these are in general, costly and not affordable for rubber small growers who produce more than 90% of the global supply of NR. Hence there is a need to evolve more sustainable and affordable disease control strategies. In Malaysia, the government has allocated around USD 10 million to assist in the spraying programme.

This workshop in Sri Lanka will review progress of the research and development activities conducted in the member Institutes. The scientists will be encouraged to take up sustainable strategies like molecular manipulations and breeding for disease resistance. Sri Lanka has demonstrated that disease resistance breeding approach is effective in the case of *Corynespora* leaf disease. Sri Lanka has rich experience in breeding and selection of high yielding clones with resistance to important leaf diseases. It is in this backdrop that Sri Lanka is selected as the venue for this workshop.

Let us hope that we will have effective discussions which can help us to set achievable targets in sustainable and affordable management of *Pestalotiopsis* leaf disease. On behalf of the Directors and Board Members of the IRRDB, I would like to express our thanks to the RRI Sri Lanka for the organization of this workshop.

Dato' Seri Dr. Abdul Aziz S. A. Kadir
Secretary General
International Rubber Research & Development Board

The Current Status of the Circular Leaf Spot Disease in Sri Lanka and the Way Forward

T.H.P.S. Fernando, M.K.R. Silva and E.A.D.D. Siriwardena
Rubber Research Institute of Sri Lanka

Rubber plantation industry plays a vital role in Sri Lankan economy. It generates export earnings (approx. USD 1 billion), sustains the livelihood of thousands of people, provides the most versatile raw material for numerous industries, supplements thousands of hectares to the forest cover, contributing to CO_2 sequestration, fuel and timber. Rubber plantations conserve water reservoirs, the bio-diversity and the cooling effect to the environment. This industry provides many other socio-economical and ecological benefits. Today, the industries and new avenues based on both plantation sector and the production sector are flourishing. Materializing this treasure in our hands, has become of utmost importance. In the face of declining rubber growing extent, saving the existing rubber lands is essential since they are the main source of latex, one of the most versatile industrial raw materials playing the key to many industries. More than 70% of the plantations are represented by the small holder sector, being their sole livelihood. The balance 30% are under the management of about nineteen regional plantation companies making a significant contribution to the economy of Sri Lanka. The productivity of the plantations should be improved to sustain the national production and to raise the income levels of the growers. Just like in all the other sectors, plant protection remains the first line of defence against the development of the industry.

Report of the New Leaf Fall Disease in Sri Lanka

With the report of the new leaf fall disease condition in 2019, the Rubber Research Institute took immediate actions to educate the Extension Staff and the growers regarding the disease, in order to isolate the disease, and to limit further spread of it. A detailed disease detection survey was undertaken by the collaboration of the Rubber Research Institute, Rubber Development Department and the Regional Plantation Companies. A national programme to combat this new disease has also been implemented to delimit the spread of the disease to new areas.

Identification of the Disease

The disease starts with a pin point or pin head sized lesions. Later they become larger forming characteristic circular lesions. Under conducive weather conditions, the small circular lesions may coalesce to form larger necrotic areas. With time, a blight condition showing yellowish leaves are observed at the lower canopies of the plants leading to a leaf fall condition (Fig. 1).

Causative Agents of the Disease: Sri Lanka's Scenario

In Sri Lanka both the causative agents have been consistently isolated from diseased leaf samples. More than 500 numbers of disease samples have been studied and around 70% of the isolates resulted with Pestalotioides group. Three genera namely: *Neopestalotiopsis* spp., *Pestalotiopsis* spp., and *Pseudopestalotiopsis* spp. were among the collection of isolates. Approx. 25% of the fungi were *Colletotrichum* species (*C. siamense*, *C. fructicola*, *C. tropicale*, *C. gigasporum*) (Fig 2). And the remaining 5% was represented by various types of fungi like *Fusarium* spp., *Botryodiploidea* spp., *Curvularia* spp. *Phomopsis* spp., and *Negrospora* spp. The pathogenicity studies have shown that even though *Colletotrichum* were less in number, they were the most pathogenic group. Pestalotioides was more abundant but majority of the isolates were non-pathogenic or very mild in their pathogenesis. However, Kock's postulates have been proven using both the pathogen groups. Mixed infection conditions are very rarely reported from rubber plantations and this is the first occasion of a synergistic action made by several fungal organisms on a rubber leaf fall disease. The present causative agents are new to all the rubber growing countries. Since this is a mixed infection condition, the disease has been named as the "Circular Leaf Spot Disease" – (CLSD).

Factors Affecting the Severity of the Disease

To reveal the factors affecting, a detailed survey has been carried out. Below factors have been observed affecting the incidence and severity of the disease. Weather factors such as rainfall and its distribution has influenced the disease severity level significantly. The clonal composition in the country has badly influenced the disease spread. The clone RRIC 121, the most severely affected clone consisting of more than 75% of the country's rubber extent. The wintering pattern also interferes the disease level and the early wintering clones show a slight escape from the disease while the late wintering clones succumbed to the disease. The prevailing low prices too have an influence since most of the growers are not following the good agricultural practices. The prices of agricultural inputs like fertilizers and pesticides have gone up considerably. The overall poor condition of the cultivations, coincides with the disease to make the cultivation unproductive. The use of inappropriate exploitation systems is playing a role while over exploitation is also observed as a serious problem in most of the plantations.

Since the Year 2019…..

In Sri Lanka, the disease was first reported during the later part of 2019 spreading to approx. 100 ha. Application of pesticides for delimiting operations restricted further spread of the disease to a considerable level. By the end of 2021, the disease had spread to over 20,000 ha where around 10,000 ha were in moderate to severe condition. Different plantation companies report various levels of crop losse but they are due to many other reasons as well. However, it should be understood that if no proper action is made to manage the other causative reasons, the natural rubber production of the country will be further affected. Moreover, this pathogen is having alternative hosts such as tea, cinnamon, papaya, guava, coconut and oil palm. There is a high possibility for the pathogen to invade the other agricultural crops as well. Department of Agriculture and other Crop Research Institutes of the country were also informed of the condition to be in alert about the spread of the disease to other crops. Based on the observations made so far, the pathogen enters into the plant at the time of refoliation. Tender leaves are highly susceptible to the pathogens. And some of the fungi are found as endophytes, showing a growth inside the plant without harming. Since these fungi are regarded as opportunistic pathogens, whenever the cultivation become poorly maintained, they have the capacity to be pathogenic. The wet weather is highly favourable for the incidence and the severity of the disease.

Screening of *Hevea* Clones Against the Disease…

This disease has been reported from almost all the rubber clones grown in the traditional rubber growing areas of Sri Lanka in different severity levels. Identification of a disease tolerant rubber clone will be the most reliable and long term solution to combat the disease. The pathogen population studies and the studies on the biology of the pathogens should be completed before performing the clonal screening programme. For the matter of urgency, a clonal screening programme has been launched by the Plant Pathology & Microbiology Department (Fig 3). The research conducted for the last two years revealed that clones like RRIC 100, RRISL 2006 and CEN 4 tolerate the disease. Further research should be made; more observations should be collected before making firm conclusions.

History of Rubber Diseases

During the past too, handling of leaf diseases of rubber plantations had not become an easy task. Powdery mildew had become very destructive at very early stages and sulphur dusting had been undertaken by the growers for the survival. During 1980's a global epidemic on Corynespora Leaf Fall Disease (CLFD) was experienced (Fig. 4) and several very high yielding clones like RRIC 103, RRIC 110 were affected. The last option a Pathologist may recommend is the host eradication and then the RRISL had recommended this option in view of saving the industry. After about two decades, RRISL introduced an internationally recognized screening tool to detect Corynespora susceptible clones. By now, the department of Plant Pathology & Microbiology and Genetics & Plant Breeding are capable in detecting CLFD resistant clones.

Management of the Leaf Fall Disease

This malody is not considered as a lethal disease. It has been identified as a secondary leaf fall condition. Hence, integrated disease management is advocated by the RRISL.

Prevention of the Disease….

Prevention will be the most effective method to manage any disease. To restrict further spread and severity, there should be a national effort. All the rubber growers, RPCs and small growers should take part in this programme otherwise the attempts will be a failure (Table 1). In rubber plantations, there is an annual natural leaf fall - wintering during the months of December – January. To prevent the buildup of a high inoculum potential, the growers are requested to collect the infected fallen leaves and to burn them under controlled conditions or a ditch can be made in between the plants and all the collected leaves should be used for composting (this is an on-going trial). Through this operation, the inocula exposed to the new flush appearing at the time of refoliation can be reduced to a significant level. RRISL provides a bio pesticidal decomposing microbial culture to be used in these ditches. Most of the plantations have not been properly fertilized during the past. Proper fertilization will affect the vigour of the plants and will improve the resilience and strength to tolerate the disease.

Other good agricultural practices like weeding, management of the cover crops etc. too should be followed. It has been observed that in all the cultivations there are weak plants that will never reach the tappable level. Being the runts, these plants easily catch the disease, propagate them spreading to the surrounding trees.

It has been proven that these weak plants have a direct relationship to early disease incidences and also to the severity level. These weak plants should be removed to lower the natural inoculum potential. Moreover, this operation will improve ventilation and lower the density of the plantations. The abandoned or neglected cultivations also need to be cleared since they will fascilitate the propagation of the pathogen.

Chemical Controlling of the Disease

The fungicide application has been recommended where essential under very severe conditions (disease hot spots). This operation will lower the disease severity, avoid any die-back condition and reduce the leaf fall. Interim recommendations have been made for chemical control of the disease. Two systemic fungicides namely carbendazim - (10 g per liter) and hexaconazole - (10 ml per liter) are recommended for chemical controlling (Fig. 5).

The timing for fungicide applications is very important (Fig. 6). All the immature plantations can be protected as their height of the canopy is easily reachable for chemical applications. Two types of mist blowers have been identified to be used in chemical applications. The mist blower carried by four men taking the mist to more than 50 ft height is appropriate for the mature rubber plantations. The single man carrying mist blower is capable in reaching a height around 40 –50 ft. Chemical controlling alone will not be sufficient to manage the disease. Overall agronomic practices that have been recommended by the Rubber Research Institute such as weeding, fertilizer application, correct tapping system are helpful in the disease management. All stakeholders are requested to get the support of the Rubber Development Department or the Rubber Research Institute to identify the disease and to make proper steps in the disease management.

Biological Controlling of the Disease

Identification of biological controlling agents for the disease is important since they will make the management strategies more environment friendly. RRISL has already identified biological controlling agents and under laboratory conditions, they are very effective (Fig. 7). In Sri Lanka, native endophytic micro-organisms have been isolated and the research is in progress to test the efficacy under field conditions.

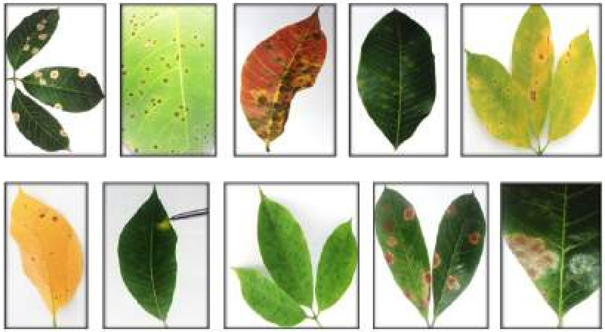


Fig. 1: Diversity of the symptoms

Cultural & Reproductive Morphology: Pestalotioides Genera

Cultural Reproductive Morphology: Colletotrichum spp.

Fig. 2: Cultural and Reproductive Characteristics *Pestalotioides* & *Colletotrichum*

Fig. 3: Screening of *Hevea* Clones

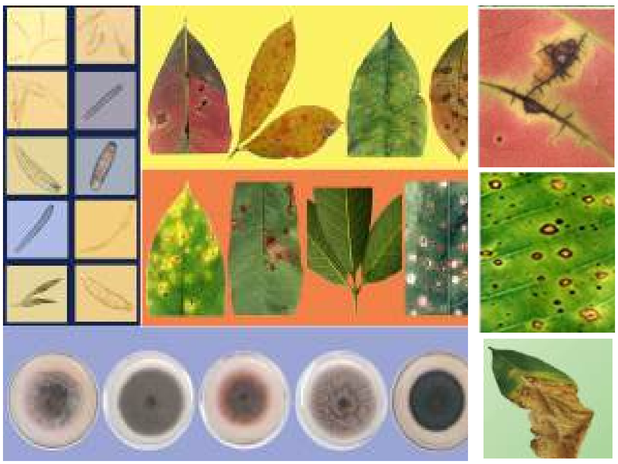


Fig. 4: Corynespora Leaf Fall Disease

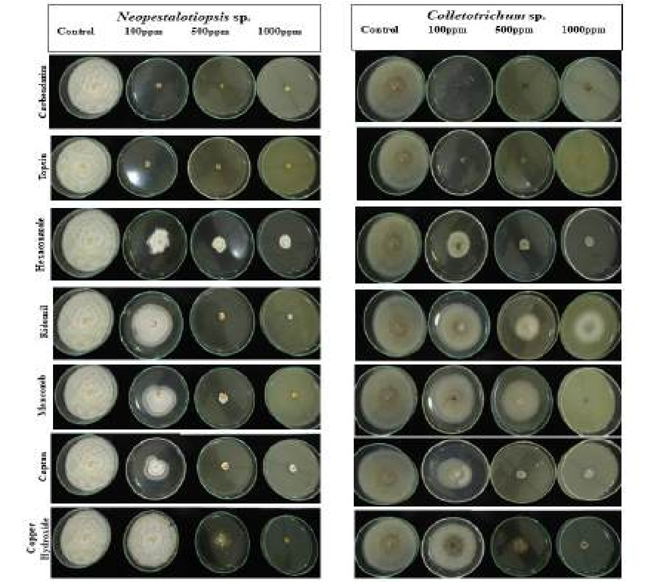


Fig. 5: Identification of effective fungicides-Poisoned food technique

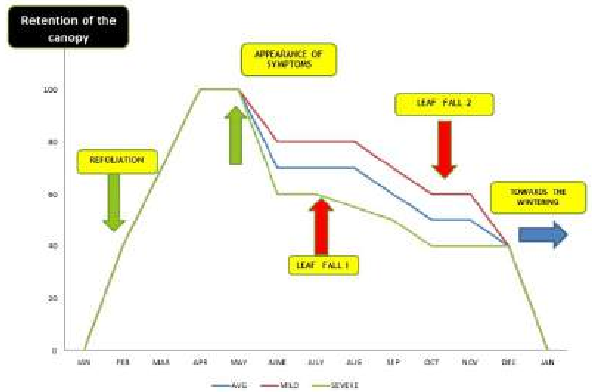


Fig. 6: Progressive changes in the disease incidence & severity

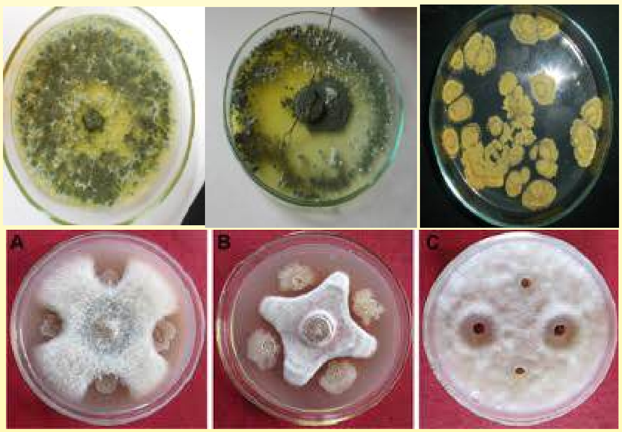


Fig. 7: Identification of Biological Controlling agents against CLSD pathogens

Spraying / Fogging fungicides against CLSD

Factors Affecting the Severity of the Disease

To reveal the factors affecting the disease severity, a detailed survey has been initiated. Weather factors such as high rainfall and its distribution have influenced the severity level very badly. Among the existing clones, RRIC 121 has been categorized as a severely affected clone. In Sri Lanka, RRIC 121 represents more than 75% of our cultivations. This is one of the reasons for the high incidence of the disease in the country. Hence, growers should diverse their clones reducing the crop risk. Current tapping systems have not been correctly followed by many growers. It has been reported that there is a high incidence of Tapping Panel Dryness in our rubber plantations due to the adoption of tapping irregularities. Such irregularities give extra pressure on the trees and naturally they succumb to the diseases easily. Most of the plantations are up kept very poorly and the overall sanitation of them is not in a good condition. Abandoned plantations are also seen and these plants easily catch the disease, spreading the inocula to the surrounding areas. The growers should always contact the Rubber Research Institute and Rubber Development Department for advices in improving the overall conditions of their plantations and that will definitely help to reduce the severity level of the disease. A national plan has been set to manage the disease in Sri Lanka and all the stake holders, Extension staff and Researcher should implement this as a joint effort.

NATIONAL PLAN TO COMBAT THE LEAF FALL DISEASE IN THE RUBBER PLANTATIONS OF SRI LANKA [2023 - 2025]

Strengthening the Implementation of the Recommended Strategies

RRISL - RDD - MPI

Goal: National Programme supports research and the existing knowledge on the Circular Leaf Spot Disease of rubber plantations and to develop effective disease management strategies.

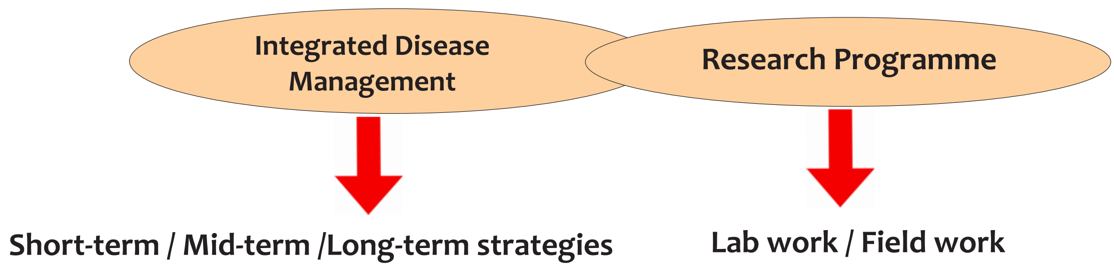
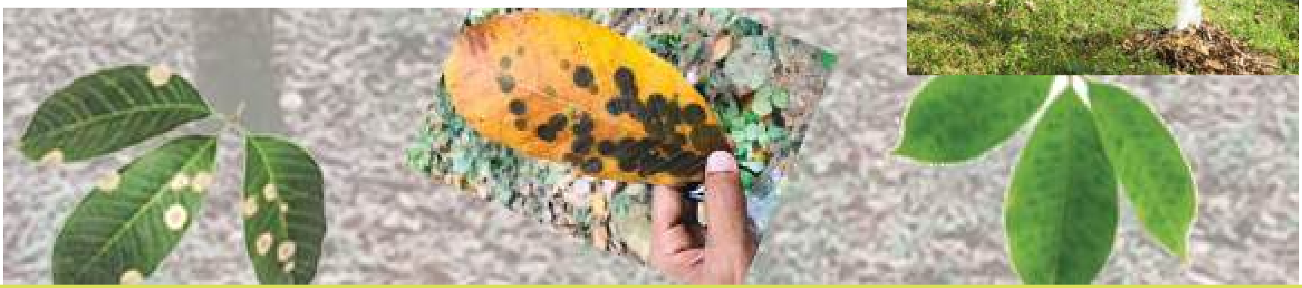


Table 1 : National Programme to be conducted to combat CLSD 2023-2025

Action	Activities to be undertaken	Details	Responsibility
Reduction of the inoculum potential	Collection of all the fallen leaves and debris in to ditches in between the rubber plant rows for composting	Especially for smallholder farmers	RDD RRISL RPCs
	Biopesticide for destroying the pathogen & microbes for the acceleration of composting	RRISL will provide the microbe consortium to accelerate the decaying	RRISL RDD
Disease avoidance	Plant disease escaping rubber clones RRIC 100 / RRISL 2006 / CEN 4	Recommendation and to provide the clones to RPCs	RRISL RDD
Removal of runts from the plantations	Removal of weak plants and plants beyond reaching the tappable level	Avoid points of inocula Lower the plant density to improve ventilation	RDD RPCs
Quarantine legislation Nurseries	Adoption of recommended measures in transportation of plants or planting materials to disease free areas	Removal of infected leaves. Burn them. Fungicide application before transportation	RRISL RDD
Recommended fertilizer application	Application of fertilizer to improve tree vigour / resilience to the disease	To establish demonstration plots	RDD RRISL
Management of weeds to avoid harbouring pathogens	Exclude weeds with pathogens Lower the humidity levels to suppress pathogens growth	To establish demonstration plots	RDD RRISL
Disease Management - Chemical & Biological controlling	**Chemical controlling** Use of fungicides where more than 60% of leaf fall is observed	**Carbendazim 10g / L & Hexaconazole 10ml / L** Cont inuation of the RRISL trials	RDD RRISL
	Biological controlling Under pilot scale trials	Continuation of the trials	RRISL
Popularization of the activities	Posters / Leaflets / newspaper articles / media conferences / training programmes	Awareness programmes for implementation	RDD RRISL

National Programme contd.

Action	Activities to be undertaken	Details	Responsibility
Developing a surveying system - remote sensing to mark the disease hot spots -	Demarcation of disease hot spots / vulnerable areas / disease free areas Establishment of geographical distribution of the disease Guide for a site specific chemical controlling programme	To be undertaken during the months of Sept- October	RRISL RDD RPCs
Screening of clones against the disease	Lab conditions Four nurseries Natural field conditions	Identification of disease tolerant clones Eg. RRIC 100, RRISL 2006, CEN 4	PP & MB RRISL
Breeding for resistance and keeping the clonal balance	Field programmes Introduce more disease resistant / tolerant clones	To introduce promising clones since RRIC 121 currently occupy [illegible]	RRISL G & PB RPCs
Modified fertilizer recommendations to withstand the spreading disease	Field trials on going At least the recommended fertilizer application should be adopted	Since there is an additional leaf fall condition, additional nutrients will be essential	RRISL SPN
To establish the crop lose due to the disease	Field trials Currently the average crop los due to the disease has been estimated as 10%	RRISLto survey, collect data and analyze	PS BC & P PP & MB
Training of Extension staff / Technical staff	RDD / ASD / RPCs / Support from the PA/ Summarizing the Google form / Weather factors	RRISLto conduct training programmes	RDD / ASD PP & MB BM



Current Research & Development Programme Conducted by RRISL Collaboratively with other National/ International Organizations & Universities to combat Circular Leaf Spot Disease

- Conduct a joint disease survey – RRISL/ RDD/ RPCs
    - To trace out the total disease extent in the country / To identify disease hot spots
- Improvement of strategies to reduce the inoculum potential (Agronomic/ Biological/ Chemical Practices)
- Induction of tree resistance through immunity buildup (Agronomic/ Biological/ Chemical Practices)
- Biological controlling methods – Using native biological controlling agents
- Reduction of the disease causing fungus load in the field
    - Construction of in-situ ditches and burrowing of fallen diseased leaves in them
    - Burning of fallen diseased leaves
- Studies on the life cycle characters of the associated pathogens – provide proper information to streamline the disease management protocols
- New pesticide formulations for higher effectiveness
    - With different fungicides & their concentrations – *In-vitro* & field experiments
    - In different solvents (Water/ Agriculture oil based/ White oil/ Diesel)
- New application techniques to reach high tree canopy & to improve the effectiveness
    - Use of the drone technology for fungicide application
    - Use of the fogging technology for fungicide application
- Studies on the impact of weather factors and the climate change on disease development
- Clonal screening programme –to identify disease-tolerant clones in the recommended clone list / among the clones in the pipeline
- Breeding for resistance to introduce disease resistance new clones
- Studies on the avenues of manipulating fertilization on the disease management
- To evaluate the socio economic impacts of the disease and to trace out the crop drop due to the disease & to identify other factors affecting the crop drop

Facing Challenges in Diagnosis & Management of Rubber Diseases

C.K. Jayasinghe
Fellow, International Rubber Research & Development Board

As with any other agricultural crop the consequence of the number of diseases increased with the domestication of the rubber tree and to-date more than hundred pathogens have been identified as capable of attacking the rubber tree. One of the interesting features of the disease scenario is the considerable change taken place in the relative importance of the diseases during the last several decades. Significant contribution towards this change was the production and well acceptance of the clones tolerant to common diseases. Unfortunately, some of these high yielding breeds succumbed to new diseases creating unrest among the growers.

Some of the challenges faced by the plant protectionists are changing scene of the disease scenario of the rubber tree, breaking down of the disease resistance, invasion of new pathogens from neighbouring countries & other continents, difficulties encounted in spraying chemicals, complicated symptom production, withdrawal of fungicides due to their high toxicity and complications in screening for disease tolerance. However, today Pathologists are well equipped with basic information on the maladies of the rubber tree thanks to intensive research carried out for more than a century. Further, more than 20 invaluable tools have been offered during the recent past with the view of enhancing the knowledge of plant protectionists in the world with the courtesy of IRRDB.

Climate change is a new threat as weather plays as important role in development and spread of all significant maladies of the rubber tree. The relationships have been well established for historical diseases like Oidium leaf fall, Gloeosporium leaf fall, Phytopthora leaf fall, South American leaf blight, Bark rot and White Root Disease since the middle of the 20^{th} century. Research activities are in progress to establish the effect of weather on Corynespora leaf fall, a deadly disease of recent origin.

The only hope in long-term management of canopy diseases is the production of disease resistant genetic materials. It is happy to note that not only leading rubber growing countries but also underprivileged members of the IRRDB has taken steps to confer the disease resistance to new genetic materials using outstanding disease resistant clone like RRIC 100.

Management of Pestalotiopsis/Circular Leaf Spot Disease in Rubber Plantations: Global Scenario

C. Kuruvilla Jacob

Fellow, International Rubber Research & Development Board

Sporadic occurrence of Pestalotiopsis leaf disease on rubber was first reported from Johor, Malaysia during 1975. The symptoms described closely resembled that of Anthracnose disease reported in 1961 from Inda although the pathogen identified then was *Glomerella cingulata*, the perfect stage of *Colletotrichum* spp. Detailed morphological and molecular studies on the pathogen were taken up only during 2018 subsequent to the epidemic scale spread of the disease in Indonesia and Malaysia during the period 2016 to 2018. The pathogen was identified as *Pestalotiopsis* spp. based on the conidial characteristics and was later confirmed by molecular studies. Although a similar epidemic disease was reported during the same period from India and Thailand, the causal organism was identified as *Colletotrichum* spp.

Epidemic occurrence of Pestalotiopsis leaf disease was reported from Sri Lanka, Vietnam and Philippines where Colletotrichum was also reported to be associated in some cases. A sporadic occurrence Neopestalotiopsis with similar symptoms was reported from China. In Indonesia 387,000 ha was affected by Pestalotiopsis while in Malaysia, Sri Lanka and Philippines 73000, 40000 and 36000 ha respectively were reported to be affected. Infection by Colletotrichum covered 124,000 ha and 8,500ha in Thailand and India respectively. Rainy season favoured the incidence and spread of the disease at all regions. It extended from June to October at all the locations except Indonesia where the rains occur during February to June. A rainfall spell of >30 cm and humidity >80% favour the disease spread.

The symptoms appear as circular brown/grey lesions with or without a scabby centre and wavy/yellow margin. *Pestalotiopsis* conidia has distinct appendages on terminal cell while the conidial cells of *Colletotrichum* is cylindrical with smooth terminal cells, devoid of appendages. In the *in vitro* studies on fungicides at various concentrations, Triazoles, Carbendazim and Thiophanate-methyl caused inhibition of the pathogen. Mancozeb is not effective even at 1000 ppm.

Repeated spraying with systemic fungicides was found effective in small scale field experiments conducted in Indonesia, Malaysia and Sri Lanka. About 4 to 6 rounds of fogging of systemic fungicides is widely practised in Indonesia but the leaf retention is not satisfactory and the crop loss is heavy (>40%). Drones were used on experimental scale for spraying fungicides, but single drone spray was not effective for disease control. In India. Spraying of copper oxy chloride dispersed in spray oil which is recommended for the control of Abnormal Leaf Fall disease could give protection against circular leaf spot disease also.

A sustainable alternative for disease control is crown budding of susceptible high yielding clones with resistant crown clone. This has been tried on experimental scale in India. Crown budding afforded protection against all the major leaf diseases including Colletotrichum. But crown budding after planting in the main field is very labour intensive and its establishment is difficult rendering the technique not feasible on a large-scale planting . Crown budding in the nursery stage and subsequent planting in the field has been observed to be easier and effective. We could achieve 98% success in field establishment by this method. It will be fully effective if the nursery is close to the main field, as damage during transport can be avoided.

Results of field trials for evaluation of the influence of increased dosage of fertilizers were reported from Indonesia. A 25% extra dose of K application reduced disease incidence by 9%. A 25% extra dose of N supply after the disease incidence triggered faster re-foliation and reduced the adverse effects of the disease. However, surveys conducted in Malaysia could not reveal any relationship between fertilizer application and disease incidence/severity.

Although scanty reports on field tolerance of cultivated clones against the leaf spot disease is available, there are no systematic studies to evaluate the rubber clones .The IRRDB germplasm collections available in our institutes are also not evaluated yet for disease resistance . This remains as a major gap in our knowledge.

New molecular tools like detection of QTL and their use for inheritance of disease resistance traits in the cross-bred progeny (F1 generation) for the leaf diseases of rubber including Colletotrichum leaf disease is being done in India. However, these studies are in very early stages and there is a long way to go before any transgenic plants with the resistance genes are evolved. The field evaluation of transgenic clonal population of rubber incorporated with Mn SOD gene, now being done in India, offers hope.

Other gaps in our knowledge include lack of understanding of the steps in the host- pathogen interaction, absence of quick screening methods to locate resistant clones and lack of knowledge on effective biocontrol techniques. A cost- effective disease management protocol is not available for recommendation to the rubber growers. These lacunae point to future research goals and should be regarded as challenges for our scientists.

Planters' Perspectives on the Circular Leaf Spot Disease: Sri Lankan Scenario

Manoj Udugampola
Chief Operating Officer – Rubber (Pussellawa Plantations Ltd) / Chairman – Colombo Rubber Traders Association

Introduction and Impact:

Pestalotiopsis leaf disease, also known as Circular Leaf Spot Disease (CLSD) caused by the fungus *Pestalotiopsis* spp., can have significant negative effects on rubber estates and is a matter of significant concern for rubber planters in Sri Lanka.

The disease was first detected in commercial plantations in Sri Lanka in latter part of year 2019. The Rubber Industry plays a crucial role in the country's economy, and any threat to its productivity demands attention. The disease's impact on rubber plantations could be detrimental, leading to reduced latex yield, compromised tree health, and economic losses. Therefore, CLSD could directly impact the economic viability of rubber plantations. The plantations in Sri Lanka already struggling due to decreased natural rubber prices in Colombo Rubber Auctions as well as at the Global Rubber Market, would face a severe blow if the leaf disease reduce the productivity and production of the rubber estates.

Challenges Faced:

1. **Climate and Environment:** Sri Lanka's tropical climate, with high humidity and rainfall, creates an ideal environment for the growth and spread of the Leaf Disease. This challenge is exacerbated during the rainy seasons, making disease management a constant struggle.
2. **Sustainability Concerns:** Overreliance on chemical treatments raises environmental and sustainability concerns. Balancing disease control with sustainable agricultural practices is a complex challenge that requires innovative solutions. The chemicals recommended for disease control by Rubber Research Institute of Sr Lanka (RRISL) – Carbendazim and Hexaconazole too are quite expensive.
3. **Limited Resources:** Many rubber plantations, particularly smaller ones, may lack the financial resources and technical expertise needed for effective disease management. This limitation can hinder timely and efficient response measures. The larger plantations too find it difficult to find suitable spraying equipment specially for tall trees in mature rubber plantations. The Spraying machines

Adaptation and Innovation: Planters are continually adapting to the evolving challenges posed by Pestalotiopsis leaf disease. They are exploring innovative practices and technologies.

Collaboration and Knowledge Sharing: Planters are keen on collaboration and knowledge sharing. They often participate in workshops, seminars, and research initiatives that provide insights into the disease's dynamics, management strategies, and best practices. Work very closely with RRI of Sri Lanka.

Government Support and Research: Looking at the current scenario, Planters seek the support of the Government and all Agricultural Institutions of the country other than the RRI of Sri Lanka for support. Adequate funding for research and extension services is crucial to developing locally relevant strategies for disease management.

Education and Awareness: Planters play a pivotal role in educating estate staff workers about the disease, its symptoms, and the importance of timely reporting with the assistance of RRI of Sri Lanka.

Long-Term Outlook: While the challenges posed by Pestalotiopsis leaf disease are substantial, Sri Lankan rubber planters remain resilient. By combining traditional knowledge with emerging scientific insights, they are striving to ensure the sustainability of rubber cultivation in the country.
In conclusion, from a Sri Lankan planter's perspective, Pestalotiopsis leaf disease presents multifaceted challenges that demand a holistic approach involving cultural practices, sustainable management strategies, collaboration, and innovation. The aim is to secure the health of rubber plantations, maintain latex production, and contribute to the country's agricultural economy.

The Status of the Circular Leaf Spot Disease (Pestalotiopsis) in IRRDB Member Countries

Tri Rafani Febbiyanti
International Rubber Research & Development Board

New Leaf fall disease (LFD) was first detected in 2016 in North Sumatra, Indonesia. Then, this disease was reported from other rubber producing countries.

In Indonesia, in 2018 the attack area reached 22,000 hectares, growing to 103,000 hectares in 2018. By April 2019, became 382,000 Ha in June 2019 (Febbiyanti, 2019). In Malaysia, the development of LFD was reported in November 2017 which attacked smallholder rubber plantations in Labis, Kulai and Setindan Kluang Johor Plantations (Adam, 2019). In 2020, the attacked area due to LFD in Sri Lanka was 20,000 hectares, in Thailand was 122,537 hectares and in was India 300 hectares.

Table 1: LFD affected areas in each country

Country	Detect year	Area affected (ha) by the year
Indonesia	2016	390,000 (2022)
Malaysia	2017	73,000 (2021)
India	2017	8,500 (2022)
Thailand	2019	124,000 (2022)
Sri Lanka	2019	30,000 (2023)
Vietnam	2021	10,000 (2022)
Philippines	2022	36,000 (2023)

Fig. 1:
Symptoms of new leaf fall disease

Symptom of this disease is characterized by the formation of spots on the mature leaves. Then the spots continue to develop so that the tissue around the spots undergoes necrosis (Figure 1). The spot size varies between 0.5 to 3 cm in diameter. The leaves turn yellow sporadically and then the leaves fall off. Severe infection can cause leaf fall to continue until the plant canopy becomes thin.

Leaf fall disease causing pathogen: *Pestalotiopsis* sp. is an airborne pathogen that spreads very quickly, mostly attacking older leaves, attacking all clones and also attacking all ages of plants. This disease causes continuous /simultaneous leaf drop which causes the plant canopy to become thin, defoliate up to 75-90% and finally there are no leaves in the plant canopy.

Pestalotiopsis sp. is a weak pathogen that can infect plants through wounds. Fungal spores (conidium) are dispersed by the wind, but at close range, the spores can be carried by splashing water and insects (Selmaoui et al., 2014). Furthermore, *Pestalotiopsis* sp. are also endophytic in many plants (Metz et al., 2000) and are opportunistic pathogen that can attack some ornamental plants (Pirone 1978). *Pestalotiopsis* species were also reported to attack several plants (Calluna, Erica, Pieris and Rhododendron) and the number of infected plants continued to increase (Hopkins & McQuilken, 2000).

Losses caused by this disease, namely the decline in production reached more than 25% -30%. Based on data from the Indonesian Rubber Research Institute, Sembawa, the decline in production in May 2018 was around 27%, then in June 2018 it was 45.8%, compared to 2017. Losses due to this disease in 2019 led to a decrease in production of up to 41%.

Disease development and different levels of damage by pathogen of LFD in rubber plantation are influenced by the rubber clone resistance, pathogen virulence and environmental conditions such as soil, altitude and climatic factors. The severity of the disease will be high if the rainfall reaches 300 mm/month with an average air humidity of more than 80%. Elnino and Lanina phenomenon conditions also affect the severity of this disease and loss of production in rubber plants.

Management of LFD

Good agricultural practices are recommended to keep rubber tree strong and healthy. The practice involves manuring, weed control, pest and disease control.

* The use of disease-tolerant clones: RRIC 100, RRISL 2006, IRR 112, CEN4, PB235, BPM1.
* Avoid tapping irregularity.
* Prevent tree stress.
* Apply fungicides.

Apply chemical fungicides

Fogging :

- Fogging with hexaconazole-250ml+500ml water and 2-litre diesel+ 50ml emulator per hectare/need to apply at night- early morning when there is dew.
- Apply this when it's at phase B- the bronze stage of the leaves during refoliation season.
- Every two weeks until attaining 20% of the canopy.

Spraying :

- Spraying with hexaconazole, 1 to 2-litre fungicide per hectare and propiconazole, thiophanate-methyl, 1 to 2 litre per hectare (ground spraying).
- Every two weeks for spraying interval.

Dusting :

- Dusting sulfur when leaf flush (0-21 days).
- Dusting can be given at a dose of 5-7 kg/ha, 1 week intervals with 4 applications and dusting is carried out at 02.00-06.00 hours (night –early morning).
- Sulfur criteria used. 1) Fine powder, more than 90% pass through 300 mesh sieve, 2). Moisture content is less than 3%, 3) Sulfur content is more than 90%.

Fig. 2: Fungicide application

Status Report of New Colletotrichum Circular Leaf Spot Disease of Rubber (*Hevea brasiliensis*) in India and Its Management

Shaji Philip and E.Edwin Prem
Rubber Research Institute of India

Leaf fall due to new Colletotrichum Circular Leaf Spot (CCLS) disease was reported during May to November from the traditional rubber growing areas of Kerala, India from the year 2017 onwards. A Heavy leaf fall was observed in the trees of mature and immature infected plantations. The initial symptom is pin spot with yellow/brown halo initially, which turns into white discoloured round spot on the mature leaf and later leading to leaf fall. The spot size varies between 1-2 cm diameter. Abnormal Leaf Fall Disease (ALF) and CCLS occur together in many locations, but the extent of leaf fall that can be attributed to each disease is unclear as of now. The disease was first noticed in a private mature plantation near Paika in Kottayam district during 2017. Heavy leaf fall was noticed, after prophylactic spraying of oil-based Copper oxychloride to control abnormal leaf fall. The fallen leaves exhibit two to three circular leaf spots. Disease spread was observed in subsequent year (2018) in the nearby areas (Palai, Erattupetta, Chettuthode and Chengalam). In 2019, the spread of the disease was observed from Punalur to Thrissur which caused severe leaf fall in estates and small holdings. During 2020, the disease was noticed in the large estates of Kanyakumari, Kannur district and Sullia regions of South Karnataka. In 2021, the disease was observed during summer rains in May in the plantations of North Malabar, Kanyakumari and South Karnataka districts. Leaf fall was observed in three spells. A disease map was developed for CCLS disease.

CCLS samples were collected from different hot-spot regions and the pathogen was isolated and the pathogenicity was confirmed. The fungus was identified by sequencing 7 genes viz. Actin, Calmodulin, Glyceraldehyde 3 Phosphate dehydrogenase, Glutamin Synthatase, Chitin Synthase, ITS and Tubilin. The isolates are belonging to be *Colletotrichum siamense* and *C. fructicola*. Whole genome sequencing of the pathogen was also carried out. The genome size is 57.46 MB. The genome has 98% completeness, a total of 16,254 protein coding genes were predicted. A total of 993 proteins were predicted having CAZymes function. The genome analysis is in progress.

The same disease symptoms were observed in other hosts in the rubber plantation. The fungus was isolated from the infected leaves and are belonged to *Colletotrichum* spp. The cross infection of isolates from other hosts on rubber leaves gave same symptom and rubber isolates also produced same symptom on other hosts. The faster infectivity and sporulation were observed on other hosts on inoculation, this may be a reason for the faster spread of pathogen during favorable season. In 2021, based on our survey, all the recommended clones were susceptible to CCLS disease. The disease begins after summer rains and record two to three disease peak spells. We found that prophylactic spraying of oil-based COC could effectively control CCLS as well as ALF in mature plantations and Mancozeb (0.2%) for immature area. The results are promising and a recommendation will be made after completion of multi-locational trials during 2023. (A package to control both ALF and CCLS). The diseases may extend immaturity period in immature plants and reduce latex volume in tapping trees. During 2021, Rubber Board has undertaken a massive spraying in 1000 ha area (hot-spot) affected with the CCLS disease. The results were promising. Plant Pathology division has also made random survey in the sprayed area of Taliparampa, Sreekandapuram, Thrissur, Kanjirapally and Pala regions. We have found very good leaf retention in the oil-based copper oxychloride sprayed areas. *In vitro* screening of bacterial endophytes was carried out with the CCLS –pathogen and two isolates gave good antagonism. Considering the importance of the new circular leaf spot disease, the prophylactic spraying is being extended during 2023 also. This year, Rubber Board of India supplied inputs to growers for spraying 10000 ha (prophylactic spraying of Oil based COC). Testing of new molecules and delivery devices are progressing. We have standardized the crown budding technique using the crown FX 516 which is tolerant against leaf diseases. The nursery crown budding is recommended to growers. Field crown budding of popular clones with FX 516 is under progress. Our present attempt is to develop crown budded plants in root trainer cups.

Farmer Cooperatives – Cornerstone to Bring Excellence and Sustainability in Rubber Agriculture

Jacob Mathew
Fellow, International Rubber Research & Development Board

Smallholders represent the major chunk of rubber growers in Kerala's economy, the major rubber-producing state of India. Until 1986, small rubber growers were unorganized and rubber processing was unscientific. Therefore, the quality of the product was very poor. Hence, they get low price for their product. In order to solve this problem, Rubber Board of India took the first initiative in 1960s by starting Rubber Marketing Cooperative Societies. However, it could not reach out to resource-poor growers in rural areas. The political and bureaucratic control in these cooperatives was a hindrance to promote the self-help concept. In order to solve this and to promote the self-help concept, the Rubber Board promoted the formation of small voluntary associations of small growers registered under the Charitable Societies Act called Rubber Producers Societies (RPS) in 1986.

In India, according to recent estimates, there are about 2304 RPS, which function as self-help groups that aim the economic and social empowerment of rubber growers. It is a non-political group and works on a democratic line. Administration is carried out through an elected Director Board headed by a President and Directors are elected rotationally. RPS assists in the transfer of new technologies to members. It also assists common marketing of members' rubber grade-wise and at remunerative prices. This is considered as one of the greatest merits of the RPS. This has improved the living condition of small rubber growers as they get better prices for their products. RPS also establishes and runs common crop processing facilities that help members in upgrading the quality of rubber. Provision has also been given for the treatment of the effluent generated during sheet processing for biogas generation. Members of RPS receive of various inputs from the Rubber Board and other possible sources and distribute them among eligible members. There are also processing companies and trading companies in the rubber-growing areas in Kerala. Most of them are private companies jointly owned by the Rubber Board and RPS in the concerned areas.

Most recently, Farmer Producer Companies have been promoted by the Govt. of India with an objective to make the farmer entrepreneurs through agribusiness activities. A few rubber farmer producer companies have been formed in India and started rubber products manufacturing businesses using their own crop of their plantations.

Support from the Rubber Board and various civil service organizations is being given for capacity building in good agricultural and scientific processing practices to the members of these societies. Under the present crisis in the rubber plantation sector, such empowerment programs, drive farmers to adopt good farming practices and ancillary income generation opportunities for revenue generation from plantations. Adoption of quality raw material production practices also would realize more income, whereby, rubber production could be more sustainable and economically viable.

Even though farmer cooperatives have their own limitations, the formation of RPS has improved the welfare of small rubber growers. The quality of rubber produced has significantly been improved through group processing. This has increased the income of the small rubber growers which has also improved their living status. RPS has also encouraged a cooperative spirit and provides quality and expert help at their doorsteps. Farmer Producer Companies which is the hybrid version of cooperative and private limited company concepts would make rubber farmers entrepreneurs eliminating involvement of intermediate participation in trading.

Cooperative movements in the rubber plantation sector help the struggling farmers to be more competitive and self-reliant. All in all, cooperation helps rubber producers get more with less.

The Examination of Leaves Infected with Pestalotiopsis Leaf Disease Using a Field Emission Scanning Electron Microscope

Murnita Mohmad Mahyudin
Malaysia Rubber Board

ABSTRACT

Pestalotiopsis Leaf Fall Disease (PLD)is a new disease of rubber. Its effect to the rubber industry is detrimental, causing almost 100% defoliation and more than 50% yield reduction. It has spread to almost every part in Malaysia, affecting more than 73,000 hectares of rubber areas. The factors that trigger the outbreak is unknown and warrant further research. Experiments are initiated involving studies on the pathogen identity, presence, behaviour and pathogenesis. All these are performed by observing the spore abundance, dispersion, survivability and symptom development. Observing the infected leaves under a microscope is one of the approaches used to identify the PLD pathogen. The images of spores infecting the foliage under compound and stereo microscopes were extremely small, making them difficult to view and identify. Therefore, observation using a field emission scanning electron microscope (FESEM) was attempted. We expect to observe dominant spores on the surface of the infected leaves, which can be used as an indicator or as a clue to the identity of the causal pathogen. In this experiment, FESEM imaging uncovered numerous spores of various fungi on the leaf surface. More fungi were found on newly emerging lesions than on older lesions. Hypha networks were also noticed in all samples, including healthy ones However, penetration of hyphae was observed on diseased leaves only. The majority of spores observed in this study were commonly found during isolations of PLD-infected leaves. Based on observation, they were believed to be *Colletotrichum* sp., *Bipolaris* sp., *Pestalotiopsis* sp., and *Letendraea* sp. The round-shaped spores may have been *Nigrospora* sp or *Guignardia* sp. Generally, spores observed by FESEM were similar to those obtained during diseased leaf isolation. The objective of this paper is to share the most recent findings from the investigation into the causative agent that causes the Pestalotiopsis Leaf disease that affects *Hevea brasiliensis* in Malaysia.

INTRODUCTION

Pestalotiopsis Leaf Fall Disease (PLD)is a new disease of rubber. Its effect to the rubber industry is detrimental, causing almost 100% defoliation and more than 50% yield reduction. It has spread to almost every part of Malaysia, affecting more than 73,000 hectares of rubber areas. The factors that trigger the outbreak are unknown and warrant further research.

Hitherto, scarcity of compelling information on the influence of environmental factors has caused cost-effective and socially acceptable preventive actions from being devised. Meanwhile, limited reference and information available for this disease and its causal pathogen (s) make it rather difficult for MRB to quickly devise eradication and management strategies.

In order to design effective control strategies and disease management, knowledge on the causal agent and disease cycle is paramount. Epidemiology study helps to understand the synergism among host, pathogen and environment in disease occurrence and development. In this study, the disease aetiology and contribution of the environmental factors to disease outbreak as well as the behavior of the pathogen in the field would be elucidated. The environmental factors would possibly play a very significant role in the disease outbreak, considering *Pestalotiopsis* sp has been co-existing with rubber trees for such a long time.

The important part of the project is elucidating the microorganism that is causing the disease. Experiments are initiated involving studies on the pathogen identity, presence, behaviour and pathogenesis. All these are performed by observing the spore abundance, dispersion, survivability and symptom development. A spore trap will be set up at selected locations to study the spore abundance and dispersion, while symptom and disease development would be carried out by observing tagged leaves. Microbiome presence on the leaf surface will be observed too, microscopically.

Disease diagnosis was performed by proving the Koch's Postulates using the fungi obtained from the isolations of infected leaves. Results from spore trapping served to validate the choice of fungi for the purpose of artificial inoculations. However, more often than not, artificial inoculations have proven to be ineffective in achieving desirable outcomes. As a result, the focus has shifted towards investigating the microbial community present on phyllophanes.

By the end of the study, we hope to get some information on the causal pathogen, its behaviour and life cycle, factors influencing the disease occurrence and development as well as clonal susceptibility to the disease. However, this paper will solely focus on the observation of fungi present in the diseased leaves.

MATERIALS AND METHODS

Field Emission Scanning Electron Microscope (FESEM) observation.

Observing the infected leaves under a microscope is one of the approaches used to identify the PLD pathogen. Previously, several observations were made using both stereo and compound light microscopes. However, the images of spores infecting the foliage were extremely small, making them difficult to view and identify particularly those whose cells were partially buried in leaf cells.

Therefore, observation using a field emission scanning electron microscope (FESEM) was attempted. FESEM produces images that are three to six times clearer and less electrostatically distorted than the scanning electron microscope. We expect to observe dominant spores on the surface of the infected leaves, which can be used as an indicator or as a clue to the identity of the causal pathogen.

Leaf samples with two stages of PLD lesions were sent for microscopic viewing (Figure 1). In MRB, the service is provided by G-TAC, and the method employed is an in-house test method.
The samples were chemically fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer, followed by washing with 0.1M phosphate buffer, and finally dehydrated with a graded series of ethyl alcohol prior to the drying process using a Critical Point Dryer (CPD). CPD is the most commonly used dehydrating method for biological sample preparation. The specimens were then prepared for examination by evaporatively coating them with an ultrathin layer of platinum in a high vacuum environment. This provides a conducting layer that enables examination using JEOL JSM 7601F Plus FESEM. FESEM was operated at a 1 kV accelerating voltage at a variable working distance and a 0° tilt angle using the lower electron image secondary detector (LEI).

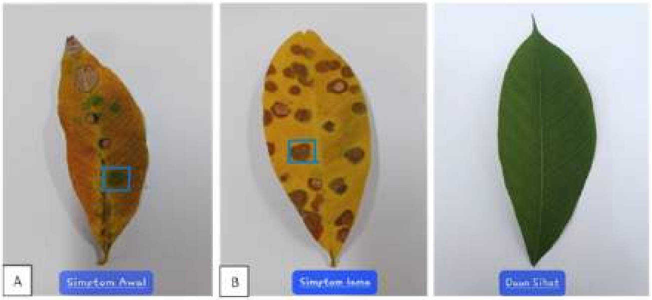


Fig. 1: Two stages of lesions used in this study in comparison with a healthy leaf.
A) The newly emerging lesions that were greenish in colour.
B) The typical dark brownish lesions.
C) Healthy leaf.

RESULTS AND DISCUSSIONS

Although light microscopy is a useful tool for observing plant–microbe interactions, it lacks the resolution to observe these interactions in fine detail. Other microscopes, such as confocal scanning, can be used but require organisms to be tagged with fluorescent reporters. SEM allows the researcher to examine plant–microbe interactions at high resolution without transforming the plant or microbe with a fluorescent reporter.

In SEM, a highly focused beam of electrons moves across a sample. In this experiment, FESEM imaging uncovered numerous spores of various fungi on the leaf surface. They varied considerably in size and shape. More fungi were found on newly emerging lesions than on older lesions. Hypha networks were also noticed in all samples, including healthy ones (Figure 2). However, penetration of hyphae was observed on diseased leaves only.

In the newly emerging lesions, ovoid, non-septate, short, and cylindrical spores, which are believed to belong to *Colletotrichum* sp., were more visible than other spores. Additionally, spores of *Pestalotiopsis* sp. and *Letendraea* sp. were also noticed (Figure 3).

The majority of spores observed in this study were commonly found during isolations of PLD-infected leaves. Based on observation, they were believed to be *Colletotrichum* sp., *Bipolaris* sp., *Pestalotiopsis* sp., and *Letendraea* sp. The round-shaped spores may have been *Nigrospora* sp or *Guignardia* sp (Figure 3 and 4).

A seta was spotted in one of the images of older lesions. This could indicate the involvement of *Colletotrichum* sp in the pathogenicity. Hence, from the images, penetration of hypha into leaf cells was observed in all spores, indicating that all of them contribute to the pathogenesis. Nonetheless, this is probably a secondary infection, as these situations were observed mostly on images of older lesions.

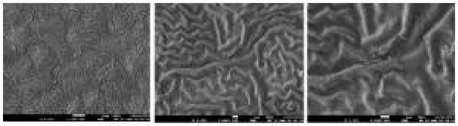


Fig. 2: Adaxial surface of a healthy leaf. There were hyphae ramifying on the leaf surface however there was no penetration since lesions were not observed.

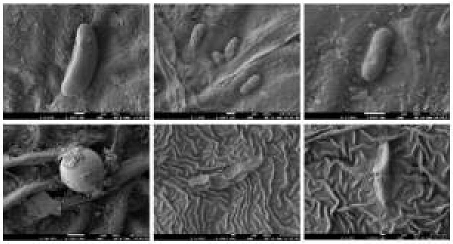


Figure 3: FESEM images of newly emerging lesions. A-C) Ovoid, non-septate, short, and cylindrical spores, probably belong to *Colletotrichum* sp. D) A round shape spore. E) Pestalotiopsis sp spore. F) Letendraea sp. spore.

The causative agent responsible for Pestalotiopsis Leaf Disease is a subject of ongoing debate among researchers. Various publications have reported divergent findings during the diagnosis of the disease. In most studies, several species of *Colletotrichum* and a few *Pestalotiopsis*-related genera were identified to be involved in the disease onset (Pornsuriya et al., 2020; Aliya et al, 2022; Kusdiana et al, 2020). However, there are other studies that have elucidated the role of other fungi in the pathogenicity. Guevara et al (2022) identified *Phyllosticta capitalensis* as the primary pathogen responsible for the disease in Colombia meanwhile, *Calonectria follicola* was associated with the disease in Thailand (Thaochan et al., 2022).
There is possibility that the disease is caused by pathogen complex since a persistent presence of a group of fungi has been observed both during the isolation of diseased leaves and through microscopic examinations. In most cases, the spores seen by FESEM were the same as those acquired from isolation of infected leaves, all this while. Multiple pathogenic species can often infect numerous plant species simultaneously (Fitt et al., 2006). Although mostly observed in arable crops, there is possibility that co-infection occurs in perennial crop as well.
During the study, attempts were made to artificially induced the lesions using the fungi commonly obtained during the isolations of infected leaves. However, the results were unsatisfactory. Infection using a single pathogen often failed to generate lesions. In certain instances, an individual microbe infection may not elicit severe disease symptoms. However, when co-infected with another microbial species, the plant may experience the development of severe disease due to synergistic interactions (Zamir et al., 2023, unpublished data). Regrettably, thus far, the anticipated outcomes have not been achieved, even in cases where various combinations of pathogens were used.

FESEM was attempted as a potential alternative, with the hope that by observing the microbial community on the phylloplane, we could deduce some conclusions on what the causative agent is. Generally, spores observed by FESEM were similar to those obtained during diseased leaf isolations. However, still, the pathogenesis could not be determined and concluded since no single or dominant spores that could be used as an indicator were observed.

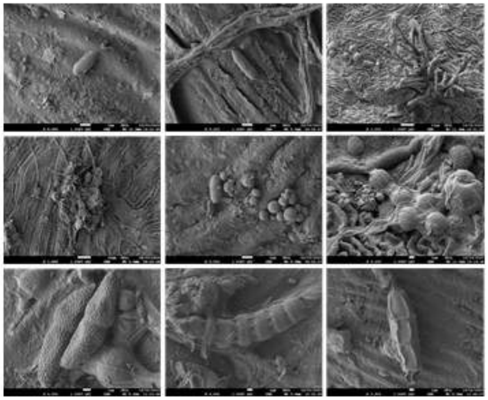


Fig. 4: FESEM images of old lesions.
A-B) Ovoid, non-septate, short, and cylindrical spores, probably belong to *Colletotrichum* sp.
C) A bristle, hair-like structure called seta which is commonly observed in *Colletotrichum* sp.
D-F) Round shape spores that may have been *Nigrospora* sp or *Guignardia* sp.
G) *Letendraea* sp. spore. H) *Bipolaris* sp spore.

CONCLUSION

The microbes observed under FESEM were comparable to those obtained when diseased leaf isolations were performed. Hence, the determination and conclusion of the pathogenicity remain elusive since there were no single or dominating spores that could serve as reliable indicators observed. Therefore, the possibility that the disease is caused by a pathogen complex cannot be disregarded, as a presence of a cluster of fungi has been consistently identified during the isolation of affected leaves and subsequent microscopic analyses.

Unravelling the Complex Interactions of Fungal Pathogens in *Pestalotiopsis* Leaf Fall Disease Of *Hevea Brasiliensis*

Mohamad Zamir Hadi bin Ismail
Malaysian Rubber Board

ABSTRACT

The emergence of *Pestalotiopsis* leaf fall disease (PLFD) has deeply impacted rubber plantations, resulting in substantial economic losses and repercussions for the country's rubber industry. In order to effectively manage this pressing issue, it is imperative to comprehend the factors influencing lesion occurrence and to ascertain the potential existence of fungal complex. This study was undertaken with the primary objectives of identifying the principal fungal pathogens responsible for the development of disease's lesion and investigating the role of co-infections in lesion occurrence. To address these objectives, a series of experiments involving five fungal isolates (*Neopestalotiopsis surinamensis, Colletotrichum conoides, Lasiodiplodia theobromae, Phyllosticta fallopiae, and Letendraea cordylinicola*) previously recognized via the Internal Transcribe Spacer (ITS) regions were conducted. These isolates were subjected to artificial *in vitro* inoculation on detached rubber leaves. Multiple combinations of these isolates were examined to assess their interactions and potential roles as causal agents of PLFD. Of paramount importance, this study unveiled the potential presence of a fungal complex associated with PLFD and provided insights into its epidemiology. The outcomes of this study contribute to a more comprehensive understanding of the disease, thus offering valuable guidance for the development of effective disease management strategies in *Hevea brasiliensis* cultivation in Malaysia. The objective of this paper is to inform the scientific community in particular the plant pathologists on the involvement of complex interactions of fungal pathogens in PLFD.

INTRODUCTION

The emergence of *Pestalotiopsis* leaf fall disease (PLFD) poses a significant threat to *Hevea brasiliensis* in Malaysia. PLFD has led to extensive defoliation, causing a substantial reduction in rubber latex yield and impacting the country's rubber industry (Adam et al., 2018; Syameel et al., 2020). The disease's outbreak has affected a significant portion of rubber cultivation in Malaysia and other rubber-producing countries. This study aims to comprehend the disease's impact and develop effective management strategies.

The pathogens responsible for PLFD infect rubber trees through wounds and natural openings, resulting in leaf spot symptoms and subsequent leaf fall. Environmental conditions, rubber tree cultivars, and cultural practices contribute to the disease's spread and severity (Adam et al., 2020). *In vitro* artificial inoculation is a widely used technique in plant pathology, but conflicting results have arisen when applied to PLFD, indicating potential complexities. Co-infection mechanisms among fungal pathogens have not been extensively studied in *H. brasiliensis*. This study seeks to address this knowledge gap by evaluating individual and combined effects of different fungal populations using an *in vitro* artificial inoculation technique with detached rubber leaves. The objectives include identifying primary fungal pathogens, investigating inoculation techniques and water sources, assessing the impact of leaf wounds, and examining co-infections' role in lesion incidence and disease severity. This research aims to uncover the fungal complex associated with PLFD and its interactions, contributing to a deeper understanding of disease epidemiology. The findings will aid in developing effective disease management strategies, such as targeted control measures, improved cultural practices, and resistant rubber clone selection. Ultimately, the goal is to mitigate PLFD's impact on rubber plantations, ensuring the sustainability of Malaysia's rubber industry.

MATERIALS AND METHODS

Five fungal isolates were individually tested to determine if pure cultures could induce lesion incidences. Moreover, different combinations of these fungal isolates (ranging from five to two fungi) were tested to assess whether interactions between the fungi contribute to PLFD development. Healthy mature *Hevea* leaves were used, and the experimental units consisted of five leaves per fungus or fungal combination, replicated three times. The leaves were sterilized, placed in sterile petri dishes, and exposed to different variables: type of water source (rainwater vs. sterile distilled water), presence of wounds on leaf surfaces (artificially wounded vs. unwounded), and type of fungus culture (spore suspension vs. mycelia on agar plugs).

For testing the effects of water source, leaves were placed in Petri plates filled with either sterile distilled water or rainwater. To examine the impact of wounds, some leaves were pricked with sterile needles before fungal application, while others were left unwounded. Two types of fungal sources, conidial suspensions, and mycelium on agar plugs, were compared for their effect on causing lesions.

The conditions were maintained at 26±1°C under fluorescent lamps during incubation. Artificial inoculation for combinations of pathogens followed similar procedures, focusing on water source and wound existence variables. Conidial suspensions of different combinations of pathogens were prepared and applied to the leaves. The study aimed to ascertain the contribution of these combinations to PLFD, considering potential interactions among the fungal isolates. The lesion incidence was evaluated based on the ability of fungal isolates to induce diseased spots on the detached rubber leaves. The assessment was made after seven days, and statistical analyses, including non-parametric Pearson's chi-squared tests, were used to assess associations, and fit between categorical variables related to lesion incidence.

RESULTS

The analysis utilized Pearson's chi-square tests and frequency tables to explore associations between categorical variables in the context of *Pestalotiopsis* leaf fall disease (PLFD) incidence. The investigation focused on fungal species, inoculation technique, water source, and wounding method's impact on lesion occurrence in healthy mature Hevea leaves. When considering fungal species proportions, a significant disparity in lesion incidence was found, supported by a low p-value (< 0.05) and a strong Bayesian Cramér's V effect size (0.65). *L. theobromae* was most likely to cause lesions (83%), followed by C. conoides (69%) and *N. surinamensis* (57%). *P. fallopiae* and *L. cordylinicola* showed lower lesion incidence (18% and 8% respectively), suggesting specific adaptations. Inoculation techniques using mycelial agar plugs or conidial suspensions exhibited no significant difference in lesion occurrence (p = 0.79). Similarly, water source (rainwater vs. distilled water) had no notable effect on lesion incidence (p = 0.61). The presence of wounds on leaf surfaces was related to lesion incidence, as wounded leaves exhibited a slightly higher proportion of lesions (41%) compared to unwounded leaves (37%). Examining combinations of fungi, significant differences in lesion incidence were observed. Certain combinations have initiated lesions more effectively than individual fungi. Notably, combinations involving *L. theobromae* displayed heightened lesion incidence, such as *L. theobromae* and *P. fallopiae* (92%), *L. theobromae* and *L. cordylinicola* (89%), and *L. theobromae* and *C. conoides* (86%). Additionally, a significant interaction was discovered between *L. cordylinicola* and *P. fallopiae*, suggesting an antagonistic relationship that hinders lesion formation. This finding suggests complex dynamics and interactions among these fungi, shedding light on the intricate nature of PLFD. The analysis unveiled distinct relationships between various categorical variables and PLFD incidence, highlighting the significance of fungal species combinations and the presence of antagonistic interactions in influencing lesion development.

DISCUSSION

The potential primary causal pathogens of *Pestalotiopsis* leaf fall disease (PLFD) were identified as *L. theobromae*, *C. conoides*, and *N. surinamensis* while *P. fallopiae* and *L. cordylinicola* play significant role when in combination mainly with *L. theobromae*. The phenomenon of plant-pathogenic fungi remaining dormant until favourable conditions arise was noted, suggesting a complex life cycle wherein these fungi can switch between harmless and infectious states (Newton et al., 2010). For example, *L. theobromae* exhibited endophytic behaviour and could shift to a pathogenic mode under abiotic stress conditions. This transition involved the degradation of certain host defense compounds and the expression of genes associated with secondary metabolic pathways (Paolinelli-Alfonso et al., 2016). Similarly, *Colletotrichum* species were noted for their hemibiotrophic lifestyle, involving biotrophic and necrotrophic stages of infection (De Silva et al., 2017). This dichotomy can lead to asymptomatic periods followed by necrotic phases, where the fungi destroy host cells. *Neopestalotiopsis* species were classified as endophytes, and their ability to transition to a saprobic phase under different host conditions was highlighted (De Silva et al., 2021). This flexibility could explain why these species primarily attack older leaves of rubber trees, causing visible lesions. In terms of inoculation techniques, while conidial suspensions were initially expected to be more effective due to potential sporulation and germination, the study found no significant difference between using mycelial agar plugs or conidial suspensions for inoculation. This suggests that the method of inoculation might have limited influence on lesion incidence. Regarding water source, the assumption that rainwater could encourage individual fungi to induce lesion incidences was disproven. Wet conditions, regardless of the water source, were sufficient for pathogenic fungi to cause lesions, indicating that as long as a wet environment is provided, pathogens can thrive. The impact of wounds on leaf surfaces was observed, with wounded leaves being more susceptible to developing lesions caused by pathogenic fungi. Wounding not only creates entry points for fungi but also releases chemical compounds or nutrients from the damaged tissue, potentially facilitating fungal growth.

In terms of co-infection and complex pathogen interaction, the study uncovered evidence of interactions between various fungal species in causing lesions. This phenomenon, known as co-infection, is not unique and has been observed in other plant-pathogen systems (Xu et al., 2005; Abdullah et al., 2017). It was suggested that certain fungi may act as predisposing factors, weakening the host defense systems and facilitating subsequent secondary infections by other fungi. The study concludes that co-infections might play a role in PLFD occurrence, highlighting the need for further research to understand the dynamics and mutual effects of these complex interactions.

A proposed method for identifying causal pathogens of PLFD involves a multi-step approach, including fungal isolation, clustering based on morphology, molecular identification, in vitro pathogenicity testing, and real-world environmental testing. Overall, the discussion provides a comprehensive analysis of the study's findings, linking them to existing knowledge of fungal behaviours, infection mechanisms, and complex interactions. It emphasizes the complexity of plant-fungus interactions and highlights the need for further investigation to unravel the intricacies of PLFD and similar plant diseases.

CONCLUSION

This study provides valuable insights into *Pestalotiopsis* leaf fall disease (PLFD) affecting *Hevea brasiliensis* in Malaysia. Key fungal species, including *L. theobromae*, *N. surinamensis*, *C. conoides*, *P. fallopiae*, and *L. cordylinicola*, were identified as significant contributors to lesion incidence and disease progression. The presence of multiple fungal species suggests their collective influence on disease severity, underlining the importance of studying interactions among fungal pathogens. These findings underscore the necessity of controlling primary fungal pathogens and acknowledging the potential synergistic effects of co-infections. The study marks the first comprehensive report of a potential fungal complex linked to PLFD. The identified isolates pave the way for future research aiming to replicate PLFD symptoms under real environmental conditions, whether as primary pathogens or co-infections. This investigation contributes to a deeper understanding of PLFD's epidemiology and assists in devising effective disease management strategies for *H. brasiliensis* plantations in Malaysia and beyond.

Sustainable Soil Fertility Management of the Rubber Growing Soils of Sri Lanka and Efforts Taken to Control Disease Severity

R. P. Hettiarachchi, H. A. R. K. Jayawardana, and L. A. T. S. Liyanaarachchi
Rubber Research Institute of Sri Lanka

The rubber-growing soils mainly belong to Red Yellow Podzolic (RYP) and Reddish Brown Latosolic (RBL) great soil groups and are classified as Ultisols according to the USDA soil taxonomy (Mapa et al., 1999). The fertility of rubber-growing soils is continuously declining due to the cultivation of rubber as a monocultural cropping system for more than a hundred years. The degradation of soil physical, chemical and biological properties is stimulated by soil erosion, reduction of soil organic matter, alteration of pH and nutrient removal by crops as well as by leaching. In order to increase soil fertility and land productivity, it is essential to apply fertilizers and manure with matching land management. The Rubber Research Institute of Sri Lanka (RRISL) recommends fertilizer mixtures containing the major nutrients required by the rubber tree *viz* N (nitrogen), P (phosphorus), K (potassium) and Mg (magnesium) based on long-term field experiments (Yogaratnam *et al.,* 1984a; Samarappuli *et al*., 1993; Dissanayake *et al*., 1992 &1994 and Dharmakeerthi *et al*., 1997). At present, mineral fertilizers are considered the major source for maintaining the soil's chemical fertility. However, their widespread use has also focused on the possibilities of environmental pollution. Meanwhile, fertilizer prices have skyrocketed and that may badly affect the fertility management of rubber growing soils. Therefore, soil fertility has to be balanced through the efficient use of plant nutrients and through improved soil management practices.

Numerous workers have reported the efficiencies of organic manuring in increasing N, P, K and Mg levels and cation exchange capacity (CEC) of soil by increasing organic carbon content (Samarappuli, 1992b and Samarappuli *et al*., 1998). Improvement of water infiltration, water holding capacity, bulk density and soil porosity (Samarappuli *et al*., 1998) and reduction of soil erosion (Samarappuli and Kannangara, 1992) are some of the additional benefits achieved by organic manuring. While some of the plant nutrient requirements can be met through the application of organic materials available on the farm or in the community, such materials are frequently insufficient to replenish the entire dose of plant nutrients. Based on grower preference, nutrient resources availability and the results based on long-term experimental data, several recommendations were prepared for alone application of organic, inorganic and their combined use in an integrated manner (Hettiarachchi, 2022).

Among the organic sources, the application of biochar as a soil amendment and liquid organic fertilizer could improve the growth of nursery, immature rubber plants with some soil fertility parameters (Dharmakeerthi *et al*., 2010; Dharmakeerthi *et al*., 2012 and Dharmakeerthi *et al*., 2013).

The influence of leguminous cover crop plants on soil fertility improvement is found to be of considerable importance.

Furthermore, instead of considering single nutrients as a tool for judging plant nutrient status, the use of dual plant nutrient ratios has been initiated. Hence, the combination of the methods of diagnosis and Recommendation Integrated System (DRIS), Nutrient Balance Index (NBI), Compositional Nutrient Diagnosis (CND) and sufficiency, deficiency and excessive levels of plant nutrients were developed for efficient fertility management and maximizing the growth of rubber plants (Annual Review, 2022). This is brought about by the addition of organic matter and mineral nutrients to the soil through the natural decay of fallen leaves, stems, and dead roots (Yogaratnam *et al.,* 1984a). These materials mostly having low C/N ratio would be mineralized rapidly and quickly releasing their nutrients to the soil for uptake by rubber. In addition, it improved moisture storage capacity, CEC and decreased the bulk density of soils. Some time back the *Mucuna bracteata* was introduced to Sri Lanka and it performs better than that of the traditional cover crop *Pueraria phaseoloides*.

Among the organic fertilizer sources, biofertilizers have been recognized as an economical alternative to chemical fertilizers, and are less bulky compared to organic fertilizers. Considerable attention has been focused recently on biofilm biofertilizers and their potential could be observed to increase nutrient availabilities, some soil fertility parameters, plant growth of immature rubber and dry matter accumulation of rubber nursery plants and decrease nutrient losses in rubber growing soils (Hettiarachchi *et al*., 2014 and 2021).

Considering the consequent negative repercussions of currently using nutrient sources, attention was drawn to think of alternative novel approaches in fertilizer use. The use of slow-release fertilizers (SRFs) has been shown to reduce the risk of nutrient losses from the crop root zones, as the nutrient release rate is synchronized with crop nutrient demand (Hauck, 1985; Goertz, 1991; De Silva *et al.,* 1996, Chen and Wei., 2018 and Tubeileh, *et al*., (2023). Although SRFs could have more benefits in nutrient use, to date these products have been demonstrated to be exceedingly expensive. Therefore, the application of crops spread over a large area is an expensive operation and will not be practicable.

Considering above mentioned difficulties and constraints associated with different fertility management practices, attempts were made to introduce slow-release techniques i.e. Fertilizer Encapsulated Coir Brick (FECB) and Reusable Fertilizer Porous Tube (RFPT) for rubber plantations. Their application could significantly enhance the growth of immature rubber plants, their foliar nutrients and some soil fertility parameters compared to conventional recommended fertilizer application. Considering the measured assessments throughout the period of thirty-six months showed that there is a possibility of using a single application of FECB and RFPT as a substitute for the conventional split application of recommended fertilizers for immature rubber plants (Hettiarachchi, *et al*., 2019a, 2019b, 2022 and 2023).

It is widely recognized that nutrition can influence disease in crops and it is an important component of disease control (Huber and Wilhem, 1988). Gottstein and Kuc (1989) showed that dibasic and tribasic phosphate salts could induce systemic protection against anthracnose in cucumbers. Sweeney *et al*., (2000) showed that P had a moderately suppressive effect on wheat leaf rust. In addition, soil applied sulphur (S) was found to increase resistance against a variety of fungal pathogens on different crops (Salac, 2005; Schunug *et al*., 1997; Klikocka *et al*., 2005). Potassium deficient plants tend to be more susceptible to infection than plants receiving an adequate supply of potassium (Walters, 2007). The effects of silicon (Si) in reducing the incidence and severity of plant disease have been known for some time (Fauteux *et al*., 2005). Therefore, experiments were designed to evaluate any effect on the severity of the new leaf fall disease in rubber with different fertilizers including micronutrients and the beneficial element Si during the nursery stage of rubber. Further, experiments are being conducted with increased dosage of fertilizer in split applications in immature rubber instead of the conventional fertilizer recommendation. Application of organic source including Si; partially burned paddy husk, and different split applications of inorganic fertilizer on the disease is also under investigation. In addition, observation was undertaken to find the nature of the relationship that exists between the development of the new leaf fall disease in rubber and the major soil nutrient status in the field as a case study. However, there were scattered distributions of nutrient-high and nutrient-low areas in both disease-low and disease-high areas without any prominent relationship according to the spatial variability maps developed using GIS. As a perennial crop, rubber plants in the field take about 2-3 years duration to show the response of added fertilizer to them. This situation and the high variation of the disease severity with time have made it complex to identify the exact relationship between Nutrient management in rubber plantations and the severity of the new leaf fall disease.

Integrated Approach to Boost the Sri Lankan Rubber Plantation Industry

G.P.W.P.P. Seneviratne
Consultative Committee member, Rubber Research Institute of Sri Lanka

Sri Lanka being the country that introduced the rubber tree to the South East Asia has the longest history in rubber planting, dating back to 1876. Despite its mostly expected economical benefits, both in terms of rupees to the growers and dollars to the country, the environmental benefits received from rubber plantations have never been valued or rather appreciated enough within the country. Sri Lanka is rich in its biodiversity and rubber plantations contribute lavishly to protect flora and fauna, including some endergenious species. The cooling capacity of rubber trees exceeds that of many tree species. Its low demanding nature seems helping its existence in the country as present rubber cultivation is restricted to lands where nothing else can be grown. But, for further existence of the commercial plantation industry, it needs productivity improvement as early as possible. Our productivity levels are low due to not following recommended practices by the growers partly connected to poor extention services. The exact productivity figure is however masked by the incorrect statistics available on area under rubber. Rubber Research Institute ever since its establishment in 1909 has issued recommendations based on research findings. Obviously, the recommendations get changed from time to time and the growers are expected to adopt them for better performance. But the adoption rate of recommendations is very low and due to perennial nature of the crop the consequences are long term and irreversible.

Clone composition

At present, there are 44 rubber clones recommended under three groups. The clone composition is very imbalanced at present having a single clone, RRIC 121 covering 73% of the rubber extent. As 64% of the rubber lands are under smallholder farmers and only 4-5 clones are recommended for them, it may take a long period to see a balanced clonal composition. As an urgent remodel action, new clones were added to group I of the clone recommendation from 2022 onwards for the smallholder farmers to grow.

Plants and planting

A foolproof technogy is available to produce high quality budded plants in small size polybags in less than one year. It is highly recommended to establish clearing with the on set of monsoon rains and also to plant 10% extra plants within the clearing to fill vacancies during the first 3 years. Delay in planting causes heavy casualties and not maintaining additional 10% results in rubber clearings having weak trees and poor stands.

Exploitation and Tapping Panel Dryness

There are many stress-causing factors as far as rubber tree is concerned. High intensity tapping, drought, floods, diseases, malnutrition, and mechanical damages are some. Trees can withstand these factors only to a certain extent and then react for their existence. For over-harvesting, the reaction or the only precautionary action available to the trees is to dry out the tapping cut. If this over-harvesting continues, the tree will stop giving any latex and often there's no recovery. This condition is known as Tapping Panel Dryness or TPD. If over harvesting is continued in a field, gradually more and more trees will be affected by TPD and eventually, growers are affected by low crop or low productivity.

Since 2017 onwards, more growers shifted to low-frequency harvesting methods using yield stimulants mainly to solve problem of the "tapper shortage" and to lengthen the life span of virgin panels. The rule of thumb with Ethaphon is that the crop harvested should not exceed that of normal d2 harvesting. This cannot be checked or detected by RRI but is known to the owners of the fields. But this can be found out by the rate of TPD in the field, which is irrecoverable in most cases.

The potential and productivity

The potential yield of most of the recommended clones in Sri Lanka is in the range of 2500-3000 kg/ha. But, potential yields of Rubber clones are conditional! There are rubber fields in both the smallholder sector and in estates managed by Regional Plantation Companies which record 2000 - 3000 kg/ha rubber. Despite using high-yielding clones for re-planting and new planting programmes, the national rubber productivity in Sri Lanka has been below 1000 kg/ha/y for decades. Among the key reasons for the low productivity, low stand, poor growth, and incorrect harvesting practices are in the top. The real issue faced by the rubber Industry is the low productivity due to the presence of a high percentage of dry trees resulting very low stand of productive trees. The presence of weak trees or runts mainly due to poor immature upkeep and the use of poor quality plants too contribute to the low productivity of rubber trees. The closest reason for dry trees or trees with Tapping Panel Dryness is over-exploitation. Immediate remedial measures should be taken to prevent this from further increase.

Role model

When the factors which contribute to the high yields of individual fields available already are analyzed, it is always revealed that the stand per hectare in such lands is complete and the growth condition of the trees is satisfactory and uniform and up to RRISL standards.

More importantly, such clearings do not have any TPD affected trees which proves proper and recommended harvesting, especially the frequency of harvesting. Under every other day tapping known as d2 tapping allows 160 tapping days per year. But many fields reporting over 2500 kg/ha/year have been tapped only about 100-110 days with no yield stimulants or rain guards. This is a message to carry. Higher frequencies result in lower crops or lower grams per tree per tapping. Lower frequencies (without yield stimulants) induce lower stress on the trees.

Achieving the goal

The main reason for not obtaining the potential yield from clones is that the farmers have not understood the fact that the potential high yield of all improved clones is conditional. In other words, if only good agro-management practices are adopted the potential crop of that particular clone is achievable. Therefore, as long as we move away from good agricultural practices (GAP) we move away from harvesting potential yields from such clearings. Agro-management practices during the immature phase determine the quality of the mature clearing and the productivity level of that clearing. Once the clearing is in the mature phase i.e., after 5-6 years, very few options are available to improve the quality of the rubber field. The growth phase completes in 5-6 years and infilling is not possible after 2-3 years. Once the growth phase is over, the annual growth rate becomes very low, 2-3 cm per year. Therefore, for a clearing to reach tappability within 5-6 years, the following is important : Quality of the plants (not the clone), Condition of the land (slope, availability of terraces, drains, and cover crops), Agro-management during the immature phase (specially maintaining the stand and the nutrient management).

Way out

Establishing rubber clearings while adopting RRISL recommendations with the correct stand of high-quality plants and maintaining them up to harvesting age under best agro management practices will be the only way out of the low price or unpredicted price fluctuations. Neither we can influence the escalating COP nor the rubber price. If rubber land is properly established, maintained, and harvested as per the RRISL recommendations productivity of above 2500 kg/ha/year can easily be achieved. Then the price per kilo will not impact the profitability or sustainability. Further, the harvesting method should be to harvest only what the tree can give. Overexploitation can harm the rubber plantations within a very short period and also permanently.

Nutritional condition of the trees

Sri Lanka, the fertility levels and other physical properties of rubber growing lands are so low mainly due to replanting rubber 4-5 times in the same lands without proper rehabilitation and mainly without adopting soil conservation practices. Supplementary addition of organic fertilizers had been recommended for rubber but adoption rates are extremely poor. This situation was aggravated by the current situation of the unavailability of inorganic fertilizers and their high prices. Low nutritional levels during the immature phase result in irrecoverable damage to the trees associated with high TPD levels within a few years of harvesting. Low productivity is the final result.

Factors affecting the country's rubber production

The rubber production in the country from the year 2016 is given in the Table 1 and as it can be seen, there is a variation in the rubber production. There are various contributory factors for the increase or the decrease from one to the other though the trend is decreasing from 2016 to 2022 and the reduction is about 10.3%. The new leaf fall disease was detected in Sri Lanka in 2019 and the crop reduction from 2019 to 2022 is 5.2%. It should be stressed that the reduction of the crop is due to many factors, and the excessive harvesting is the most devastating of all as tapping panel dryness condition is irreversible in most of the time. Disease can be reversed through improved agro management practices.

Table 1: Annual rubber production in Sri Lanka (Thousand Metric tons)

Year	Rubber Production
2016	79.10
2017	83.07
2018	82.56
2019	74.75
2020	78.21
2021	76.88
2022	70.87

All recommended agronomic practices should be adopted specially to have the full stand of the trees in clearings below 2 years.

The ideal investment for the rubber industry is to make arrangements for the prospective growers to plant rubber using only “best quality rubber plants” and applying rubber fertilizer. Farmers are strongly advised to use slow-release techniques to apply fertilizer which requires only half the recommended quantity for normal application, as recommended by the RRISL.

Identification of Causal Organisms / Cultural and Reproductive Characters

T.H.P.S. Fernando and A.H.M.N.R. Aberathne
Rubber Research Institute of Sri Lanka

SCIENTIFIC PATH IN STUDYING THE BIOLOGY OF THE PATHOGENS

Diagnosis-Detective Skills are Necessary /Studying the Symptoms / Microscopic Examination / Isolation of Pathogens /Molecular / Serological Studies

Study the Symptomatology of the Disease

Number of samples collected
Different rubber growing areas
Different rubber clones
Different development stages

Study the New Population of the Causative Agents

Isolation of Causative Micro-organisms

Symptoms investigation
Incubating the samples moist chamber
Lesions / Scraping under the microscope
Timing of sterilization

Pure Cultures / Single Conidia / Storage with Proper labeling

Collection of Pathogen Cultures

Proving the Kock's Postulates Establishment of Pathogenesis

Study the New Population of the Causative Agents

Identification of the Pathogens

Morphological characterization

Molecular characterization

Study the Factors Affecting Conidia Production, Germination & Viability of Pathogen Population

Factors Affecting Growth of the Pathogen
Incubation period / Culture Media / Light conditions

Factors Affecting Conidia Production of the Pathogen
Culture Media /Incubation period / Light Conditions / Temperature / PH

Factors Affecting Conidia Germination of the Pathogen
Water / Incubation period / Temperature / Relative Humidity / PH

Factors Affecting the viability of Conidia Production of the Pathogen
Incubation period / Dryness / UV / Temperature / Relative humidity / PH

Study the Epidemiological Parameters on the Pathogen Population

Effect of Environmental Factors Affecting the disease severity
Rain fall / Sunshinee hours / relative humidity – morning & evening /
Temperature – min & max / Results from the Spore Traps

⬇

Use of population representative pathogen isolates
Fungicide screening programme

⬇

Use of population representative pathogen isolates
Hevea clonal screening programme

Screening of Fungicides and Chemical Management of the Circular Leaf Spot Disease in Sri Lanka

M. K. R. Silva and S.C.P. Wijayaratne
Rubber Research Institute of Sri Lanka

Finding means of managing the disease via chemical controlling is of uttermost importance in the development of any disease management protocol. The screening of fungicides is the first segment of the chemical management programme and has to be carried out in three levels: *in-vitro* level, nursery level and the field level.

1. *In vitro* screening of potential fungicides

Identification of potential fungicides is needed to be done through reference and a market survey. Screening of selected fungicides under laboratory conditions should be performed using three techniques: poisoned food technique, soil fungicide screening test and conidial germination inhibition test. During the *in vitro* tests, the selection of the suitable pathogen isolates is critical. Collection of the isolates of the current pathogen population, representing all the rubber growing areas and different clones is carried out. Then, the pathogenicity of the available set of pathogen isolates should be screened, clustered and based on the clusters, a set of pathogen isolates to be screened against is selected.

Poisoned Food Technique (PFT)

The potential fungicides in a series of concentrations have to be used to identify the effective range. The fungicide is incorporated into the autoclaved agar just before solidification, mixed well to get the desired final concentration. Sterilized distilled water is substituted for the fungicide in the control set. Into each Petri dish, the fungicide amended medium is dispensed. At the centre of each dish, a mycelial disc, taken from the periphery of actively-growing culture of the pathogen, is placed. Colony diameters are measured at the full fungal growth in the control plates.

Soil Fungicide Screening Test (SFST)

Top soil is air-dried and sieved to get an even particle size. A portion of soil (around 15 g) is placed in boiling tubes and autoclaved. The fungus is grown on the growth medium. A mycelial disc (around 10 mm diameter) obtained from the growing edge of the culture is transferred onto the soil surface of each tube. Thereafter, another 15 g of sterile soil is placed over the mycelial disc. Afterwards, 10 ml of the desired fungicide solution in different concentrations is gently poured into separate tubes. In the control experiment, 10 ml of sterilized distilled water is substituted for the fungicide. The open ends of the tubes are covered with aluminum foil, and are incubated for 24 hours at room temperature. Then the tubes are emptied, and the mycelial discs are washed with sterile distilled water to remove any adhering soil particles. The discs are then placed, mycelial surface down, on the growth medium in a Petri dish and incubated and observed for mycelial growth. At the end of incubation, the colony diameter is measured, and at each reading, the actual diameter is obtained after subtracting the diameter of the mycelial disc from each diameter reading. The mean of the two-diameter readings perpendicular to each other is taken as the average mycelial diameter of each plate. Percent inhibition of growth in each of the treatments are calculated with respect to the control by the equation given below.

The percentage of inhibition over control: $I = \{(C - T) / C\} \times 100$

Where, I = Percentage Inhibition over control

C = Growth of pathogen in control

T = Growth of pathogen in treatment

Conidial Germination Inhibition Test (CGIT)

Aqueous solutions of fungicides in different concentrations are prepared by dissolving appropriate amounts of each test fungicides in Mc' Cartney bottles. Conidial suspensions are prepared and the cultures are flooded with 10 ml sterile distilled water and the colony surface is mechanically disturbed with a paint brush to suspend conidia. The resulting suspension is filtered through muslin cloth and the concentration of the suspension is adjusted to 10×10^4 conidia / ml with sterilized distilled water. One ml of the conidia suspension and 1.0 ml of the test fungicide concentration is mixed separately in Mc'Cartney bottles and 0.02 ml drops of the mixture are placed on a glass slide. Conidial suspension in sterile distilled water is used as the control. For each concentration, six slides are used. Incubation is carried out in 100% relative humid chamber at $28 \pm 2°$ C. Percent conidial germination is measured after 12 hours of incubation.

2. Screening of potential fungicides under nursery condition

Before approaching the field-level testing of fungicides and their concentrations, the nursery-level testing is generally employed to determine the effect of selected fungicides on disease control. In addition, this screening, provides with a sound platform to evaluate the effectiveness of planned fungicide treatments on different leaf maturity stages, different disease development stages and different intervals of fungicide applications.

The fungicides to be tested under polybag conditions can be determined based on the effectiveness of the *in vitro* experiments. Polybaged plants are established under a mature rubber clearing with high disease severity, and therefore the natural inoculum potential is guaranteed. Then the fungicides are applied according to different treatments before the appearance of the lesions on the leaves. The interval of fungicide application has to be according to the treatment plann.

At the development of the lesions, the lesion percentage of polybag plants in each treatment is estimated as follows:

$$\text{Lesion Percentage} = \frac{\text{Number of leaflets with CLSD lesions}}{\text{Total number of leaflets}} \times 100$$

Thereafter, the advancement of the severity of the disease is assessed in regular intervals to evaluate the effectiveness of the relevant treatment.

3. Field screening of potential fungicides

It is apparent that the action of foliar application of fungicides into mature rubber plantations itself is a challenging task. In general, the success of any fungicide application protocol on a foliar disease of rubber not merely depends on fungicide formulation and their concentration but also on all four of the following factors,

i) The fungicide/(s) applied should be effective against the pathogen
ii) The concentration of the appropriate fungicide should be effective against the pathogen
iii) The time phase/(s) of application should be optimized against the pathogen (this will be based on the life cycle characters of the pathogen/(s) & leaf maturity stages of the host plant)
iv) Spraying technology should be optimized to reach the affected foliage and should facilitate the effective absorption

Therefore, these parameters are needed to be optimized in the field-level screening experiments so that the planned fungicide application treatments have to be carried out accordingly.

At the evaluation of the incidence and severity of the disease, sampling has to be carried out as to judiciously represent the whole treatment plot. Eg: A treatment plot consisting of approximately 400 trees is divided into 4 sections and in each section, it needed to go along the rows & get the reading in every 10th tree and on each sample tree, the disease level is denoted as per the following table:

Lesion Status (Disease Incidence)	Disease severity	Index of the tree
No Lesion	No Leaf Fall	0
Lesions on few leaves	No Leaf Fall	1
Lesions on <25% of the canopy	Leaf Fall <25%	2
Lesions on 26%to 50% of the canopy	Leaf Fall 26%to 50%	3
Lesions on51% to 75% of the canopy	Leaf Fall 51%to 75%	4
Lesions on almost all the leaves	Leaf Fall76% to 100%	5

Therefore, the Average Disease Severity Index (ADSI) of the treatment plot is calculated according to the following formula:

$$\text{ADSI} = \frac{[(0 * n1) + (1 * n2) + (2 * n3) + (3 * n4) + (4 * n5)] + (5 * n6)]}{N}$$

n1 = No. of plants representing index 0
n2 = No. of plants representing index 1
n3 = No. of plants representing index 2
n4 = No. of plants representing index 3
n5 = No. of plants representing index 4
n6 = No. of plants representing index 5
N = Total number of trees

Status of the Management of the disease in Sri Lanka

With the adoption of the above-mentioned screening protocols, fungicide systems with an effectiveness towards the disease were identified especially; under the *in vitro* and nursery-level experiments. At the field-level experimentation, the disease severity data of the fungicide spraying showed that the spraying had resulted a delay of disease incidence and severity and however, it has not imposed a significant effect on the disease development throughout the time. Under the height of this situation, RRISL has made interim recommendations on the disease management and advocate the methods to survive with the disease sustaining the rubber plantation industry, while the research programme is being continued for further improvements of the recommendations. However, at the same time, it should be noted that the application of chemicals would help to lower the disease severity, avoid dieback conditions and also to reduce the leaf fall incidence.

The current interim recommendation on the chemical management of the disease is given below.

- **Nursery stage (Poly bag & budwood)**

It is recommended to apply below fungicides alternatively.

Carbendazim (50% WP) in 2-3g/l

Hexaconazole (50g/l SC) in 2-3 ml

The frequency of applications should be adjusted according to the weather condition and the severity of the disease.

- **Immature Cultivation**

When the leaf fall is exceeding 10%, it is recommended to carry out a fungicide application. Enter

Carbendazim (50% WP) in 10g/l or

Hexaconazole (50g/l SC) in 10ml/l

- **Mature Cultivations**

It has been recommended to protect the mature plantations that have shown more than a 60% leaf fall during the previous year (September – October), with 03 rounds of fungicide spraying.

The first fungicide application could be done after the refoliation, when the plants are with apple green leaf stage. The second fungicide application is to be done after all the leaves have turned to semi-mature –mature leaf stages while the third fungicide application can be delayed up to a late stage just before the onset of the monsoon rains (somewhere around in the month of May, where the monsoonal rains start during the May-June period).

Some details of the chemical management strategies are given in the following table.

Fungicide	Dilution rate	Requirement/ha/ application	Requirement /ha
Carbendazim	10g/l	750g (capacity 15L x 5 tank)	1kg 500g (for 2 applications)
Hexaconazole	10ml/l	750ml Capacity 15L x 5 tank)	750ml (for 1 application)

Some general instructions of the adoption of these protocols are given below:

- Avoid rainy days while spraying fungicides & ensure to have a minimum of 5 – 7 hours dry spell after spraying.
- Apply the fungicides as early as possible in the morning.
- Chemical spraying should be well calibrated to facilitate proper spraying.
- Ensure to spray at least 05 spray tanks (75L) to cover 01ha of a mature cultivation. When using drones, it could be reduced to 45L-60L per hectare.
- Sprayers should be well calibrated to facilitate proper spraying.

At the practical application of the fungicides in the field there are several challenges to be dealt with:

- The tall nature of the trees (some trees grow up to a height of 25-30m)
- The dense canopy of the trees
- Undulating terrain of rubber lands
- Lack of appropriate machinery
- High cost of labour & chemicals
- Environmental concerns

Therefore, in order to overcome these challenges different application technologies have been introduced and some are under experimentation.

1. ***ASPEE Turbo Mist blower -*** (Four-men carried type)

Country of origin – India
Chemical tank 13L
4 stroke engine
Single cylinder petrol engine
4Hp (Horse power)
Weight 56kg

2. ***Cifereli Mist blower –*** (Knap sack type sprayer)

Country of origin – Italy
Chemical tank capacity 15L
2 stroke engine
Single cylinder petrol engine
5Hp (Horse power)
Weight 12kg

3. ***Drones***

Country of origin – China
Chemical tank capacity 20L
Operated by rechargeable battery

4. ***Fogging machines***

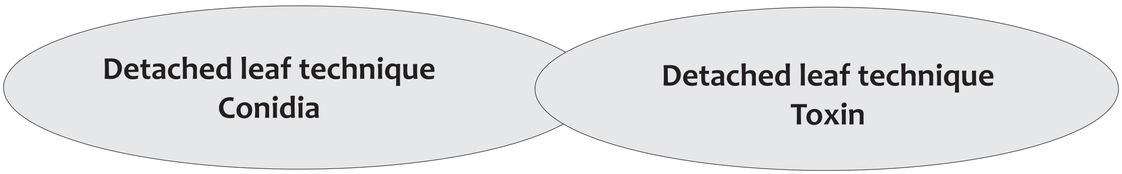
Country of origin – Korea
Chemical tank capacity 10L
Chemicals dissolved in – Diesel/O

Screening of *Hevea* Clones to Identify Disease Resistant /Susceptible Clones

T.H.P.S. Fernando, E.A.D.D. Siriwardena and S.C.P. Wijayaratne
Rubber Research Institute of Sri Lanka

The Circular Leaf Spot Disease (CLSD) caused by *Colletotrichum* spp. and Pestalotioides genera has caused global epidemic in many Asian rubber growing countries. Almost all the rubber clones are susceptible to the disease in various intensities. Presently in all rubber growing countries, there is a degree of uncertainty about the disease tolerance of recommended clones. Chemical control is not economically feasible to manage the disease in mature clearings in many rubber growing countrie and the only long-term solution is to cultivate disease tolerant clones.

Screening of clones – *In Vitro*



Detached leaf technique - conidia

Detached leaf method described by Brown & Soepena (1994) was slightly modified and used. Leaves of the late copper brown stage of the test clones were inoculated with the fungus by placing six, 20 µl drops of a conidia suspension (1 X 10^4 conidia/ml) on either side of the mid rib. The inoculated leaves were incubated at RT under 100% RH. Observations were made after three days of incubation and the lesions were grouped into one of the five categories based on the size of the lesions. Lesion groupings were, Group I – no reaction; Group II – pin point sized lesions; Group III – pin head sized lesions; Group IV – moderately extended lesions with slight growth of mycelium; Group V – more extended lesions with profusely grown mycelium. The testing of each clone should be repeated three times.

Leaf puncture bioassay - Toxic metabolites

Czapek dox liquid medium (100 ml) in 500 ml flasks were inoculated with three mycelial plugs (5 mm diameter). The cultures were incubated without shaking for 12 days at RT and at the end of the incubation period, the cultures were filtered through Whatman No 1 filter paper, Syntex funnels under vacuum and then through Millipore filters. The resulting culture filtrate was used as the source of the toxin.

Screening of clones in a bud wood nursery

Bud wood nursery experiments were established in different rubber growing agro-climatic regions of Sri Lanka. 50 clones clones under consideration for recommendation. The highly susceptible clones were also planted randomly to obtain the necessary inoculum for the test plants. The site selected was also adjacent to fields with susceptible clones thus ensuring the continued exposure of test plants to natural inoculum. The test clones were inspected for the incidence of CLFD for three consecutive years at 6month intervals.

Screening of clones under field conditions

Mature field clearings were selected from estates / RRISL collaborative trials, small scale and large scale plantings to all the test clones recommended by the RRISL. All clones were screened half yearly at 12 locations.

Assessment of disease incidence

A scoring system shown below was used to assess the disease incidence and severity.

Sampling techniques

Ten randomly selected plants per each clearing and another five plants per every 100 trees of the clearing were examined. To randomly select the test plants, each field was divided in to quadrants. The test plants were selected along the directions randomly.

Development of average disease severity index (ADSI)

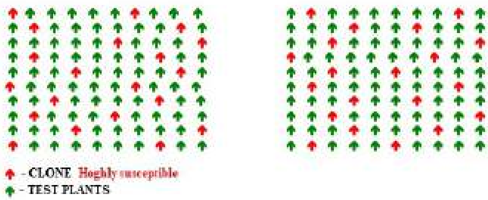
ADSI was calculated using the following formula

$$ADSI = \frac{[(0 \times n1) + (1 \times n2) + (2 \times n3) + (3 \times n4) + (4 \times n5)]}{N}$$

n1 = number of plants representing score index 0
n2 = number of plants representing score index 1
n3 = number of plants representing score index 2
n4 = number of plants representing score index 3
n5 = number of plants representing score index 4
N = Total number of plants examined

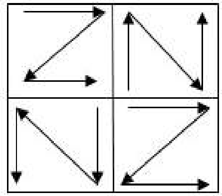
Ranking of clones was carried out based on the average disease severity index as follows

ADSI value	Description
0	No disease (Resistant)
0.01 - 1.00	Light infections (Mild)
1.01 - 2.00	Moderate infections (Moderate)
2.01 - 3.00	Severe infections (Severe)
3.01 - 4.00	Very severe infections (Very severe)



Layout of the test clones established in the bud wood nursery experiment with susceptible clone

Different sizes of lesions produced by two clonal materials assayed by detached leaf technique using toxic metabolite



The The directions of selection of test plants in quadrants

gories of lesions based on size. Group I – no reaction;
p II – pin point sized lesions; Group III – pin head sized lesions;
p IV – moderately extended lesions with slight growth of mycelium;
p V – more extended lesions with profusely grown mycelium

Different degrees of wilting caused by the toxic metabolite (leaf wilt bio assay)

Breeding Programmes to Strengthen the Disease Management

Samanthi Withanage
Rubber Research Institute of Sri Lanka

From the first introduction of *Hevea brasiliensis* to Sri Lanka in 1976, *Hevea* breeding has paved the way to improve the planting material genetically. Today, genetically improved material has the potential to be around 3,500 Kg/ha/year under commercial conditions. This is more than a ten fold increase in rubber yield since the introduction of the *Hevea* breeding programme in Sri Lanka, which has built up different breeding objectives with time. This initial seedling population has shown large variability in the yield. Until the 1920's, no significant effort had been made to improve this preliminary material.
However, the main objective of developing *Hevea* planting material in Sri Lanka at the beginning (1935 to 1954) was kept as increasing yield. The yield of selections from this phase was satisfactory and showed above average girth. However, the progeny of the 1941 HP program, which had parents PR 107, PB 86, Tjir 1, and Tjir 16, were susceptible to Phytophthora bark rot and Canker diseases. In 1952, steps were taken to exchange more clones among rubber growing countries and imported RRIM (Rubber Research Institute of Malaysia) 500 and 600 series from Malaysia, TR (Terres Rouges), IRCI, PR, GT, and AVROS clones from the Far East. With this progress in the mid-1950's, the rubber growing area was extended to the area above 300 m (1000 ft.) mean sea level, but the development of Powdery Mildew caused successive defoliation. Even in lowland areas, the yield dropped due to leaf fall by powdery mildew. With this bad experience, the next phase of *Hevea* breeding (1955 -1969) was started. Most RRIC clones (RRIC 1– RRIC 99) were to develop high yielding clones tolerant to Powdery Mildew and Phytophthora diseases.

Therefore, the breeding objectives were focused on high yield, vigour, and disease tolerance. However, when planning the breeding program during period, it was realized that the clone LCB 870 (Lands Caoutchouc Bedrijeven) was the only clone showed Powdery Mildew resistance. In the meantime, RRIC 52 (selected from Tjikadoe seedlings) was identified to exhibit resistance to Powdery Mildew with approx 20% leaf fall. Following the first "Non-Wickham introduction of *Hevea* material to Sri Lanka in 1955, it was focused to incorporate South American Leaf Blight (SALB), Oidium, and Phytophthora resistance to the local clones. In 1957, attempts were made to combine characteristics such as high yield, wind resistance, and vigour with the Powdery Mildew resistance of RRIC 52.

In the mid 1980's, a sudden susceptibility of two promising clones, RRIC 103 and RRIC 104, to Corynespora leaf disease (CLD) removed from the recommendation and even eradicated from the plantations, followed the clone RRIC 110 again during mid 1990's. Therefore, the measures were taken to strictly assess the genotypes established from 1974 HP to 1981 HP for CLS disease. Many promising genotypes were removed by having at least one susceptible parent in their parentage. Therefore, in breeding programs after 1986, utmost care was taken to select parents based on CLD resistance. However, out of twenty-seven RRISL 200 series clones, 18 had to be removed from the clone recommendation mainly due to their susceptibility to Corynespora leaf fall disease. Although the clones RRISL 201 and 208 perform well, they have recorded moderate and mild susceptibility to Corynespora leaf fall, respectively. The progeny of 2005 hand pollination, which had RRIC 100 and RRIC 103 as grandparents and RRIC 52 and PB 86 as great-grandparents, was screened with microsatellite molecular markers. However, only around 40% of field screening results agreed with molecular grouping, whereas 57 % did not agree, and around 3% of genotypes did not show a clear correlation. The Breeding programe towards developing CLD resistance continues with the fourth generation self-progeny of the RRIC 52 X PB 86 hybridization.

Today, fairly large numbers of genotypes belonging to HP progenies developed since 2000 are being tested at various stages of the breeding and evaluation process and are continuously assessed by RRISL Plant Pathologists. Focused on new circular leaf spot disease, all hand-pollinated progenies, breeding pool, and non Wickham germplasm collection are being screened at field level. During this comprehensive breeding and evaluation process (Figure 01), producing a clone with a high yield with all other secondary characters is challenging. Therefore, it is often attempted to develop a high yielding vigorous clone with one or two other important secondary characteristics, such as tolerance to important foliar diseases. Accordingly, the clonal recommendations are revised with time.

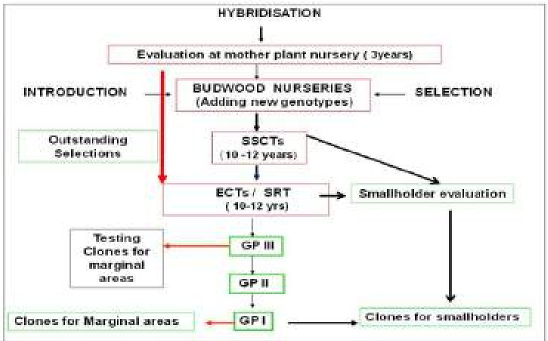


Fig. 1 : Hybridization, Evaluation & Selection of new Genotypes, and clone recommendation process

Economically Important Diseases of the Rubber Tree: Changing Scenario






The para rubber tree (*Hevea brasiliensis* Muell. Arg.) native to South America was introduced to Sri Lanka in 1876 by Sir Henry Wickham. Later, the commercial plantings were undertaken and with such domestication, the occurrence of rubber diseases also began. Mr. J.B. Caruthers, was the Government Mycologist to report the first disease from the rubber tree.

With further expansion of the cultivation, disease incidences too became prominent. Today, after a century of the discovery of the first disease, more than sixty five pathogens are recorded for the rubber tree in Sri Lanka. The relative economical importance of the diseases changed with time. Presently, about twelve number of diseases play an important role in the economy in the Sri Lankan rubber plantation industry.

Identification of Economically Important Leaf & Stem Diseases of Rubber

T.H.P.S. Fernando and A.H.M.N.R. Aberathne

Rubber Research Institute of Sri Lanka

The rubber tree is susceptible to many economically important foliar diseases; Powdery mildew, Colletotrichum leaf disease, Corynespora leaf fall disease and Phytophthora leaf disease. Chemical controlling systems have been identified for almost all of these diseases. As a result of extensive research programmes, the disease resistant *Hevea* clones have been identified. Hence integrated disease management protocols have been introduced.

Foliar Diseases

Disease	Causative fungus
Powdery mildew	*Oidium hevea* / *Erysiphe* spp.
Colletotrichum leaf disease	*Colletotrichum acutatum* & *Colletotrichum gloeosporoides*
Corynespora leaf fall disease	*Corynespora cassiicola*
Phytophthora leaf fall disease	*Phytophthora* spp.

Powdery Mildew

Powdery mildew - White powdery colonies of the fungus on semi - mature leaflets

Whitish powdery lesions later turn in to brown colour and remain on the leaf throughout the year - Old Oidium patches

Flowers are also atacked by Powdery mildew

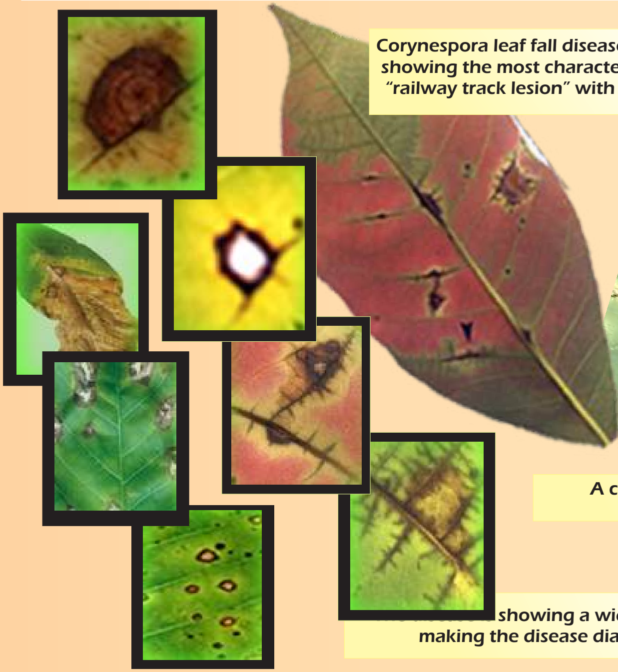
Broomstick appearance Canopy due to the leaf fall leaving the petioles

Colletotrichum Leaf Disease

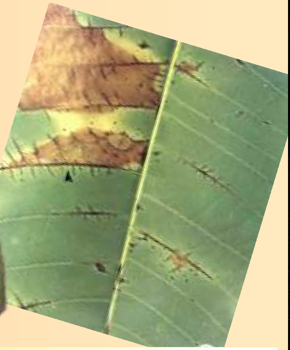
Colletotrichum infected leaves showing shriveling & tip die-back

Affected semi-matured leaves. Parts of the leaves are dropped away and blisters (raised heads) are also prominent

Corynespora Leaf Fall Disease



Corynespora leaf fall disease-affected leaves showing the most characteristic symptom - "railway track lesion" with blighted leaves



A close-up of the characteristic railway track lesion

showing a wide variety of lesion types making the disease diagnosis complicated

Corynespora-affected plants showing leaf fall condition

Abnormal Leaf Fall Disease
(Phytophthora Leaf Fall Disease)

Phytophthora affected pods showing coagulated latex drops

Affected leaflets
Dark brown lesions on petioles with coagulated latex drops in the midst of the lesions

Carpet of mature leaves on the ground in an epidemic year

The canopy depletion due to Phytophthora infection

The newly-recommended clones showing resistance towards abnormal leaf fall disease. Note the green canopy throughout the year

Stem & Branch Diseases

Phytophthora bark rot is the only economically important disease of the tapping panel or bark of the trees. Presently, most of the clones are resistant towards this disease. Adoption of rain guards also protects the panel from this disease. Ustulina stem rots are mostly seen on wounded trees. This disease is not very common in Sri Lanka but cause considerable loss when affected. Pink disease is not considered as a serious disease of rubber plantations in Sri Lanka.

Disease	Causative fungus
Phytophthora bark rot	*Phytophthora* spp.
Ustulina stem rot	*Ustulina deusta*
Pink disease	*Corticium salamonicolor*

Phytophthora Bark Rot

Characteristic symptom of the Bark Rot (Black Stripe) caused by *Phytophthora* spp. Note the sunken lesions just above the tapping panel

Bark Rot (Black Stripe) caused by *Phytophthora* spp. The bark is removed to show the vertical black stripes on the wood

Ustulina Stem Rot

A mature tree affected with *Ustulina* spp. with grey-white fruit bodies

A wind-damaged tree due to the ustulina disease

The prominent characteristic double lines on the infected wood

Pink Disease

Initial symptoms on the bark with latex oozing and cobweb stage on branches

Pink coloured encrustations on branches; the second stage of the infection

Identification of Economically Important Rubber Root Diseases

M.K.R. Silva and D.A.N. Mallikaarachchi
Rubber Research Institute of Sri Lanka

Disease	Causative fungus
White root disease	*Rigidoporus microporus*
Brown root disease	*Phellinus noxius*
Black root disease	*Xyllaria thwatsii*
Patch Canker disease	*Pythium* spp. & secondary organisms

White Root Disease

White root disease has become the most destructive disease in Sri Lanka & in many other rubber growing countries. This disease has become an important economic factor in the rubber plantation industry during the recent past as it is the main cause for reducing tree stand in rubber plantations. Maintenance of the recommended stand per hectare plays a vital role in obtaining higher productivity levels.
Rigidoporus microporus is a soil borne basidiomycete. It affects trees of any maturity killing them and subsequently spreading towards the adjoining trees. Despite of the effective disease management protocols available, it has been continuously highlighted that the incidence of the disease is showing an increasing trend. The reasons behind this have been understood.

- Currently, rubber cultivations in traditional rubber growing areas are mostly in their fourth or fifth generation. Hence, with time, the inoculum potential has built up.
- The number of alternative hosts for this pathogen has also built up. For example tea, cinnamon, forest trees, fruit crops, creepers have succumbed to this disease.
- In spite of the fact that growers are well educated of the land clearing protocols to be done at the time of uprooting & land clearing, they never practice them.
- Low rubber prices have always influenced on the implementation of agronomic practices.
- The current financial crisis faced by the rubber growers has discouraged the growers to adopt the recommended management protocols.
- The beneficial soil micro-organisms (inhabiting antagonistic micro-organisms) and the decaying micro flora have been depleted due to the intensive agricultural practices.
- The pathogen has also changed with time becoming more aggressive.

Foliar Symptoms : Yellowing & Buckling of leaves and pre-mature flowering

Appearance of yellow to orange coloured bracket like fructifications

Whitish rhizormorphs firmly adhered on to the root surface

Brown Root Disease

The causative agent is *Phellinus noxius.* This disease is significant in non traditional rubber growing areas like Moneragala, Padiyathalawa, Maha Oya and Vauniya. The causative agent is a facultative parasite and hence can live for a long time on decaying wood. The disease spread is mainly through root contacts. The diagnostic characteristic is the production of dark brown gummy mycelium with a heavy encrustation of sand and stones. During the later stages, dark brown, typical bracket like fruit bodies appear on the decaying stumps.

Bracket-like fruit bodies on a dead stump due to Brown root disease

Bracket-like fructifications Upper surface is brownish purple in colour

Infected root pieces

Black Root Disease

The causative agent is *Xylaria thwaitsii.* This disease is prominent in several areas located in the intermediate or dry zones of the country. The causative fungus is extremely slow-growing and exposure to sunlight is detrimental towards it.

The mycelium appears black in colour and later forms black patches. Fruit bodies arise on the soil surface as knob-like structures surrounding the plants.

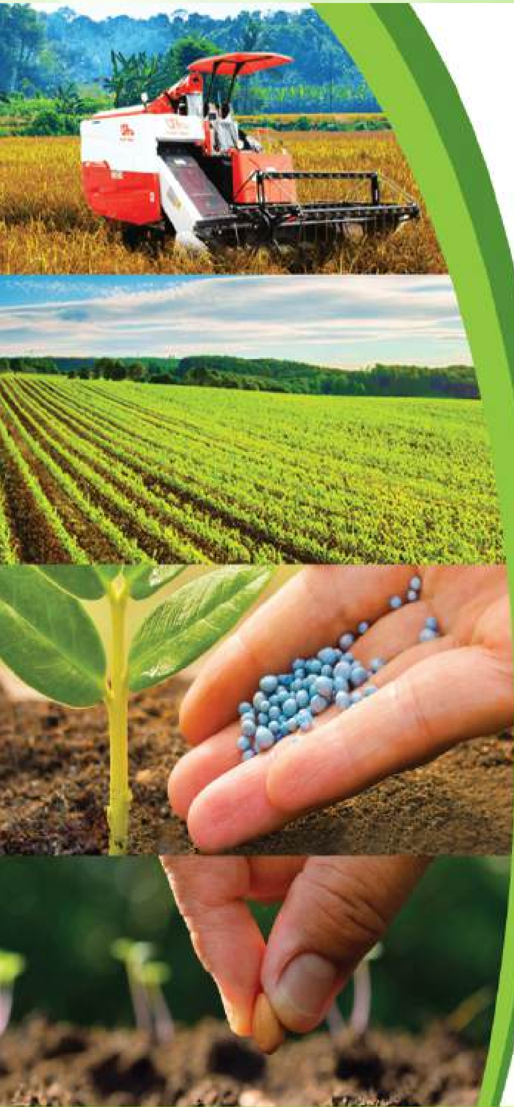
Blackish & finger-like clusters of fructifications of Xylaria thewatssii

A black root disease-affected tree

Patch Canker Disease

Affected plant showing latex exudation at the colar region forming latex pads

The primary causative agent is *Pythium* spp. and this disease is associated with several secondary micro organisms. The symptom of this disease is the formation of latex pads at the collar region by coagulation of latex . At the same time, invasion of secondary micro organisms forms extensive death of bark at the collar region some times leading the plant to death. Water-logging conditions, planting in rocky areas, mal-planting practices or any mechanical damage at the collar region may predispose the plants to this disease.



To Keep Rubber Diseases Away
We Need Your Cooperation